

Fibroblast Growth Factors/Fibroblast Growth Factor Receptors as Targets for the Development of Anti-Angiogenesis Strategies

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Abstract: Angiogenesis, the process of new blood vessel formation from pre-existing ones, plays a key role in various physiological and pathological conditions, including embryonic development, wound repair, inflammation, and tumor growth. The 1980s saw for the first time the identification, purification, and sequencing of the two prototypic heparin-binding angiogenic fibroblast growth factors (FGF) 1 and 2. Since then, 22 structurally-related members of the FGF family and different classes of FGF receptors have been identified. Several experimental evidences point to a role for various FGFs in the neovascularization process that takes place in inflammation, angioproliferative diseases, and tumor growth. Thus, the FGF/FGF receptor system represents a target for the development of anti-angiogenic therapies. Purpose of this review is to summarize the different modalities that have been approached to impair the pro-angiogenic activity of the FGF/FGF receptor system and discuss their possible therapeutic implications.

Key Words: Angiogenesis; endothelium; FGF; FGF receptors; inhibitors.

1. THE FGF/FGF RECEPTOR SYSTEM IN ANGIOGENESIS

Angiogenesis is the process of new blood vessel formation from pre-existing ones. Neovascularization is involved in embryonic development, wound repair, and inflammation [1]. Also, the local, uncontrolled release of angiogenic growth factors contributes to neovascularization that takes place during angiogenesis-dependent diseases, including cancer [2].

The 1980s saw the purification of the pro-angiogenic proteins fibroblast growth factor-1 (FGF1) and FGF2 [3]. Since then, 22 structurally-related members of the FGF family have been identified [4]. Among them, FGF1, FGF2, FGF4, FGF5, and FGF8 have been demonstrated to be endowed with angiogenic potential [5]. FGFs are pleiotropic factors that act on different cell types, including endothelial cells (ECs), by interacting with tyrosine kinase (TK) FGF receptors (TK-FGFRs), heparan-sulfate proteoglycans (HSPGs), integrins, and gangliosides. Several experimental evidences point to a role for FGFs in tumor angiogenesis, inflammation, and angio-proliferative diseases (discussed in [5]). Thus, the FGF/FGF receptor system may represent a target for anti-angiogenic therapies.

FGFs induce a complex "pro-angiogenic phenotype" in cultured ECs (Fig. (1)) that recapitulates the angiogenesis process *in vivo*, including expression of proteases, integrins, and cadherins and the stimulation of EC proliferation and migration (summarized in [6]).

Extracellular matrix (ECM) degradation, mainly by the plasmin-plasminogen activator (PA) system and matrix metalloproteinases (MMPs), represents an important step of the angiogenic process [7]. FGFs upregulate urokinase-type PA (uPA) and MMPs production in ECs [8, 9]. uPA converts plasminogen into plasmin that degrades different matrix proteins and activate MMPs [10].

FGF2 stimulates chemotaxis/chemokinesis in ECs [11]. When cultured on permissive three-dimensional matrix, ECs invade the substratum and organize capillary-like structures with a hollow lumen [12]. FGF2 enhances this response in collagen I [13] and fibrin [14] gels in a CD44- [15] and integrin- [16] dependent manner. Also, FGF2 promotes EC reorganization on Matrigel [17] that requires MMPs [18] and uPA [19] activity as well as $\alpha_6\beta_1$ integrin engagement [20], thus underlying the tight cross-talk among FGFs and the integrin receptor system (see below).

EC migration and proliferation are limited by lateral cell-cell adhesion and ECM interactions [21] mediated by cadherin and integrin engagement. Interestingly, FGF2 regulates the expression of different cadherins [21] and integrins [22] and the production of various ECM components in ECs [23], contributing to the maturation of the new blood vessels (Fig. (1)).

The angiogenic activity of various members of the FGF family has been demonstrated *in vivo* in different experimental models, including the chick embryo chorion-allantoic membrane assay [24], the avascular rabbit [25] or mouse [26] cornea assays, and the subcutaneous Matrigel implantation assay [27]. In these experimental models a potent angiogenic response can be obtained by the delivery of FGFs as recombinant proteins, *via* retroviral, adenoviral, lentiviral, and adeno-associated viral vector transduction, or *via* implantation of FGF-overexpressing cell transfectants. The latter approach allows the continuous delivery of FGF produced by a limited number of cells, thus mimicking more closely the *in vivo* situation [28]. For instance, the release of 1.0 pg FGF2 per day from viable cells triggers an angiogenic response in the chick embryo chorion-allantoic membrane assay quantitatively similar to that elicited by 1.0 μ g of the recombinant molecule [29]. These considerations may impact the design of FGF-antagonist strategies.

FGFs establish a complex interaction with EC surface [5]. As stated above, FGFs interact with TK-FGFRs and HSPGs [5]. Also, FGFs may require the engagement of the integrin receptor $\alpha_v\beta_3$ [30] and of cell surface-associated gangliosides [31] (Fig. (2)).

The four members of the TK-FGFR family [TK-FGFR1 (*flg*), TK-FGFR2 (*bek*), TK-FGFR3, and TK-FGFR4] are encoded by distinct genes and their structural variability is increased by alternative splicing [32]. TK-FGFR1 is expressed by ECs *in vivo* [33] and *in vitro* [6]. Less frequently, cultured ECs can express TK-FGFR2 [34], whereas the expression of TK-FGFR3 or TK-FGFR4 has never been reported in endothelium. The interactions of FGFs with TK-FGFRs occur with high affinity [dissociation constant (K_d) = 10-550 pM] and causes receptor dimerization and autophosphorylation of specific tyrosine residues located in the TK-FGFR intra-cytoplasmic tail. This in turn leads to the recruitment of intracellular messengers/adaptors that bind to phosphorylated tyrosine residues on the activated receptor [for further details see [35] and (Fig. (2))].

HSPGs are associated with the surface of ECs at densities ranging between 10^5 - 10^6 molecules/cell. They consist of a core protein and of glycosaminoglycan (GAG) chains represented by

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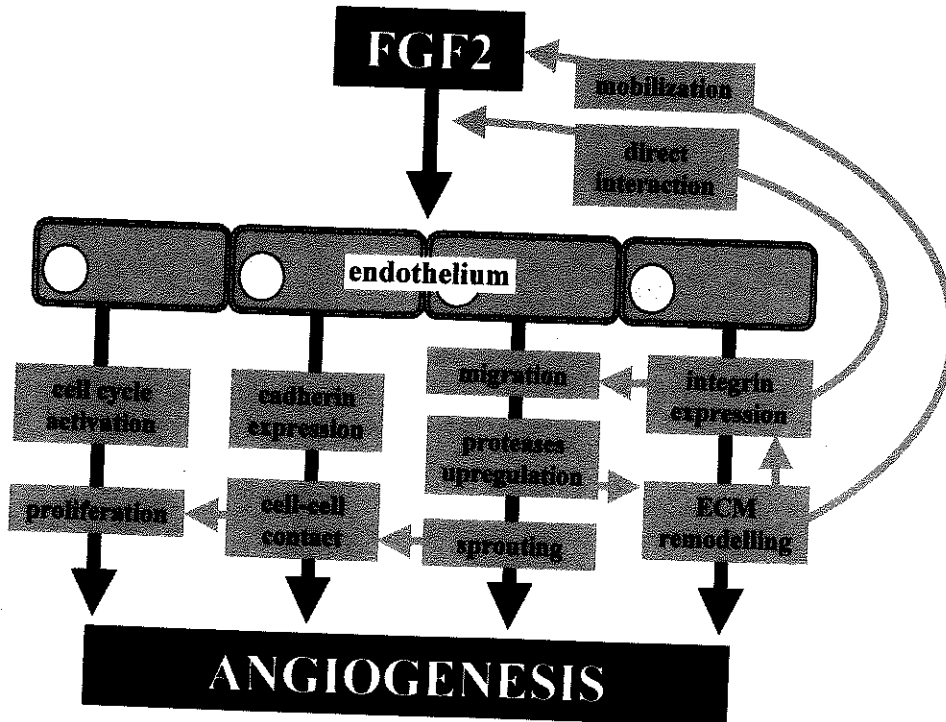


Fig. (1). Events triggered by FGF/FGF receptor interaction in ECs that contribute to the acquisition of the angiogenic phenotype *in vitro* and neovascularization *in vivo*.

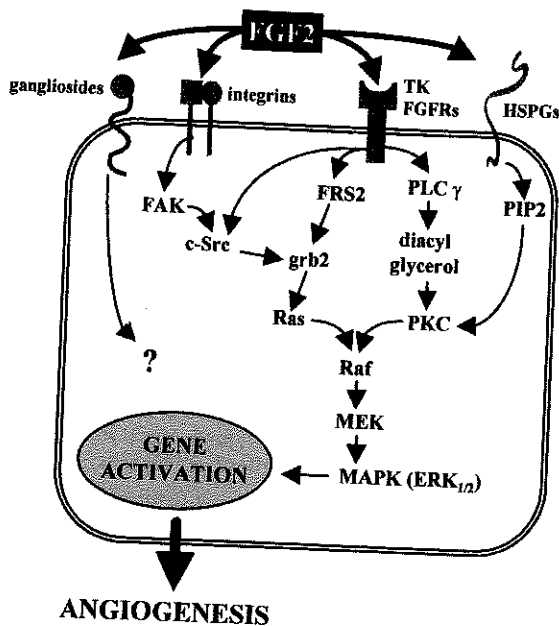


Fig. (2). Signal transduction pathways triggered by the interaction of FGF2 with EC integrins, TK-FGFRs, and HSPGs. Only second messengers converging to the Raf/MEK/MAPK pathway are shown. For more details about the second messengers activated by TK-FGFRs, integrins, and HSPGs see [35], [288], and [289], respectively. No data are available about the possibility that FGF2/ganglioside interaction may directly activate intracellular second messengers.

unbranched heparin-like anionic polysaccharides [36]. The interaction of HSPGs with FGFs occurs with low affinity ($K_d = 2-200$ nM) and is mediated by the negatively charged sulfated groups of the GAG chain [37] that bind to basic amino acid motifs present in the growth factor molecule [38]. FGF/HSPG interaction

modulate angiogenesis *in vitro* and *in vivo* by direct activation of phosphatidylinositol 4,5-bisphosphate (PIP₂) and protein kinase C (PKC)- α [39] that eventually lead to the activation of mitogen activated protein kinases (MAPKs) [40]. Also, HSPGs promote FGFs internalization [41] and present FGFs to TK-FGFRs in a proper conformation, thus facilitating the formation of productive HSPG/FGF/TK-FGFR ternary complexes [42]. Finally, HSPGs act as a reservoir for extracellular FGFs that are protected from degradation [43] and accumulate in the microenvironment to sustain a long-term stimulation of ECs [44]. Interestingly, FGF2 regulates the synthesis and release of proteases and glycosidases that digest HSPGs and induce the mobilization of free HSPG/HSPG chains [45]. Also, ECM degradation leads to the mobilization of entrapped FGF2 (Fig. (3)) with consequent activation of an angiogenic response [46]. The capacity of FGFs to complex HSPGs (as well as other ECM or serum components [5]) may modify their accessibility to neutralizing antibodies or antagonist compounds.

Integrins are transmembrane receptor heterodimers comprised of α and β subunits that mediate cell adhesion to a variety of adhesive proteins of the ECM [47]. Integrins regulate also the response of ECs to growth factors, including FGF2 [48]. In particular, $\alpha_v\beta_3$ integrin is expressed on ECs where it plays a central role in neovascularization. For this reason, $\alpha_v\beta_3$ is considered a target for the development of anti-angiogenic therapies [49]. Similar to classical adhesive proteins, FGF2 binds $\alpha_v\beta_3$ [30] with a K_d equal to 20 nM (M. Rusnati, unpublished observations). Consequently, immobilized FGF2 promotes EC adhesion and spreading, leading to uPA upregulation, cell migration, proliferation, and morphogenesis [50]. $\alpha_v\beta_3$ /FGF2 interaction and EC adhesion to immobilized FGF2 lead to the assembly of focal adhesion plaques containing $\alpha_v\beta_3$ and TK-FGFR1 [50]. Consistently, a direct $\alpha_v\beta_3$ /TK-FGFR1 interaction is required for a full response to FGF2 [51]. Unlike TK-FGFRs, integrins lack intrinsic TK activity. Yet, an early event during integrin signaling is the tyrosine phosphorylation of the non-receptor TK focal adhesion kinase (FAK) [52] that, in turn, leads to the activation of the RhoA GTPase and/or pp60^{src} [53-55]. In ECs, this signal transduction pathway can be activated upon integrin engagement by adhesive proteins and leads

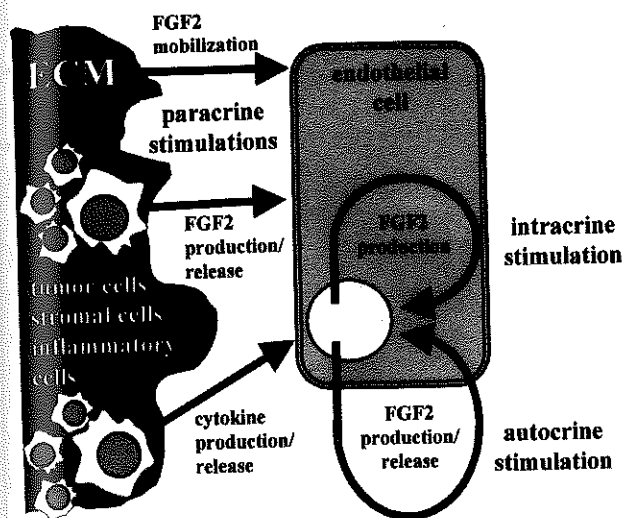


Fig. (3). Different mechanisms of action of FGFs. FGFs released by producing cells or mobilized from ECM activate ECs via a paracrine mode of action. Alternatively, cytokines can stimulate ECs to produce FGFs that, in turn, will act at the intracellular level (intracrine stimulation) or in an autocrine manner via an extracellular loop of stimulation.

to nuclear translocation of NF- κ B [56] and MAPK activation [50] (Fig. (2)). Accordingly, FGF2 induces FAK phosphorylation in ECs (M. Rusnati, unpublished observations).

EC adhesion and activation by immobilized FGF2 may have relevance *in vivo* since FGF2 accumulates as an immobilized protein in the ECM, mainly by binding to HSPGs. Accordingly, heparin-bound FGF2 retains its cell-adhesive capacity [57]. Thus, HSPGs may facilitate the interaction of ECM with FGF2 that, in turn, promotes EC adhesion and activation.

Gangliosides are neuraminic acid-containing glycosphingolipids mainly found associated to the EC membrane, where they modulate cell growth, adhesion, and cell-cell interaction [58]. Gangliosides bind FGF1, FGF2, and FGF4 via negatively charged neuraminic acid residues [31, 59]. Consistently, the ganglioside GM₁ expressed on the EC surface binds FGF2 with a K_d equal to 3 nM, acting as a functional FGF2 co-receptor [31]. Even though no data are available about the involvement of gangliosides in FGF signaling, ganglioside-rich lipid rafts have been implicated in the modulation of signal transduction and biological activity of different growth factors [60]. Indeed, the specific GM₁ ligand cholera toxin B subunit acts as FGF2 antagonists in ECs [31].

The complex signal transduction pathways activated by the engagement of EC receptors by FGFs (see Fig. (2)) is mirrored by the complexity of the elicited angiogenic phenotype, raising the possibility that different intracellular signals are responsible for the various steps of the angiogenic process. However, the inhibition of the activation of a single second messenger may be sufficient to hamper the whole angiogenic program (see Table 4 and 5).

FGFs can act on ECs via a paracrine mode consequent to their release by inflammatory, tumor and stromal cells and/or by their mobilization from the ECM. On the other hand, FGFs play autocrine/intracrine roles in ECs (see [61] and references therein). Relevant to this point, the single-copy human *fgf2* gene encodes multiple FGF2 isoforms (from 18 to 24 kDa) that play different functions possibly related to differences in their release and/or subcellular localization [62]. Indeed, high molecular weight FGF2 isoforms contain a nuclear localization sequence, are mostly recovered in the nucleus, and lead to cell immortalization when overexpressed in ECs. In contrast, 18 kDa FGF2 is mostly cytosolic

[63] and induces a transformed phenotype in EC transfectants [64]. Taken together, the data suggest that endogenous FGFs produced by cells of the endothelial lineage may play important autocrine, intracrine, or paracrine roles in angiogenesis and in the pathogenesis of vascular lesions (Fig. (3)).

2. INHIBITING THE FGF/FGF RECEPTOR SYSTEM

Theoretically, the angiogenic activity of FGFs can be neutralized at different levels (Fig. (4)): i) by inhibiting FGFs production/release; ii) by sequestering FGFs in an inactive form in the extracellular environment; iii) by inhibiting the expression of the different FGF receptors in ECs; iv) by masking FGF receptors, thus preventing their engagement by FGFs; v) by interrupting the signal transduction pathway(s) triggered by FGFs in ECs; vi) by neutralizing FGF-induced effectors/biological responses whose function is essential in mediating the angiogenic potential of FGFs. All these approaches have been challenged experimentally and will be described below.

2.1. Inhibiting FGF Production

As already mentioned, various cell types, including leukocytes, tumor, and stromal cells, produce FGFs (leading to paracrine EC activation) and/or cytokines that stimulate FGF synthesis in ECs (leading to autocrine/intracrine EC activation) (see Fig. (3)). In both cases, the inhibition of FGF production will lead to inhibition of neovascularization. This has been achieved with different approaches (Table 1), including chemotherapeutics, that inhibit FGF production by killing FGF-producing tumor cells, and by transfection with FGF antisense cDNAs or with dominant negative cDNAs encoding for second messengers involved in the regulation of FGF synthesis (Table 1).

2.2. Inhibiting FGF Receptor(s) Expression

The blockage of FGF activity can be achieved by hampering the expression of the various FGF receptors on EC surface, including TK-FGFRs, HSPGs, integrins, and gangliosides.

FGF2-dependent proliferation and migration of ECs are abolished by transfection of ECs with a TK-FGFR1 antisense cDNA [40]. Accordingly, liposome-mediated gene transfer of the TK-FGFR1 antisense cDNA blocks intratumoral angiogenesis in human melanomas grafted in nude mice [65]. Also, the synthetic retinoid fenretinide inhibits FGF2-induced angiogenesis *in vivo* and EC proliferation *in vitro* by reducing the expression of TK-FGFR2 on the EC surface [66]. Finally, EC surface expression of TK-FGFR1 and TK-FGFR2 can be inhibited by antibodies directed against $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins or by exposure to fibrin [67].

Lead exposure causes HSPG down-regulation, leading to inhibition of EC responsiveness to FGF2 [68]. Also, anti-angiogenic antithrombin inhibits EC proliferation by down-regulating the surface expression of the HSPG perlecan [69]. Accordingly, overexpression of perlecan antisense cDNA suppresses the autocrine and paracrine functions of FGF2 in fibroblasts [70]. Heparinase removes HSPGs from ECs, abolishing their capacity to migrate in response to FGF2 [40]. Similarly, the GAG 6-O-endosulfatase inhibits neovascularization induced *in vivo* by FGF2 [71].

EC morphogenesis on three-dimensional fibrin gel or Matrigel is suppressed by down-regulation of $\alpha_v\beta_3$ expression obtained by specific DNazymes [72], raising the possibility that a similar inhibitory effect might be observed also for FGF2-dependent activities.

Finally, specific inhibitors of the synthesis of complex gangliosides, including fumonisins B₁, D-threo-1-phenyl-2-decano-yl-amino-3-morpholino-1-propanol, and D-1-threo-1-phenyl-2-hexadecanoylamino-3-pyrrolidino-1-propanol, affect EC proliferation triggered by FGF2 [31].

Table 1. Inhibition of FGF Production in Tumor and ECs

Cell Type	Experimental Approach	Inhibitor	Reference
tumor cells	modulation of gene expression	FGF2 antisense cDNA transfection	[65]
		STAT1 knockout	[175]
		dominant negative STAT3 transfection	[73]
		dominant negative Akt transfection	[73]
	chemotherapeutics	taxane IDN 5109 (BAY59-8862)	[176]
		docetaxel	[177]
		epidermal growth factor receptor TK inhibitor ZD1839 (Iressa)	[178]
		doxycycline	[179]
		thalidomide	[180]
		zoledronic acid	[181]
	second messenger inhibitors	JAK inhibitor AG490	[73]
		PI3K inhibitor LY294002	[73]
		PKA inhibitor 8-chloro-cyclic AMP	[182]
	natural products	genistein	[183]
		fumagillin and its analog TNP-470	[184]
		curcumin	[132]
		green tea (epigallocatechin-3-gallate)	[134]
	endogenous molecules	dipeptidyl peptidase IV	[185]
INF- α		[186]	
ECs	modulation of gene expression	c-jun antisense cDNA transfection	[187]
		dominant negative ERK _{1/2} transfection	[188]
		dominant negative JNK transfection	[188]
		anti-early growth response-1 (Egr-1) DNA-cleaving deoxyribozymes	[189]
		anti-FGF2 antisense oligonucleotides	[190]
	second messenger inhibitors	PI3K inhibitor LY294002	[191]
		PKC inhibitor calphostin C	[192]
	natural products	green tea (epigallocatechin-3-gallate)	[134]

2.3. Inhibiting FGF Interaction with EC Receptors

In the presence of FGFs and their EC receptors, it is still possible to block neovascularization by sequestering FGFs in the extracellular environment or by concealing the receptors to their ligands.

2.3.1. Sequestering FGFs in the Extracellular Environment

Classically, the interaction with target cells can be prevented by means of specific antibodies raised against the growth factor. This is the case also for FGF2, whose functions can be inhibited by neutralizing antibodies in different experimental conditions [73, 74].

Once released in the extracellular environment, FGFs interact with several partners that modulate their bioavailability, stability, local concentration, interaction with EC receptors, and intracellular fate [5]. The identification of these molecules and the biochemical characterization of their FGF-binding/antagonist capacity may allow the design of selective inhibitors. Since the bulk of data refer to FGF2, we will focus on this member of the FGF family, even though many of the interactions described below may apply to various FGFs.

Several ECM components or their degradation products affect FGF-driven angiogenesis (Table 2). Thrombospondin-1 (TSP-1), a modular glycoprotein secreted by different cell types, including

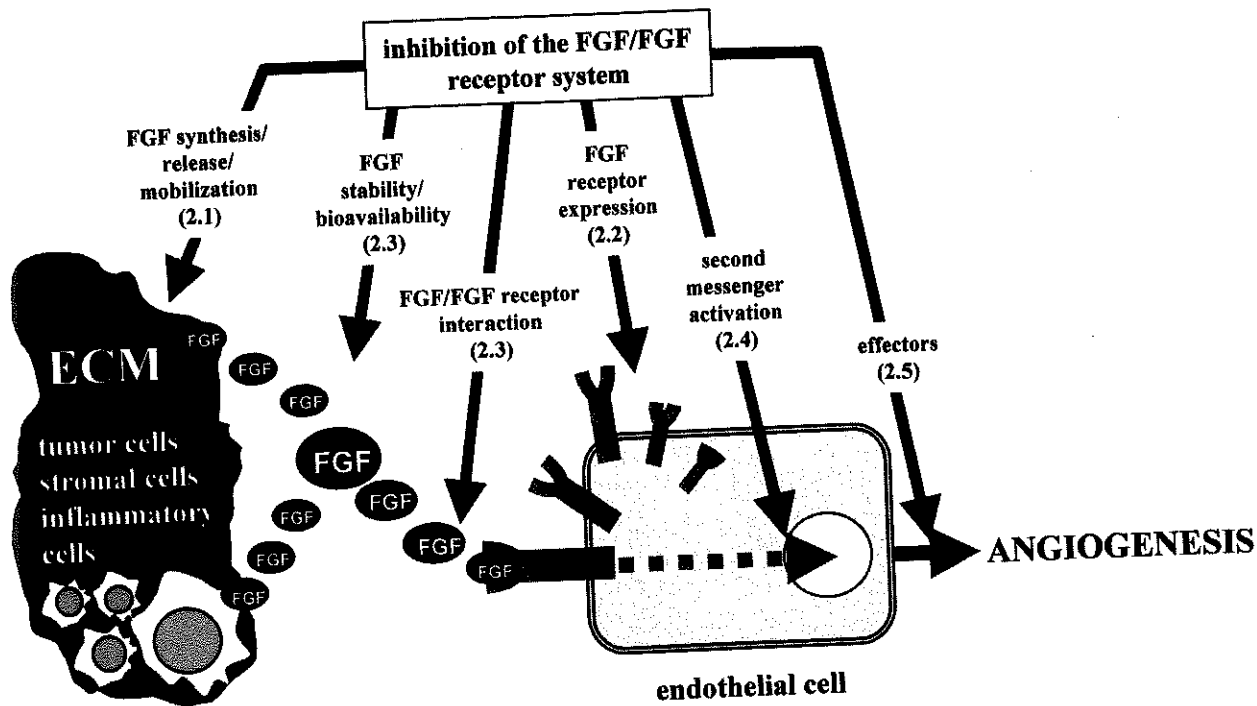


Fig. (4). Anti-FGF strategies for the development of anti-angiogenic therapies. Intracellular and extracellular FGF inhibitors can act on different targets. The numbers in brackets refer to the paragraphs in the text where the different classes of inhibitors are described in more details.

ECs, is composed of multiple domains that bind to soluble factors, receptors, and ECM components including HSPGs and integrins [75]. TSP-1 is a potent endogenous inhibitor of angiogenesis and this effect is due, at least in part, to its capacity to bind FGF2 [76]. The interaction is mediated by the COOH-terminal, anti-angiogenic 140 kDa fragment of TSP-1. TSP-1 prevents the interaction of FGF2 with HSPGs and TK-FGFRs. Accordingly, TSP-1 inhibits the mitogenic and chemotactic activity of FGF2 in ECs. TSP-1 also prevents the accumulation of FGF2 in the ECM and favors the mobilization of matrix-bound FGF2, generating inactive TSP-1/FGF2 complexes [77]. Thus, free TSP-1 acts as a scavenger for matrix-associated FGF2, affecting its location, bioavailability, and function, whereas ECM-associated TSP-1 acts as a "FGF2 decoy", sequestering the growth factor in an inactive form.

Fibstatin is a fibronectin fragment that binds FGF2, thus inhibiting its capacity to trigger cell proliferation, migration, and tubulogenesis in cultured ECs and angiogenesis and tumor growth *in vivo* [78].

A variety of serum components affect FGF activity in ECs (Tab. 2). α_2 -Macroglobulin (α_2M) is a 718 kDa homotetrameric protein present in human plasma where it acts as a broad-specific proteinase inhibitor. To exert its activity, α_2M undergoes major conformational changes that lead to the activated form α_2M^* . Both α_2M and α_2M^* bind a variety of cytokines and growth factors, including FGF1, FGF2, FGF4, and FGF6 [79]. The binding of α_2M to FGF2 occurs with high affinity and leads to sequestration of the growth factor in the extracellular environment, thus inhibiting FGF2/EC interaction, protease-inducing activity [80], and mitogenic capacity [79].

Long-pentraxin 3 (PTX3) is a 45 kDa glycosylated protein predominantly assembled in 10-20 mer multimers [81]. Its COOH-terminal domain shares homology with the classic short-pentraxin C-reactive protein whereas its NH₂-terminal portion does not show significant homology with any other known protein [82]. PTX3 is synthesized and released by activated mononuclear phagocytes and ECs [82] and acts as a soluble pattern recognition receptor with unique functions in various physio-pathological conditions. These

functions relay, at least in part, on the capacity of PTX3 to bind different structures [83]. In particular, PTX3 binds FGF2 with high affinity [83], preventing its binding to cell surface TK-FGFRs and HSPGs with a consequent inhibition of cell proliferation and migration. Also, PTX3 inhibits FGF2-dependent neovascularization and tumorigenesis *in vivo* [83]. PTX3 exists both as a free or ECM-immobilized molecule [84]. Relevant to this point, FGF2 and PTX3 retain their binding capacity independently of their free or immobilized status [83]. Thus, as described for TSP-1, free PTX3 may have access to ECM-bound FGF2 by acting as a scavenger for the stored growth factor, whereas ECM-associated PTX3 may act as a "FGF decoy", sequestering the growth factor in an inactive form.

Platelet factor 4 (PF4) is a well known inhibitor of angiogenesis ([85] and references therein) that binds FGF1 [86] and FGF2 [85], thus inhibiting their interaction with HSPGs, cell internalization, and mitogenic activity [85]. The observation that PF4-derived peptides can be modified to obtain a significant increase in their FGF2-binding and antagonist activity underlies the possibility that peptides from FGF-binding proteins represent a potential class of anti-angiogenic agents with defined mode(s) of action [87]. Like PF4, platelet derived growth factor (PDGF) BB binds FGF2 [88] and inhibits FGF2-dependent neovascularization [89]. Similarly, the chemokine CXCL13 (formerly known as B cell-attracting chemokine 1) binds FGF2, displaces the growth factor from ECs, impairs the formation of functional FGF2 homodimers, and inhibits FGF2-dependent survival of ECs [90]. Also the chemokine BRAK/CXCL14 inhibits FGF2-dependent migration of ECs *in vitro* and angiogenesis *in vivo*, even though its mechanism of action is still unknown [91].

A soluble form of the extracellular portion of TK-FGFR1 (xcFGFR1) was identified in body fluids [92] and in endothelial ECM [93]. xcFGFR1 binds FGF2 and prevents FGF2/TK-FGFR1 interaction [94]. Accordingly, xcFGFR1 inhibits signal transduction triggered by FGF1, FGF2, and FGF3 by forming heterodimers with cellular TK-FGFR1 [95] and inhibits FGF2-dependent proliferation in ECs [8].

Table 2. Endogenous Inhibitors of FGFs in ECs

Localization	Molecule	Mechanism of Action
intracellular	homeobox gene GAX	inhibition of NF- κ B activation [193]
	sprouty proteins	inhibition of TK-FGFR signaling [194]
	heat shock proteins (Hsp) 70 and 90	pAkt, c-Raf-1, and ERK _{1/2} down modulation [195]
ECM	collagen I	unknown [196]
	TSP-1	FGF2 sequestration [76], CD36 engagement [155], integrin occupancy (?), HSPG occupancy (?)
	alphastatin (fibrinogen fragment)	unknown [197]
	endostatin	cytoskeleton organization [198], Shb activation [199]
	fibstatin (fibronectin fragment)	FGF2 sequestration [78]
blood	CXCL13	FGF2 sequestration [90]
	CXCL14	unknown [91]
	PDGF	FGF2 sequestration [88]
	α_2 M	FGF2 sequestration [79]
	PTX3	FGF2 sequestration [83]
	heparin	FGF2 sequestration [42]
	gangliosides	FGF2 sequestration [59]
	PF4	FGF2 sequestration [86], HSPG occupancy [109], unknown [153]
	xcFGFR1	FGF2 sequestration [94], formation of heterodimers with TK-FGFR1 [95]
	histidine-rich glycoprotein	HSPG occupancy [109], tropomyosin engagement [200]
	antithrombin	HSPG down-regulation [69]
	thromboxane	inhibition of TK-FGFR1 internalization [201]
	angiostatin (fragment of plasminogen)	inhibition of ERK cascade [202]
	prolactin (16 kDa fragment)	unknown [203]
	vitamin D3-binding protein	CD36 engagement [204]
	ghrelin	inhibition of TK/MAPK cascades [205]
	lysophosphatidylcholine	inhibition of ras/ERK _{1/2} cascades [206]
	cleaved HMW kininogen	tropomyosin engagement [207]
	IL-4	alteration of cell cycle [208]
	IL-12	unknown [209]
	IP-10	unknown [210]
	pigment epithelium-derived factor	inhibition of Fyn [211]
	vasculostatin (fragment of brain angiogenesis inhibitor-1)	unknown [212]
	vasostatin	unknown [213]
	kininostatin (fragment of kininogen)	inhibition of cyclin D1 expression [214]
	kallistatin	HSPG occupancy, inhibition of FGF-induced proteases [112]
	TGF- β 1	unknown [14]

Localization	Molecule	Mechanism of Action
	TIMP-2, 4	inhibition of FGF-induced proteases [215]
	IFN- γ	TK-FGFR down-regulation [216]
	IL-1	TK-FGFR down-regulation [216]
	TNF- α , β	unknown [217]
	somatostatin	unknown [218]
	retinoids	unknown [66]
	apolipoprotein(a)	unknown [219]
extracellular micro- environment	heparan sulfate 6-0-endosulfatase	HSPG desulfation [71]
	heparinase	HSPG degradation [40]
	semaphorin-3F	inhibition of ERK _{1/2} cascade [220]

FGFs bind free heparin, a negatively charged GAG released in the blood stream during inflammation. At variance with HSPGs, that act as FGF co-receptors (see above), free heparin sequesters FGFs in the extracellular environment exerting an antagonist effect. However, due to its anticoagulant activity and its capacity to bind a wide array of growth factors, cytokines, enzymes, and proteases, unmodified heparin can not be used as an anti-angiogenic drug. This prompted a series of studies aimed at identifying heparin derivatives and/or heparin-like molecules endowed with a more specific FGF antagonist activity and a more favorable therapeutic window (reviewed in [96]). A list of polyanionic compounds able to bind FGFs and to inhibit their biological activity in ECs is shown in Table 3.

It must be pointed out that polyanionic compounds may exert also co-stimulatory effects on FGF activity depending on various experimental conditions, including: i) the member of the FGF family under investigation and/or the utilized biological assay; ii) the molar ratio of the FGF:polyanion interaction and medium composition [97]; iii) the EC type under study ([42] and references therein); iv) the structural properties of the polyanion under test [98]. Taken together, these considerations call for an extreme caution in the design of this class of anti-angiogenic compounds and in the evaluation of their biological activity.

Given the structural similarity among the various members of the FGF family and the heparin-binding capacity shared by a variety of angiogenic growth factors and cytokines, it may be difficult to envisage the design of selective polyanionic antagonists. Nevertheless, recent observations have shown the possibility to achieve a certain degree of specificity by selective structural modifications of the *E. coli* K5 polysaccharide [99, 100]. It must be pointed out, however, that the "multitarget" activity of certain polyanionic compounds may increase their efficacy *in vivo*. Indeed, tumor angiogenesis and growth are often the result of the synergistic action of more than one angiogenic growth factor ([5] and references therein). Relevant to this point, pentosan polysulfate (PPS) efficiently inhibits the biological activity of the angiogenic HIV-1 transactivating factor (Tat) [101] as well as of FGF2 [102]. Interestingly, phase I and II clinical trials have shown that PPS leads to stabilization of Kaposi's sarcoma [103], a lesion in which HIV-1 Tat and FGF2 act synergistically [104].

A peculiar class of polyanionic compounds is represented by sialo-gangliosides that act as functional FGF2 co-receptors when associated to the EC surface [31]. During tumor growth, sialo-gangliosides are shed in the microenvironment, where they bind and sequester FGF2, inhibiting its EC interaction and mitogenic

activity [59]. Sialo-gangliosides may therefore represent the basis for the design of novel anti-angiogenic FGF-antagonists.

2.3.2. Masking FGF Receptors

Neutralizing anti-TK-FGFR antibodies have been shown to block FGF2-mediated angiogenesis *in vivo* [105]. Also, TK-FGFRs can be bound by synthetic peptides and masked to their ligands. For instance, the interaction of FGF2 with TK-FGFR1 can be inhibited by peptides derived from the amino acid sequence 112-155 of the growth factor [8]. Also, a structural analysis carried out on FGF2 identified a region encompassing residues 48-58 as involved in FGF2 dimerization. Accordingly, the derived peptide FREG-(48-58) prevents dimerization of the growth factor and its interaction with TK-FGFR1, thus inhibiting TK-FGFR1 phosphorylation, FGF2-dependent EC proliferation and migration *in vitro* and angiogenesis *in vivo* [106]. Furthermore, a polyclonal antibody directed against FREG-(48-58) blocks FGF2 action *in vitro* [106]. In contrast, a FGF2 peptide derived from the amino-terminal extension of the high molecular weight 24 kDa FGF2 isoform plus the first 31 amino acids from the canonic 18 kDa isoform, inhibits FGF2-dependent migration of ECs without affecting FGF2/TK-FGFR1 interaction nor extracellular regulated kinase_{1/2} (ERK_{1/2}) activation [107]. Finally, FGF/TK-FGFR interaction can be disrupted by protamine, an arginine-rich polypeptide that inhibits FGF2-dependent proliferation of ECs [8] possibly by binding and masking TK-FGFRs [108].

Besides masking TK-FGFRs, protamine interacts with and masks HSPGs [108]. Similarly, the histidine-rich glycoprotein and PF4 bind and mask cell surface HSPGs, hindering these receptors to FGF2 and FGF1 [109]. Also, the anti-angiogenic collagen XVIII fragment endostatin prevents FGF2/HSPG interaction [110]. In keeping with these observations, a liposome-based peptide vaccine targeting the heparin-binding domain of FGF2 generates a specific anti-FGF2 antibody that inhibits FGF2 binding to HSPGs and FGF2-dependent angiogenesis *in vivo* [111]. Finally, kallistatin, a serpin originally identified as a specific inhibitor of tissue kallikrein, inhibits FGF2-induced proliferation, migration, and adhesion of cultured ECs and neovascularization *in vivo* possibly by hindering HSPGs to FGF2 binding [112].

Besides TK-FGFRs and HSPGs, integrins may represent a target for anti-angiogenic compounds. For instance, synthetic peptides representing two regions of the FGF2 molecule [FGF2(61-73) and FGF2(82-101)] inhibit FGF2-dependent proliferation of ECs [113]. These regions contain an Asp-Gly-Arg (DGR) sequence that is the inverse of the integrin-recognition sequence RGD present in many adhesive proteins. Actually, the two FGF2-derived peptides

Table 3. Heparin-Like Polyanionic Compounds that Inhibit FGF2 Activity in ECs

Polyanionic Compound	Inhibited EC Response
sulfated malto-oligosaccharides	proliferation, morphogenesis [221]
sulfated beta-(1->4)-galactooligosaccharides	angiogenesis [222]
RG-13577 (non sulfated aromatic compound)	proliferation, morphogenesis [223]
heparin-derived oligosaccharides	proliferation [97], angiogenesis, tumor growth [224]
fucoidan	proliferation, migration [225], morphogenesis, integrin expression [226]
suramin	motogenesis [105]
suramin derivatives	angiogenesis, proliferation [227], migration, uPA expression [228], tumorigenesis [229]
PPS	proliferation, migration [102]
TMPP (porphyrin analogue)	morphogenesis [230]
K5 derivatives (chemically sulfated polysaccharides from <i>E. coli</i>)	proliferation, FGF2-dependent cell-cell interaction, morphogenesis, angiogenesis [99], cell adhesion [57]
suleparoid (heparan sulfate analog)	angiogenesis [231]
undersulfated glycol-split heparins	proliferation, FGF2-dependent cell-cell interaction, angiogenesis [232]
synthetic sulfonic acid polymers	FGF2-dependent cell-cell interaction [233], proliferation, angiogenesis, morphogenesis [234]
β -cyclodextrin polysulfate	angiogenesis [235]
ATA (aurintricarboxylic acid)	angiogenesis [236]
PS-ODN (phosphorothioate oligodeoxynucleotides)	morphogenesis, angiogenesis [237]
gangliosides	proliferation, angiogenesis [238]
carrageenan	proliferation [143]
inositol hexaphosphate	angiogenesis [239]

inhibit $\alpha_v\beta_3$ -mediated EC adhesion to immobilized FGF2 without affecting FGF2/TK-FGFR interaction [30]. Accordingly, RGD-containing tetra or eptapeptides, and monoclonal anti- $\alpha_v\beta_3$ antibodies inhibit FGF2-dependent EC adhesion, proliferation, and uPA production [30, 113]. Following these observations, we have demonstrated that RGD-peptidomimetics inhibit FGF2-dependent neovascularization and tumorigenesis *in vivo* [114, 115]. A similar mechanism of action may be shared by disintegrins, a class of naturally occurring integrin antagonists that inhibit different aspects of FGF2 biology [116].

Finally, the cholera toxin B subunit inhibits FGF2-dependent proliferation of ECs by binding the cell surface GM₁ ganglioside [31].

2.4. Inhibiting FGF Receptor Signal Transduction

Intracellular signals activated by FGFs in ECs (Fig. (2)) might be considered as a target for angiogenesis inhibitors [35]. Actually, FGF activity can be inhibited *in vitro* and *in vivo* by synthetic compounds (Table 4) and selective dominant negative mutants or antisense cDNAs (Table 5) targeting various signal transduction pathways triggered by FGFs. Also, different endogenous inhibitors of angiogenesis have been shown to affect FGF signaling (Table 2). Among them, several cytokines modulate EC activation and/or neovascularization induced by FGF2. It is possible to hypothesize that these cytokines, by interacting with their cognate receptors on ECs, may interfere with the signal transduction pathway(s) activated by the angiogenic growth factor. However, the therapeutic exploitation of this approach is greatly limited by the fact that several among the second messengers activated by FGFs during pathological neovascularization are implicated in various physiological processes. Their inhibition may thus cause undesired side effects.

2.5. Inhibiting FGF-Activated EC Responses/Effectors of Angiogenesis

FGFs induce a complex "pro-angiogenic phenotype" in ECs characterized by an increase in ECM degradation and in EC motility, proliferation, and morphogenesis (see Fig. (1)). These processes are mediated by distinct effectors induced/activated by FGFs, and their blockage may result in the inhibition of FGF-dependent angiogenesis.

For instance, in order to degrade ECM, FGFs upregulate the production of several proteases in ECs (see above). Tissue inhibitors of MMPs (TIMPs) and synthetic MMP inhibitors [117] inhibit FGF2 neovascularization [118]. Interestingly, a MMP-independent mechanism of inhibition of FGF-dependent angiogenesis has been proposed for TIMP-2 [118]. Also, MMP production and FGF2-dependent angiogenesis can be inhibited by endogenous mediators, like interferons (IFNs) [119]. Similarly, PA/plasmin inhibitors affect FGF2-dependent angiogenesis *in vitro* and *in vivo* [120]. Finally, inhibition of proteases has been proposed to contribute to the FGF2-inhibitory effect exerted by kallistatin [112].

The epidermal growth factor-like domain of murine uPA alone or fused to the Fc portion of human IgG acts as high-affinity urokinase receptor antagonist and inhibits FGF2-induced angiogenesis *in vivo* [121]. Accordingly, medroxyprogesterone acetate exerts an angiostatic effect by increasing the expression of PA inhibitor-1, thus counteracting the uPA-inducing activity of FGF2 [122].

The properties of neovasculature differ from those of quiescent endothelium. Vascular targeting agents exploit differences in cell proliferation, permeability, maturation, and reliance on tubulin cytoskeleton to induce selective blood vessel occlusion and destruction [123]. In particular, microtubule-destabilizing agents, including combretastatin-derived prodrugs and analogues, disrupt rapidly proliferating and immature tumor endothelium, leading to reduced blood flow and hypoxia [124]. Interestingly, microtubule-destabilizing agents, e.g. combretastatin A-4 and vinblastine, may also show a distinct anti-angiogenic activity [125]. Accordingly, the *trans*-resveratrol derivative 3,5,4'-trimethoxystilbene acts as a microtubule-destabilizing agent endowed with both anti-angiogenic FGF2-antagonist activity and vascular targeting capacity [126]. Similarly, microtubule-stabilizing agents, including paclitaxel and taxane derivatives [127, 128], affect FGF2-triggered angiogenesis *in vitro* and *in vivo*. Also, by preventing the formation of stress fibers, the antifungal polyether macrolide goniocidin-A inhibits FGF2-induced migration and morphogenesis in ECs, leaving unaffected their proliferation [129]. These findings are of importance

Table 4. Chemical Inhibitors of FGF2-Mediated Intracellular Signaling

Inhibitor	Second Messenger	Inhibited EC Response
SU5416	FGFR-TK	survival [240], angiogenesis [241], EC monolayer wound repair ^a
SU5402	FGFR-TK	proliferation [240]
Z24	FGFR-TK	angiogenesis [241]
PD173074	FGFR-TK	morphogenesis, angiogenesis [242]
CP-547,632	FGFR-TK	proliferation, angiogenesis [243]
PD 098059	ERK _{1/2}	proliferation [50], survival [244], uPA expression [245], MMP3 expression [246], migration [247], CD13 expression [248], morphogenesis [248], angiogenesis [248], survival, integrin activation [249], Egr-1 expression [250], KDR expression [251]
U0126	ERK _{1/2}	morphogenesis [245], survival [252], MMP3 expression [246], motogenesis ^a
apigenin	ERK _{1/2}	proliferation [253]
SB203580	P38	morphogenesis [254]
LY294002	PI3K	survival [252], CD13 expression, morphogenesis [248], migration [162], proliferation [253], cytoskeleton organization [255], motogenesis ^a , FGF2 production [191]
neutralizing antibodies	PI3K	proliferation [256]
apigenin	PI3K	proliferation [253]
Bis I	PKC	survival [252]
GO6983	PKC	survival [252]
GFX	PKC	KDR expression [251]
chelerythrine	PKC	proliferation [257]
H7	PKC	proliferation [258], survival [259]
NSC 639366	PKC	migration, uPA expression, angiogenesis [260]
calphostin C	PKC	angiogenesis [261], FGF2 production [192]
manumycin A	Ras	CD13 expression [248], morphogenesis [248], proliferation [262]
FTS	Ras	proliferation [262]
FPT inhibitor III	Ras	proliferation [253]
tyrphostin 23	Pan-TK	proliferation [50], EC monolayer wound repair ^a
genistein	Pan-TK	proliferation [263]
herbimycin A	Pan-TK	proliferation [263]
PP1	c-Src	migration [264], morphogenesis [262]
PP2	c-Src	angiogenesis, morphogenesis, cytoskeleton organization [265]
neutralizing antibodies	PLC- γ	proliferation [266]
aristolochic acid	PLC- α 2	migration [267]
ONO-RS-082	PLC- α 2	migration [267]
rapamycin	p70 ^{src}	proliferation [253]
C3	RhoA	ICAM-1 expression [268]
Grb2-Src homology 2 domain binding antagonist	Grb2	proliferation, migration, angiogenesis [269]

(Table 4) Contd....

Inhibitor	Second Messenger	Inhibited EC Response
forskolin	cAMP	proliferation [270]
8-bromo AMPc	cAMP	proliferation [270]
ML-9	AKT	angiogenesis [271]
CAI	Ca ⁺⁺ influx	proliferation, adhesion, MMP-2 expression [272]
pertussis toxin	G-proteins	migration [267]

^a it refers to the capacity of an EC monolayer to repair a mechanical wound in response to FGF2 (Urbinati C., personal communication).

when considering that combining vascular targeting agents with angiogenesis inhibitors may result in additive or synergistic effects on the inhibition of vascularization and tumor growth [130].

Finally, apoptosis-inducing agents can inhibit the action of FGF2, possibly counteracting its mitogenic activity. This is the case of betulinic acid, a pro-apoptotic mitochondria-damaging pentacyclic triterpenoid, that inhibits FGF2-induced EC invasion and tube formation [131].

2.6. Inhibiting FGF/FGF Receptor Activity with Nutraceuticals and Other Drugs

Numerous bioactive plant compounds (often referred to as nutraceuticals) and natural marine products have been tested for their potential clinical applications. Some of these compounds are currently under study for their anti-FGF and anti-angiogenic potential, including curcumin from *Curcuma longa* [132, 133] and epigallocatechin-3-gallate from green tea [134]. The *Gleditsia sinensis* fruit extract inhibits the angiogenic activity of FGF2 *in vivo* [135]. *Citrus pectin* inhibits the formation of the productive heparin/FGF2/TK-FGFR1 ternary complex, probably by interacting directly with the growth factor and competing for heparin binding [136]. The 1,2,3,4,6-penta-O-galloyl-beta-D-glucose from *Galla Rhois* inhibits proliferation and tube formation induced *in vitro* by FGF2 as well as its angiogenic activity *in vivo* [137]. Resveratrol, found in grapes and wine, inhibits FGF-driven angiogenesis *in vitro* and *in vivo* [138]. Finally, 4-O-methylgallic acid isolated from the dietary legume *Canavalia gladiata* inhibits FGF2-stimulated invasion and tube formation by ECs [139]. The antineoplastic compound apidine, a new marine-derived depsipeptide, inhibits angiogenesis elicited by FGF2 *in vivo* and FGF2-dependent EC proliferation *in vitro* [140]. Philinopside-A, a novel sulfated saponin isolated from the sea cucumber *Pentacta quadrangulari*, and the Chinese folk medicine-derived phytochemical 11,11'-dideoxyverticillin from fungus *Shiraia bambusicola* are potent inhibitors of TK-FGFR1 activity [141, 142]. Psammalin-A is a phenolic natural product isolated from a marine sponge that suppresses the invasion and tube formation of ECs stimulated by FGF2. Carrageenan-1 is a natural polysulphated carbohydrate that inhibits FGF2 mitogenic activity in ECs [143]. Also, a naturally occurring agent isolated from cartilage, referred to as Neovastat (AE-941), inhibits FGF2-dependent angiogenesis *in vivo* [144].

Interestingly, several drugs developed for the treatment of tumor-unrelated diseases have been shown to be endowed with FGF-antagonist activity. Spironolactone, a mineralocorticoid receptor antagonist mainly used in the treatment of heart failure, inhibits neovascularization triggered by FGF2 *in vivo* [145]. Transilast, an anti-allergic drug, inhibits FGF2-dependent EC proliferation [146]. Bisphosphonate drugs inhibit osteoclastic bone resorption and are widely used to treat skeletal complications. Zoledronic acid, a new generation bisphosphonate, inhibits FGF2-induced EC proliferation and neovascularization *in vivo* [147].

Cidofovir, approved for the treatment of cytomegalovirus retinitis in AIDS patients, inhibits FGF2-dependent tumorigenesis [28]. Indomethacin, a nonsteroidal anti-inflammatory drug, inhibits angiogenesis *in vivo* by affecting FGF2-induced EC proliferation [148]. Cerivastatin, an HMG-CoA reductase inhibitor used for the treatment of hypercholesterolemia-related diseases, inhibits EC locomotion *in vitro* and angiogenesis *in vivo* [149]. SR 25989, an esterified derivative of ticlopidine, inhibits FGF1-dependent healing of a mechanical wound in confluent endothelium [150]. Triamcinolone acetonide, a corticosteroid mainly used in the treatment of intraocular disorders, inhibits EC sprouting triggered *in vitro* by FGF2 and its angiogenic activity *in vivo* [151]. Finally, a secretory phospholipase-A2 inhibitor prevents FGF2-dependent EC proliferation, migration, and morphogenesis *in vitro* [152].

3. THE MULTITARGET OPTION

Different FGF inhibitors act with a multitarget mechanism of action (Fig. (5)). PF4 binds FGFs [85, 86], masks HSPGs [109], and acts intracellularly [153]. Similarly, TSP-1 sequesters FGF2 in an inactive form [76, 77], binds $\alpha_v\beta_3$ [75] and HSPGs [154] (possibly preventing FGF2 interaction), and inhibits FGF2 activity by a CD36-dependent mechanism of action [155]. Like TSP-1, fibstatin binds heparin and integrins, suggesting that multiple interactions may be responsible for its anti-angiogenic activity [78].

RGD-containing peptides antagonize FGF2 mainly by competing for $\alpha_v\beta_3$ interaction [30]. However, their direct binding to integrins leads to a caspase-dependent apoptotic signal that contributes to EC inhibition [156]. The histidine-rich glycoprotein, besides masking HSPGs to FGF1 and FGF2, binds and transduces anti-angiogenic signals through cell surface tropomyosin on ECs [109]. Curcuminoids inhibit FGF production by tumor cells [132] and prevent FGF2-dependent protease production in ECs [133]. Kallistatin has been proposed to inhibit FGF2 activity by binding and masking HSPGs and by inhibiting protease activity [112]. The blockage of ERK_{1/2} activation by chemical inhibitors leads to inhibition of FGF2 production and of FGF2-mediated response in ECs (Table 2 and Table 5).

In tumors, FGF inhibitors with a multitarget mechanism of action, as well as the combination of FGF antagonists and classic chemotherapeutic agents, should prevent the development of drug-resistance and decrease the dosage and related toxicity of each single drug, as shown for cisplatin used in combination with Neovastat [144].

Several anti-tumor agents are endowed with an intrinsic anti-angiogenic, FGF-antagonist activity [157]. For instance, the quinazoline-derived α_1 -adrenoreceptor antagonist doxazosin, used for the treatment of prostate cancer, inhibits FGF2-induced morphogenesis in ECs [158]. Thalidomide, used for the treatment of relapsing malignant gliomas, inhibits FGF2-induced EC proliferation [159]. The same effect is exerted by the anti-estrogen tamoxifen, used as adjuvant in the treatment of breast cancer [160].

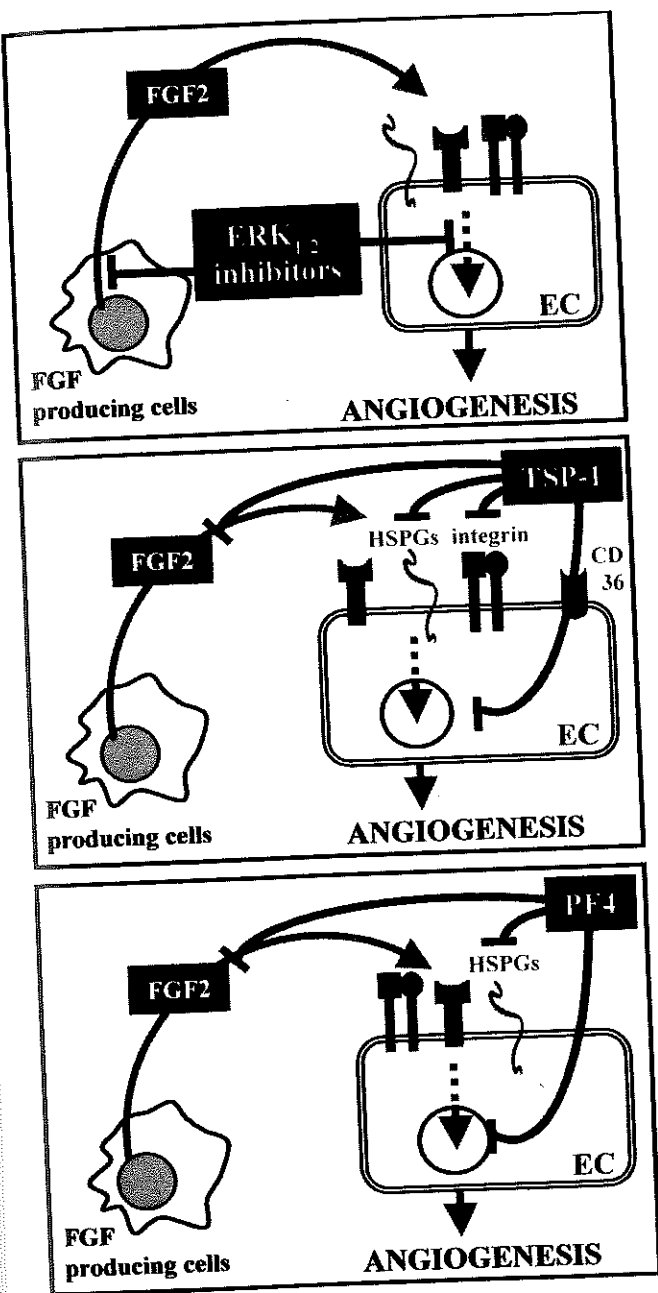


Fig. (5). Multitarget activity of selected FGF inhibitors. Possible mechanisms of action of anti-angiogenic ERK_{1/2} inhibitors, TSP-1, and PF4. See text for further details.

and by the functionally related medroxyprogesterone-acetate that inhibits the release of uPA induced by FGF2 in ECs [161]. The topoisomerase-I inhibitor topotecan possesses an indirect anti-tumor effect *in vivo* mediated by angiostimulation due, at least in part, to inhibition of FGF2-induced EC migration [162]. Aplidine, that exerts a cytotoxic effect in tumor cells and is currently tested in early phase clinical trials, possesses FGF2-antagonist activity [140]. The same dual effect has been demonstrated for Neovastat [144]. The chemotherapeutic 6-methylmercaptapurine-riboside inhibits FGF2-dependent angiogenesis *in vitro* and *in vivo* [163]. Combination of tegafur and uracil (UFT), utilized for the treatment of a variety of malignant tumors, inhibits EC proliferation induced by FGF2 [164]. The antimetabolite 6-thioguanine, utilized in the management of acute myelogenous leukemia, inhibits EC proliferation and angiogenesis triggered by FGF2 [163]. Finally, Atiprimod, an azaspirane cationic amphiphilic drug, activates

caspses and induces apoptosis in various tumor cell lines and, simultaneously, inhibits FGF2-induced proliferation and migration of ECs [165].

It must be pointed out that, due to their pleiotropic nature, FGFs may contribute to cancer progression not only as pro-angiogenic growth factors but also by acting directly on tumor cells (Fig. (6)). For instance, the co-expression of FGF7/KGF and its receptor TK-FGFR2 IIIb/KGFR correlates with the high proliferative activity and poor prognosis in lung adenocarcinoma [166]. Also, high levels of FGF8 [167] or FGF17 [168] are associated with less favorable prognosis in human prostate cancer. Thus, targeting the FGF/FGF receptor system in cancer may provide benefits not only in terms of angiostimulation but also by a direct inhibition of tumor cell proliferation (Fig. (6)). For instance, inhibition of the FGF/FGF receptor system in glioma cells by dominant negative TK-FGFR transfection [169] or in prostate cancer cells by *fgf2* gene knockout [170] results in inhibition of tumor growth by both angiogenesis-dependent and angiogenesis-independent mechanisms.

Table 5. "Modulation of Gene Expression" Approach for the Inhibition of FGF2-Mediated Intracellular Signaling"

Target	Inhibited EC Response
FGF2 ^b	cell proliferation [273], angiogenesis [65, 274]
FGFR-TK	proliferation [50], cytoskeleton organization [275], migration, angiogenesis [276], uPA expression [8]
Syndecan docking sites	proliferation, migration, morphogenesis [39]
FAK	angiogenesis [277]
c-Src	chemotaxis [264], angiogenesis [265]
Rac	proliferation [278]
Ras	CD13 expression [248], angiogenesis [277]
Raf	CD13 expression [248], survival [244], angiogenesis [277]
MEK	CD13 expression [248], proliferation, migration [206]
ERK _{1/2}	CD13 expression [248]
SH2	cytoskeleton organization [255], proliferation [279]
PKC	proliferation, morphogenesis [280]
c-FES	chemotaxis [281]
PI3K	survival [282]
PAK	angiogenesis [277]
AKT	survival [283], morphogenesis [284]
Egr-1 ^c	proliferation [189]
c-Fyn	morphogenesis [285]
Ets-1	angiogenesis [286]
NF-κB ^d	angiogenesis [287]

^aInhibition was obtained by overexpression of dominant negative forms of the indicated target with the exception for ^{b,c,d} where antisense oligonucleotides, neutralizing single-stranded DNA, and IκB-2A overexpression were used, respectively.

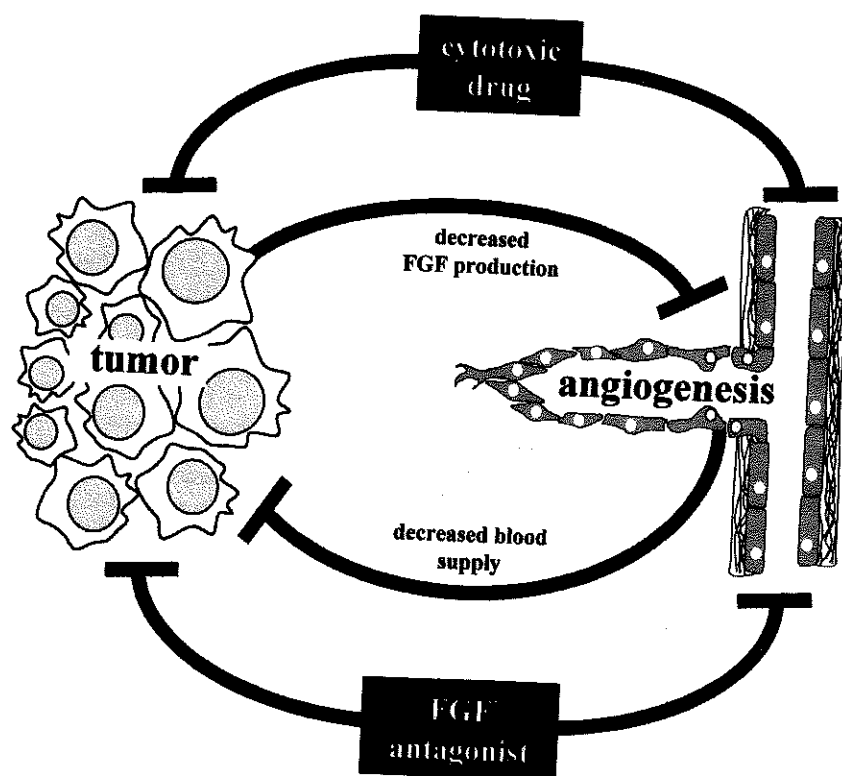


Fig. (6). Multiple effects of FGF antagonists and antineoplastic drugs on tumor growth and neovascularization. FGF antagonists can affect tumor growth indirectly by decreasing blood supply and directly by blocking FGF-dependent tumor cell proliferation. On the other hand, cytotoxic drugs can inhibit EC proliferation and decrease the amount of FGF available to ECs by killing FGF-producing tumor cells.

4. CONCLUDING REMARKS

The bulk of experimental data summarized in this review clearly indicate that the FGF/FGF receptor system may represent a target for anti-angiogenic strategies in different pathological settings, including cancer. At present, cancer clinical trials are in progress to assess the safety and efficacy of various compounds with a potential capacity to affect the FGF/FGF receptor system at different levels [171, 172]. In several cases, however, the main rationale for testing these compounds was independent of their putative FGF/FGF receptor antagonist activity. For instance, heparin derivatives have been tested in cancer patients because of their anti-thrombotic effect rather than for their capacity to bind angiogenic FGFs. Similarly, the humanized anti- $\alpha_v\beta_3$ monoclonal antibody vitaxin [173, 174] has been investigated for its ability to affect the cell-adhesive function of this integrin receptor rather than for its potential role in angiogenesis and FGF activity. Also, as stated above, numerous cytotoxic drugs can affect the FGF/FGF receptor system and angiogenesis. Novel strategies aimed at inhibiting multiple targets, including the FGF/FGF receptor system, may represent an efficacious approach for the treatment of angiogenesis-dependent diseases, including cancer.

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ABBREVIATIONS

α_2M = α_2 -Macroglobulin
ECs = Endothelial cells

ECM = Extracellular matrix
ERK = Extracellular regulated kinase
FGF = Fibroblast growth factor
TK-FGFR = Tyrosine kinase FGF receptor
FAK = Focal adhesion kinase
GAG = Glycosaminoglycan
HS = Heparan sulfate
HSPGs = HS Proteoglycans
IFN = Interferon
 K_d = Dissociation constant
MAPK = Mitogen activated protein kinase
MMP = Metalloproteinase
PF4 = Platelet factor-4
PDGF = Platelet derived growth factor
PIP2 = Phosphatidylinositol 4,5-bisphosphate
PPS = Pentosan polysulfate
PTX3 = Long-pentraxin 3
PKC = Protein kinase C
Tat = HIV-1 Transactivating factor
TIMP = Tissue inhibitors of MMP
TK = Tyrosine kinase
TSP-1 = Thrombospondin-1
uPA = Urokinase-type plasminogen activator
xcFGFR1 = Extracellular portion of FGFR1

REFERENCES

References 290-292 are related articles recently published in Current Pharmaceutical Design.

- [1] Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; 407: 249-57.
- [2] Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; 1: 27-31.
- [3] Maciag T, Mehlman T, Friesel R, Schreiber AB. Heparin binds endothelial cell growth factor, the principal endothelial cell mitogen in bovine brain. *Science* 1984; 225: 932-5.
- [4] Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. *Trends Genet* 2004; 20: 563-9.
- [5] Presta M, Dell'Era P, Mitola S, Moroni E, Ronca R, Rusnati M. Fibroblast growth factor/fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev* 2005; 16: 159-78.
- [6] Javerzat S, Auguste P, Bikfalvi A. The role of fibroblast growth factors in vascular development. *Trends Mol Med* 2002; 8: 483-9.
- [7] Liotta LA, Steeg PS, Stetler-Stevenson WG. Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* 1991; 64: 327-36.
- [8] Rusnati M, Dell'Era P, Urbinati C, Tanghetti E, Massardi ML, Nagamine Y, *et al.* A distinct basic fibroblast growth factor (FGF-2)/FGF receptor interaction distinguishes urokinase-type plasminogen activator induction from mitogenicity in endothelial cells. *Mol Biol Cell* 1996; 7: 369-81.
- [9] Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases MMP-2, MMP-9, and MT1-MMP as membrane vesicle-associated components by endothelial cells. *Am J Pathol* 2002; 160: 673-80.
- [10] Hiraoka N, Allen E, Apel IJ, Gyetko MR, Weiss SJ. Matrix metalloproteinases regulate neovascularization by acting as pericellular fibrinolysins. *Cell* 1998; 95: 365-77.
- [11] Terranova VP, DiFlorio R, Lyall RM, Hic S, Friesel R, Maciag T. Human endothelial cells are chemotactic to endothelial cell growth factor and heparin. *J Cell Biol* 1985; 101: 2330-4.
- [12] Montesano R, Orci L, Vassalli P. *In vitro* rapid organization of endothelial cells into capillary-like networks is promoted by collagen matrices. *J Cell Biol* 1983; 97: 1648-52.
- [13] Montesano R, Vassalli JD, Baird A, Guillemin R, Orci L. Basic fibroblast growth factor induces angiogenesis *in vitro*. *Proc Natl Acad Sci USA* 1986; 83: 7297-301.
- [14] Pepper MS, Belin D, Montesano R, Orci L, Vassalli JD. Transforming growth factor-beta 1 modulates basic fibroblast growth factor-induced proteolytic and angiogenic properties of endothelial cells *in vitro*. *J Cell Biol* 1990; 111: 743-55.
- [15] Henke CA, Roongta U, Mickelson DJ, Knutson JR, McCarthy JB. CD44-related chondroitin sulfate proteoglycan, a cell surface receptor implicated with tumor cell invasion, mediates endothelial cell migration on fibrinogen and invasion into a fibrin matrix. *J Clin Invest* 1996; 97: 2541-52.
- [16] Takei A, Tashiro Y, Nakashima Y, Sueishi K. Effects of fibrin on the angiogenesis *in vitro* of bovine endothelial cells in collagen gel. *In Vitro Cell Dev Biol Anim* 1995; 31: 467-72.
- [17] Kumar R, Yoneda J, Bucana CD, Fidler IJ. Regulation of distinct steps of angiogenesis by different angiogenic molecules. *Int J Oncol* 1998; 12: 749-57.
- [18] Schnaper HW, Grant DS, Stetler-Stevenson WG, Fridman R, D'Orazi G, Murphy AN, *et al.* Type IV collagenase(s) and TIMPs modulate endothelial cell morphogenesis *in vitro*. *J Cell Physiol* 1993; 156: 235-46.
- [19] Schnaper HW, Barnathan ES, Mazar A, Maheshwari S, Ellis S, Cortez SL, *et al.* Plasminogen activators augment endothelial cell organization *in vitro* by two distinct pathways. *J Cell Physiol* 1995; 165: 107-18.
- [20] Davis GE, Camarillo CW. Regulation of endothelial cell morphogenesis by integrins, mechanical forces, and matrix guidance pathways. *Exp Cell Res* 1995; 216: 113-23.
- [21] Underwood PA, Bean PA, Gamble JR. Rate of endothelial expansion is controlled by cell:cell adhesion. *Int J Biochem Cell Biol* 2002; 34: 55-69.
- [22] Klein S, Giancotti FG, Presta M, Albelda SM, Buck CA, Rifkin DB. Basic fibroblast growth factor modulates integrin expression in microvascular endothelial cells. *Mol Biol Cell* 1993; 4: 973-82.
- [23] Gerritsen ME, Soriano R, Yang S, Zlot C, Ingle G, Toy K, *et al.* Branching out: a molecular fingerprint of endothelial differentiation into tube-like structures generated by Affymetrix oligonucleotide arrays. *Microcirculation* 2003; 10: 63-81.
- [24] Ribatti D, Vacca A, Roncali L, Dammacco F. The chick embryo chorioallantoic membrane as a model for *in vivo* research on anti-angiogenesis. *Curr Pharm Biotechnol* 2000; 1: 73-82.
- [25] Herbert JM, Laplace MC, Malftrand JP. Effect of heparin on the angiogenic potency of basic and acidic fibroblast growth factors in the rabbit cornea assay. *Int J Tissue React* 1988; 10: 133-9.
- [26] Seghezzi G, Patel S, Ren CJ, Gualandris A, Pintucci G, Robbins ES, *et al.* Fibroblast growth factor-2 (FGF-2) induces vascular endothelial growth factor (VEGF) expression in the endothelial cells of forming capillaries: an autocrine mechanism contributing to angiogenesis. *J Cell Biol* 1998; 141: 1659-73.
- [27] Passaniti A, Taylor RM, Pili R, Guo Y, Long PV, Haney JA, *et al.* A simple, quantitative method for assessing angiogenesis and antiangiogenic agents using reconstituted basement membrane, heparin, and fibroblast growth factor. *Lab Invest* 1992; 67: 519-28.
- [28] Liekens S, Neyts J, De Clercq E, Verbeke E, Ribatti D, Presta M. Inhibition of fibroblast growth factor-2-induced vascular tumor formation by the acyclic nucleoside phosphonate cidofovir. *Cancer Res* 2001; 61: 5057-64.
- [29] Ribatti D, Nico B, Morbidelli L, Donnini S, Ziche M, Vacca A, *et al.* Cell-mediated delivery of fibroblast growth factor-2 and vascular endothelial growth factor onto the chick chorioallantoic membrane: endothelial fenestration and angiogenesis. *J Vasc Res* 2001; 38: 389-97.
- [30] Rusnati M, Tanghetti E, Dell'Era P, Gualandris A, Presta M. α 3 β 1 integrin mediates the cell-adhesive capacity and biological activity of basic fibroblast growth factor (FGF-2) in cultured endothelial cells. *Mol Biol Cell* 1997; 8: 2449-61.
- [31] Rusnati M, Urbinati C, Tanghetti E, Dell'Era P, Lortat-Jacob H, Presta M. Cell membrane GM1 ganglioside is a functional coreceptor for fibroblast growth factor 2. *Proc Natl Acad Sci USA* 2002; 99: 4367-72.
- [32] Johnson DE, Williams LT. Structural and functional diversity in the FGF receptor multigene family. *Adv Cancer Res* 1993; 60: 1-41.
- [33] Yoon SY, Tefferi A, Li CY. Cellular distribution of platelet-derived growth factor, transforming growth factor-beta, basic fibroblast growth factor, and their receptors in normal bone marrow. *Acta Haematol* 2000; 104: 151-7.
- [34] Dell'Era P, Belleri M, Stabile H, Massardi ML, Ribatti D, Presta M. Paracrine and autocrine effects of fibroblast growth factor-4 in endothelial cells. *Oncogene* 2001; 20: 2655-63.
- [35] Eswarakumar VP, Lax I, Schlessinger J. Cellular signaling by fibroblast growth factor receptors. *Cytokine Growth Factor Rev* 2005; 16: 139-49.
- [36] Lindahl U, Lidholt K, Spillmann D, Kjellen L. More to "heparin" than anticoagulation. *Thromb Res* 1994; 75: 1-32.
- [37] Pellegrini L. Role of heparan sulfate in fibroblast growth factor signalling: a structural view. *Curr Opin Struct Biol* 2001; 11: 629-34.
- [38] Eriksson AE, Cousens LS, Weaver LH, Matthews BW. Three-dimensional structure of human basic fibroblast growth factor. *Proc Natl Acad Sci USA* 1991; 88: 3441-5.
- [39] Horowitz A, Tkachenko E, Simons M. Fibroblast growth factor-specific modulation of cellular response by syndecan-4. *J Cell Biol* 2002; 157: 715-25.
- [40] Chua CC, Rahimi N, Forsten-Williams K, Nugent MA. Heparan sulfate proteoglycans function as receptors for fibroblast growth factor-2 activation of extracellular signal-regulated kinases 1 and 2. *Circ Res* 2004; 94: 316-23.
- [41] Rusnati M, Urbinati C, Presta M. Internalization of basic fibroblast growth factor (bFGF) in cultured endothelial cells: role of the low affinity heparin-like bFGF receptors. *J Cell Physiol* 1993; 154: 152-61.
- [42] Rusnati M, Presta M. Interaction of angiogenic basic fibroblast growth factor with endothelial cell heparan sulfate proteoglycans. Biological implications in neovascularization. *Int J Clin Lab Res* 1996; 26: 15-23.
- [43] Coltrini D, Rusnati M, Zoppetti G, Oreste P, Grazioli G, Naggi A, *et al.* Different effects of mucosal, bovine lung and chemically

- modified heparin on selected biological properties of basic fibroblast growth factor. *Biochem J* 1994; 303 (Pt 2): 583-90.
- [44] Presta M, Maier JA, Rusnati M, Ragnotti G. Basic fibroblast growth factor is released from endothelial extracellular matrix in a biologically active form. *J Cell Physiol* 1989; 140: 68-74.
- [45] Vlodayvsky I, Korner G, Ishai-Michaeli R, Bashkin P, Bar-Shavit R, Fuks Z. Extracellular matrix-resident growth factors and enzymes: possible involvement in tumor metastasis and angiogenesis. *Cancer Metastasis Rev* 1990; 9: 203-26.
- [46] Ribatti D, Leali D, Vacca A, Giuliani R, Gualandris A, Roncali L, *et al.* *In vivo* angiogenic activity of urokinase: role of endogenous fibroblast growth factor-2. *J Cell Sci* 1999; 112 (Pt 23): 4213-21.
- [47] Ruegg C, Mariotti A. Vascular integrins: pleiotropic adhesion and signaling molecules in vascular homeostasis and angiogenesis. *Cell Mol Life Sci* 2003; 60: 1135-57.
- [48] Eliceiri BP. Integrin and growth factor receptor crosstalk. *Circ Res* 2001; 89: 1104-10.
- [49] Kumar CC. Integrin alpha v beta 3 as a therapeutic target for blocking tumor-induced angiogenesis. *Curr Drug Targets* 2003; 4: 123-31.
- [50] Tanghetti E, Ria R, Dell'Era P, Urbinati C, Rusnati M, Ennas MG, *et al.* Biological activity of substrate-bound basic fibroblast growth factor (FGF2): recruitment of FGF receptor-1 in endothelial cell adhesion contacts. *Oncogene* 2002; 21: 3889-97.
- [51] Sahni A, Francis CW. Stimulation of endothelial cell proliferation by FGF-2 in the presence of fibrinogen requires $\{\alpha\}v\{\beta\}3$. *Blood* 2004; 104: 3635-41.
- [52] Schlaepfer DD, Hauck CR, Sieg DJ. Signaling through focal adhesion kinase. *Prog Biophys Mol Biol* 1999; 71: 435-78.
- [53] Palazzo AF, Eng CH, Schlaepfer DD, Marcantonio EE, Gundersen GG. Localized stabilization of microtubules by integrin- and FAK-facilitated Rho signaling. *Science* 2004; 303: 836-9.
- [54] Zhai J, Lin H, Nie Z, Wu J, Canete-Soler R, Schlaepfer WW, *et al.* Direct interaction of focal adhesion kinase with p190RhoGEF. *J Biol Chem* 2003; 278: 24865-73.
- [55] Shanna-Walia N, Naranatt PP, Krishnan HH, Zeng L, Chandran B. Kaposi's sarcoma-associated herpesvirus/human herpesvirus 8 envelope glycoprotein gB induces the integrin-dependent focal adhesion kinase-Src-phosphatidylinositol 3-kinase-rho GTPase signal pathways and cytoskeletal rearrangements. *J Virol* 2004; 78: 4207-23.
- [56] Scatena M, Almeida M, Chaisson ML, Fausto N, Nicosia RF, Giachelli CM. NF-kappaB mediates alphavbeta3 integrin-induced endothelial cell survival. *J Cell Biol* 1998; 141: 1083-93.
- [57] Presta M, Oreste P, Zoppetti G, Belleri M, Tanghetti E, Leali D, *et al.* Antiangiogenic activity of semisynthetic biotechnological heparins: low-molecular-weight-sulfated *Escherichia coli* K5 polysaccharide derivatives as fibroblast growth factor antagonists. *Arterioscler Thromb Vasc Biol* 2005; 25: 71-6.
- [58] Birkle S, Zeng G, Gao L, Yu RK, Aubry J. Role of tumor-associated gangliosides in cancer progression. *Biochimie* 2003; 85: 455-63.
- [59] Rusnati M, Tanghetti E, Urbinati C, Tufipano G, Marchesini S, Ziche M, *et al.* Interaction of fibroblast growth factor-2 (FGF-2) with free gangliosides: biochemical characterization and biological consequences in endothelial cell cultures. *Mol Biol Cell* 1999; 10: 313-27.
- [60] Miljan EA, Bremer EG. Regulation of growth factor receptors by gangliosides. *Sci STKE* 2002; 2002: RE15.
- [61] Gualandris A, Rusnati M, Belleri M, Nelli EE, Bastaki M, Molinari-Tosatti MP, *et al.* Basic fibroblast growth factor overexpression in endothelial cells: an autocrine mechanism for angiogenesis and angioproliferative diseases. *Cell Growth Differ* 1996; 7: 147-60.
- [62] Bikfalvi A, Klein S, Pintucci G, Quarto N, Mignatti P, Rifkin DB. Differential modulation of cell phenotype by different molecular weight forms of basic fibroblast growth factor: possible intracellular signaling by the high molecular weight forms. *J Cell Biol* 1995; 129: 233-43.
- [63] Florkiewicz RZ, Baird A, Gonzalez AM. Multiple forms of bFGF: differential nuclear and cell surface localization. *Growth Factors* 1991; 4: 265-75.
- [64] Quarto N, Talarico D, Florkiewicz R, Rifkin DB. Selective expression of high molecular weight basic fibroblast growth factor confers a unique phenotype to NIH 3T3 cells. *Cell Regul* 1991; 2: 699-708.
- [65] Wang Y, Becker D. Antisense targeting of basic fibroblast growth factor and fibroblast growth factor receptor-1 in human melanomas blocks intratumoral angiogenesis and tumor growth. *Nat Med* 1997; 3: 887-93.
- [66] Ribatti D, Alessandri G, Baronio M, Raffaghello L, Cosimo E, Marimpietri D, *et al.* Inhibition of neuroblastoma-induced angiogenesis by fenretinide. *Int J Cancer* 2001; 94: 314-21.
- [67] Tsou R, Isik FF. Integrin activation is required for VEGF and FGF receptor protein presence on human microvascular endothelial cells. *Mol Cell Biochem* 2001; 224: 81-9.
- [68] Fujiwara Y, Kaji T. Possible mechanism for lead inhibition of vascular endothelial cell proliferation: a lower response to basic fibroblast growth factor through inhibition of heparan sulfate synthesis. *Toxicology* 1999; 133: 147-57.
- [69] Zhang W, Chuang YJ, Swanson R, Li J, Seo K, Leung L, *et al.* Antiangiogenic antithrombin down-regulates the expression of the proangiogenic heparan sulfate proteoglycan, perlecan, in endothelial cells. *Blood* 2004; 103: 1185-91.
- [70] Aviezer D, Iozzo RV, Noonan DM, Yayon A. Suppression of autocrine and paracrine functions of basic fibroblast growth factor by stable expression of perlecan antisense cDNA. *Mol Cell Biol* 1997; 17: 1938-46.
- [71] Wang S, Ai X, Freeman SD, Pownall ME, Lu Q, Kessler DS, *et al.* QSulf1, a heparan sulfate 6-O-endosulfatase, inhibits fibroblast growth factor signaling in mesoderm induction and angiogenesis. *Proc Natl Acad Sci USA* 2004; 101: 4833-8.
- [72] Cieslak M, Niewiarowska J, Nawrot M, Koziolkiewicz M, Stec WJ, Cierniewski CS. DNazymes to beta 1 and beta 3 mRNA down-regulate expression of the targeted integrins and inhibit endothelial cell capillary tube formation in fibrin and matrigel. *J Biol Chem* 2002; 277: 6779-87.
- [73] Jee SH, Chu CY, Chiu HC, Huang YL, Tsai WL, Liao YH, *et al.* Interleukin-6 induced basic fibroblast growth factor-dependent angiogenesis in basal cell carcinoma cell line via JAK/STAT3 and PI3-kinase/Akt pathways. *J Invest Dermatol* 2004; 123: 1169-75.
- [74] Ribatti D, Urbinati C, Nico B, Rusnati M, Roncali L, Presta M. Endogenous basic fibroblast growth factor is implicated in the vascularization of the chick embryo chorioallantoic membrane. *Dev Biol* 1995; 170: 39-49.
- [75] Bornstein P, Armstrong LC, Hankenson KD, Kyriakides TR, Yang Z. Thrombospondin 2, a matricellular protein with diverse functions. *Matrix Biol* 2000; 19: 557-68.
- [76] Tarabozetti G, Belotti D, Borsotti P, Vergani V, Rusnati M, Presta M, *et al.* The 140-kilodalton antiangiogenic fragment of thrombospondin-1 binds to basic fibroblast growth factor. *Cell Growth Differ* 1997; 8: 471-9.
- [77] Margosio B, Marchetti D, Vergani V, Giavazzi R, Rusnati M, Presta M, *et al.* Thrombospondin 1 as a scavenger for matrix-associated fibroblast growth factor 2. *Blood* 2003; 102: 4399-406.
- [78] Bossard C, Van den Berghe L, Laurell H, Castano C, Cerutti M, Prats AC, *et al.* Antiangiogenic properties of fibstatin, an extracellular FGF-2-binding polypeptide. *Cancer Res* 2004; 64: 7507-12.
- [79] Asplin IR, Wu SM, Mathew S, Bhattacharjee G, Pizzo SV. Differential regulation of the fibroblast growth factor (FGF) family by alpha(2)-macroglobulin: evidence for selective modulation of FGF-2-induced angiogenesis. *Blood* 2001; 97: 3450-7.
- [80] Dennis PA, Saksela O, Harpel P, Rifkin DB. Alpha 2-macroglobulin is a binding protein for basic fibroblast growth factor. *J Biol Chem* 1989; 264: 7210-6.
- [81] Bottazzi B, Vouret-Craviari V, Bastone A, De Gioia L, Matteucci C, Peri G, *et al.* Multimer formation and ligand recognition by the long pentraxin PTX3. Similarities and differences with the short pentraxins C-reactive protein and serum amyloid P component. *J Biol Chem* 1997; 272: 32817-23.
- [82] Breviaro F, d'Aniello EM, Golay J, Peri G, Bottazzi B, Bairoch A, *et al.* Interleukin-1-inducible genes in endothelial cells. Cloning of a new gene related to C-reactive protein and serum amyloid P component. *J Biol Chem* 1992; 267: 22190-7.
- [83] Rusnati M, Camozzi M, Moroni E, Bottazzi B, Peri G, Indraccolo S, *et al.* Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* 2004; 104: 92-9.

- [84] Salustri A, Garlanda C, Hirsch E, De Acetis M, Maccagno A, Bottazzi B, *et al.* PTX3 plays a key role in the organization of the cumulus oophorus extracellular matrix and in *in vivo* fertilization. *Development* 2004; 131: 1577-86.
- [85] Perollet C, Han ZC, Savona C, Caen JP, Bikfalvi A. Platelet factor 4 modulates fibroblast growth factor 2 (FGF-2) activity and inhibits FGF-2 dimerization. *Blood* 1998; 91: 3289-99.
- [86] Lozano RM, Redondo-Horcajo M, Jimenez MA, Zilberberg L, Cuevas P, Bikfalvi A, *et al.* Solution structure and interaction with basic and acidic fibroblast growth factor of a 3-kDa human platelet factor-4 fragment with antiangiogenic activity. *J Biol Chem* 2001; 276: 35723-34.
- [87] Hagedorn M, Zilberberg L, Wilting J, Canron X, Carrabba G, Giussani C, *et al.* Domain swapping in a COOH-terminal fragment of platelet factor 4 generates potent angiogenesis inhibitors. *Cancer Res* 2002; 62: 6884-90.
- [88] Russo K, Ragone R, Facchiano AM, Capogrossi MC, Facchiano A. Platelet-derived growth factor-BB and basic fibroblast growth factor directly interact *in vitro* with high affinity. *J Biol Chem* 2002; 277: 1284-91.
- [89] De Marchis F, Ribatti D, Giampietri C, Lentini A, Faraone D, Scoccianti M, *et al.* Platelet-derived growth factor inhibits basic fibroblast growth factor angiogenic properties *in vitro* and *in vivo* through its alpha receptor. *Blood* 2002; 99: 2045-53.
- [90] Spinetti G, Camarda G, Bernardini G, Romano Di Peppe S, Capogrossi MC, Napolitano M. The chemokine CXCL13 (BCA-1) inhibits FGF-2 effects on endothelial cells. *Biochem Biophys Res Commun* 2001; 289: 19-24.
- [91] Shellenberger TD, Wang M, Gujrali M, Jayakumar A, Strieter RM, Burdick MD, *et al.* BRAK/CXCL14 is a potent inhibitor of angiogenesis and a chemotactic factor for immature dendritic cells. *Cancer Res* 2004; 64: 8262-70.
- [92] Hanneken A, Baird A. Soluble forms of the high-affinity fibroblast growth factor receptor in human vitreous fluid. *Invest Ophthalmol Vis Sci* 1995; 36: 1192-6.
- [93] Hanneken A, Maher PA, Baird A. High affinity immunoreactive FGF receptors in the extracellular matrix of vascular endothelial cells—implications for the modulation of FGF-2. *J Cell Biol* 1995; 128: 1221-8.
- [94] Bergonzoni L, Caccia P, Cletini O, Sarmientos P, Isacchi A. Characterization of a biologically active extracellular domain of fibroblast growth factor receptor 1 expressed in *Escherichia coli*. *Eur J Biochem* 1992; 210: 823-29.
- [95] Ueno H, Gunn M, Dell K, Tseng A Jr, Williams L. A truncated form of fibroblast growth factor receptor 1 inhibits signal transduction by multiple types of fibroblast growth factor receptor. *J Biol Chem* 1992; 267: 1470-6.
- [96] Presta M, Leali D, Stabile H, Ronca R, Camozzi M, Coco L, *et al.* Heparin derivatives as angiogenesis inhibitors. *Curr Pharm Des* 2003; 9: 553-66.
- [97] Barzu T, Lormeau JC, Petitou M, Michelson S, Choay J. Heparin-derived oligosaccharides: affinity for acidic fibroblast growth factor and effect on its growth-promoting activity for human endothelial cells. *J Cell Physiol* 1989; 140: 538-48.
- [98] Ishihara M, Shaklee PN, Yang Z, Liang W, Wei Z, Stack RJ, *et al.* Structural features in heparin which modulate specific biological activities mediated by basic fibroblast growth factor. *Glycobiology* 1994; 4: 451-8.
- [99] Leali D, Belleri M, Urbinati C, Coltrini D, Oreste P, Zoppetti G, *et al.* Fibroblast growth factor-2 antagonist activity and angiostatic capacity of sulfated *Escherichia coli* K5 polysaccharide derivatives. *J Biol Chem* 2001; 276: 37900-8.
- [100] Urbinati C, Bugatti A, Oreste P, Zoppetti G, Waltenberger J, Mitola S, *et al.* Chemically sulfated *Escherichia coli* K5 polysaccharide derivatives as extracellular HIV-1 Tat protein antagonists. *FEBS Lett* 2004; 568: 171-7.
- [101] Rusnati M, Urbinati C, Caputo A, Possati L, Lortat-Jacob H, Giacca M, *et al.* Pentosan polysulfate as an inhibitor of extracellular HIV-1 Tat. *J Biol Chem* 2001; 276: 22420-5.
- [102] Herbert JM, Cottineau M, Driot F, Pereillo JM, Maffrand JP. Activity of pentosan polysulfate and derived compounds on vascular endothelial cell proliferation and migration induced by acidic and basic FGF *in vitro*. *Biochem Pharmacol* 1988; 37: 4281-8.
- [103] Schwartzmann G, Sprinz E, Kalakun L, Yamagushi N, Sander E, Grivicich I, *et al.* Phase II study of pentosan polysulfate (PPS) in patients with AIDS-related Kaposi's sarcoma. *Tumori* 1996; 82: 360-3.
- [104] Ensoli B, Gendelman R, Markham P, Fiorelli V, Colombini S, Raffeld M, *et al.* Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature* 1994; 371: 674-80.
- [105] Schilling-Schon A, Pleyer U, Hartmann C, Ricck PW. The role of endogenous growth factors to support corneal endothelial migration after wounding *in vitro*. *Exp Eye Res* 2000; 71: 583-9.
- [106] Facchiano A, Russo K, Facchiano AM, De Marchis F, Facchiano F, Ribatti D, *et al.* Identification of a novel domain of fibroblast growth factor 2 controlling its angiogenic properties. *J Biol Chem* 2003; 278: 8751-60.
- [107] Ding L, Donate F, Parry GC, Guan X, Maher P, Levin EG. Inhibition of cell migration and angiogenesis by the amino-terminal fragment of 24kD basic fibroblast growth factor. *J Biol Chem* 2002; 277: 31056-61.
- [108] Neufeld G, Gospodarowicz D. Protamine sulfate inhibits mitogenic activities of the extracellular matrix and fibroblast growth factor, but potentiates that of epidermal growth factor. *J Cell Physiol* 1987; 132: 287-94.
- [109] Brown KJ, Parish CR. Histidine-rich glycoprotein and platelet factor 4 mask heparan sulfate proteoglycans recognized by acidic and basic fibroblast growth factor. *Biochemistry* 1994; 33: 13918-27.
- [110] Reis RC, Schuppan D, Barreto AC, Bauer M, Bork JP, Hassler G, *et al.* Endostatin competes with bFGF for binding to heparin-like glycosaminoglycans. *Biochem Biophys Res Commun* 2005; 333: 976-83.
- [111] Plum SM, Vu HA, Mercer B, Fogler WE, Fortier AH. Generation of a specific immunological response to FGF-2 does not affect wound healing or reproduction. *Immunopharmacol Immunotoxicol* 2004; 26: 29-41.
- [112] Miao RQ, Agata J, Chao L, Chao J. Kallistatin is a new inhibitor of angiogenesis and tumor growth. *Blood* 2002; 100: 3245-52.
- [113] Presta M, Rusnati M, Urbinati C, Sommer A, Ragnotti G. Biologically active synthetic fragments of human basic fibroblast growth factor (bFGF): identification of two Asp-Gly-Arg-containing domains involved in the mitogenic activity of bFGF in endothelial cells. *J Cell Physiol* 1991; 149: 512-24.
- [114] Kumar CC, Malkowski M, Yin Z, Tanghetti E, Yaremko B, Nechuta T, *et al.* Inhibition of angiogenesis and tumor growth by SCH221153, a dual alpha(v)beta3 and alpha(v)beta5 integrin receptor antagonist. *Cancer Res* 2001; 61: 2232-8.
- [115] Belvisi L, Riccioni T, Marcellini M, Vesce L, Chiarucci I, Efrati D, *et al.* Biological and molecular properties of a new alpha(v)beta3/alpha(v)beta5 integrin antagonist. *Mol Cancer Ther* 2005; 4: 1670-80.
- [116] Yeh CH, Peng HC, Yang RS, Huang TF. Rhodostomin, a snake venom disintegrin, inhibits angiogenesis elicited by basic fibroblast growth factor and suppresses tumor growth by a selective alpha(v)beta3 blockade of endothelial cells. *Mol Pharmacol* 2001; 59: 1333-42.
- [117] Collen A, Hanemaaijer R, Lupu F, Quax PH, van Lent N, Grimbergen J, *et al.* Membrane-type matrix metalloproteinase-mediated angiogenesis in a fibrin-collagen matrix. *Blood* 2003; 101: 1810-7.
- [118] Seo DW, Li H, Guedez L, Wingfield PT, Diaz T, Salloum R, *et al.* TIMP-2 mediated inhibition of angiogenesis: an MMP-independent mechanism. *Cell* 2003; 114: 171-80.
- [119] Bauvois B, Dumont J, Mathiot C, Kolb JP. Production of matrix metalloproteinase-9 in early stage B-CLL: suppression by interferons. *Leukemia* 2002; 16: 791-8.
- [120] Bastaki M, Nelli EE, Dell'Era P, Rusnati M, Molinari-Tosatti MP, Parolini S, *et al.* Basic fibroblast growth factor-induced angiogenic phenotype in mouse endothelium. A study of aortic and microvascular endothelial cell lines. *Arterioscler Thromb Vasc Biol* 1997; 17: 454-64.
- [121] Min HY, Doyle LV, Vitt CR, Zandonella CL, Stratton-Thomas JR, Shuman MA, *et al.* Urokinase receptor antagonists inhibit angiogenesis and primary tumor growth in syngeneic mice. *Cancer Res* 1996; 56: 2428-33.

- [122] Blei F, Wilson EL, Mignatti P, Rifkin DB. Mechanism of action of angiostatic steroids: suppression of plasminogen activator activity via stimulation of plasminogen activator inhibitor synthesis. *J Cell Physiol* 1993; 155: 568-78.
- [123] Thorpe PE. Vascular targeting agents as cancer therapeutics. *Clin Cancer Res* 2004; 10: 415-27.
- [124] Tozer GM, Prise VE, Wilson J, Cemazar M, Shan S, Dewhurst MW, *et al.* Mechanisms associated with tumor vascular shut-down induced by combretastatin A-4 phosphate: intravital microscopy and measurement of vascular permeability. *Cancer Res* 2001; 61: 6413-22.
- [125] Vacca A, Iurlaro M, Ribatti D, Minischetti M, Nico B, Ria R, *et al.* Antiangiogenesis is produced by nontoxic doses of vinblastine. *Blood* 1999; 94: 4143-55.
- [126] Belleri M, Ribatti D, Nicoli S, Cotelli F, Forti L, Vannini V, *et al.* Antiangiogenic and vascular-targeting activity of the microtubule-destabilizing trans-resveratrol derivative 3,5,4'-trimethoxystilbene. *Mol Pharmacol* 2005; 67: 1451-9.
- [127] Belotti D, Vergani V, Drudis T, Borsotti P, Pitelli MR, Viale G, *et al.* The microtubule-affecting drug paclitaxel has antiangiogenic activity. *Clin Cancer Res* 1996; 2: 1843-9.
- [128] Taraboletti G, Micheletti G, Rieppi M, Poli M, Turatto M, Rossi C, *et al.* Antiangiogenic and antitumor activity of IDN 5390, a new taxane derivative. *Clin Cancer Res* 2002; 8: 1182-8.
- [129] Abe M, Inoue D, Matsunaga K, Ohizumi Y, Ueda H, Asano T, *et al.* Goniiodomin A, an antifungal polyether macrolide, exhibits antiangiogenic activities via inhibition of actin reorganization in endothelial cells. *J Cell Physiol* 2002; 190: 109-16.
- [130] Wildiers H, Ahmed B, Guetens G, De Boeck G, de Bruijn EA, Landuyt W, *et al.* Combretastatin A-4 phosphate enhances CPT-11 activity independently of the administration sequence. *Eur J Cancer* 2004; 40: 284-90.
- [131] Kwon HJ, Shim JS, Kim JH, Cho HY, Yum YN, Kim SH, *et al.* Betulinic acid inhibits growth factor-induced *in vitro* angiogenesis via the modulation of mitochondrial function in endothelial cells. *Jpn J Cancer Res* 2002; 93: 417-25.
- [132] Shao ZM, Shen ZZ, Liu CH, Sartippour MR, Go VL, Heber D, *et al.* Curcumin exerts multiple suppressive effects on human breast carcinoma cells. *Int J Cancer* 2002; 98: 234-40.
- [133] Mohan R, Sivak J, Ashton P, Russo LA, Pham BQ, Kasahara N, *et al.* Curcuminoids inhibit the angiogenic response stimulated by fibroblast growth factor-2, including expression of matrix metalloproteinase gelatinase B. *J Biol Chem* 2000; 275: 10405-12.
- [134] Sartippour MR, Heber D, Zhang L, Beatty P, Elashoff D, Elashoff R, *et al.* Inhibition of fibroblast growth factors by green tea. *Int J Oncol* 2002; 21: 487-91.
- [135] Chow LM, Chui CH, Tang JC, Lau FY, Yau MY, Cheng GY, *et al.* Anti-angiogenic potential of *Gleditsia sinensis* fruit extract. *Int J Mol Med* 2003; 12: 269-73.
- [136] Liu Y, Ahmad H, Luo Y, Gardiner DT, Gunasekera RS, McKeehan WL, *et al.* Citrus pectin: characterization and inhibitory effect on fibroblast growth factor-receptor interaction. *J Agric Food Chem* 2001; 49: 3051-7.
- [137] Huh JE, Lee EO, Kim MS, Kang KS, Kim CH, Cha BC, *et al.* Penta-O-galloyl-beta-D-glucose suppresses tumor growth via inhibition of angiogenesis and stimulation of apoptosis: roles of cyclooxygenase-2 and mitogen-activated protein kinase pathways. *Carcinogenesis* 2005; 26: 1436-45.
- [138] Brakenhielm E, Cao R, Cao Y. Suppression of angiogenesis, tumor growth, and wound healing by resveratrol, a natural compound in red wine and grapes. *FASEB J* 2001; 15: 1798-800.
- [139] Jeon KS, Na HJ, Kim YM, Kwon HJ. Antiangiogenic activity of 4-O-methylgallic acid from *Canavalia gladiata*, a dietary legume. *Biochem Biophys Res Commun* 2005; 330: 1268-74.
- [140] Taraboletti G, Poli M, Dossi R, Manenti L, Borsotti P, Faircloth GT, *et al.* Antiangiogenic activity of apilidine, a new agent of marine origin. *Br J Cancer* 2004; 90: 2418-24.
- [141] Tong Y, Zhang X, Tian F, Yi Y, Xu Q, Li L, *et al.* Philinopsin A, a novel marine-derived compound possessing dual anti-angiogenic and anti-tumor effects. *Int J Cancer* 2005; 114: 843-53.
- [142] Tong Y, Zhang X, Zhao W, Zhang Y, Lang J, Shi Y, *et al.* Anti-angiogenic effects of Shiraichrome A, a compound isolated from a Chinese folk medicine used to treat rheumatoid arthritis. *Eur J Pharmacol* 2004; 494: 101-9.
- [143] Hoffman R, Burns WW 3rd, Paper DH. Selective inhibition of cell proliferation and DNA synthesis by the polysulphated carbohydrate l-carrageenan. *Cancer Chemother Pharmacol* 1995; 36: 325-34.
- [144] Dupont E, Falardeau P, Mousa SA, Dimitriadou V, Pepin MC, Wang T, *et al.* Antiangiogenic and antimetastatic properties of Neovastat (AE-941), an orally active extract derived from cartilage tissue. *Clin Exp Metastasis* 2002; 19: 145-53.
- [145] Klauber N, Browne F, Anand-Apte B, D'Amato RJ. New activity of spironolactone. Inhibition of angiogenesis *in vitro* and *in vivo*. *Circulation* 1996; 94: 2566-71.
- [146] Koyama S, Takagi H, Otani A, Suzuma K, Nishimura K, Honda Y. Tranilast inhibits protein kinase C-dependent signalling pathway linked to angiogenic activities and gene expression of retinal microcapillary endothelial cells. *Br J Pharmacol* 1999; 127: 537-45.
- [147] Wood J, Bonjean K, Ruetz S, Bellahcene A, Devy L, Foidart JM, *et al.* Novel antiangiogenic effects of the bisphosphonate compound zoledronic acid. *J Pharmacol Exp Ther* 2002; 302: 1055-61.
- [148] Pai R, Szabo IL, Kawanaka H, Soreghan BA, Jones MK, Tamawski AS. Indomethacin inhibits endothelial cell proliferation by suppressing cell cycle proteins and PRB phosphorylation: a key to its antiangiogenic action? *Mol Cell Biol Res Commun* 2000; 4: 111-6.
- [149] Vincent L, Soria C, Mirshahi F, Opolon P, Mishal Z, Vannier JP, *et al.* Cerivastatin, an inhibitor of 3-hydroxy-3-methylglutaryl coenzyme A reductase, inhibits endothelial cell proliferation induced by angiogenic factors *in vitro* and angiogenesis in *in vivo* models. *Arterioscler Thromb Vasc Biol* 2002; 22: 623-9.
- [150] Klein-Soyer C, Cazenave JP, Herbert JM, Maffrand JP. SR 25989 inhibits healing of a mechanical wound of confluent human saphenous vein endothelial cells which is modulated by standard heparin and growth factors. *J Cell Physiol* 1994; 160: 316-22.
- [151] Spandau UH, Sauder G, Schubert U, Hammes HP, Jonas JB. Effect of triamcinolone acetonide on proliferation of retinal endothelial cells *in vitro* and *in vivo*. *Br J Ophthalmol* 2005; 89: 745-7.
- [152] Chen W, Li L, Zhu J, Liu J, Soria J, Soria C, *et al.* Control of angiogenesis by inhibitor of phospholipase A2. *Chin Med Sci J* 2004; 19: 6-12.
- [153] Sulpice E, Bryckaert M, Lacour J, Contreres JO, Tobelem G. Platelet factor 4 inhibits FGF2-induced endothelial cell proliferation via the extracellular signal-regulated kinase pathway but not by the phosphatidylinositol 3-kinase pathway. *Blood* 2002; 100: 3087-94.
- [154] Feitsma K, Hausser H, Robenek H, Kresse H, Vischer P. Interaction of thrombospondin-1 and heparan sulfate from endothelial cells. Structural requirements of heparan sulfate. *J Biol Chem* 2000; 275: 9396-402.
- [155] Dawson DW, Volpert OV, Pearce SF, Schneider AJ, Silverstein RL, Henkin J, *et al.* Three distinct D-amino acid substitutions confer potent antiangiogenic activity on an inactive peptide derived from a thrombospondin-1 type 1 repeat. *Mol Pharmacol* 1999; 55: 332-8.
- [156] Aguzzi MS, Giampietri C, De Marchis F, Padula F, Gaeta R, Ragone G, *et al.* RGDS peptide induces caspase 8 and caspase 9 activation in human endothelial cells. *Blood* 2004; 103: 4180-7.
- [157] Ribatti D, Vacca A, Merchionne F, Presta M. Antiangiogenesis by chemotherapeutic agents. *Mini Rev Med Chem* 2005; 5: 313-7.
- [158] Keledjian K, Garrison JB, Kyprianou N. Doxazosin inhibits human vascular endothelial cell adhesion, migration, and invasion. *J Cell Biochem* 2005; 94: 374-88.
- [159] Gelati M, Corsini E, Frigerio S, Pollo B, Broggi G, Croci D, *et al.* Effects of thalidomide on parameters involved in angiogenesis: an *in vitro* study. *J Neurooncol* 2003; 64: 193-201.
- [160] Gagliardi AR, Hennig B, Collins DC. Antiestrogens inhibit endothelial cell growth stimulated by angiogenic growth factors. *Anticancer Res* 1996; 16: 1101-6.
- [161] Ashino-Fuse H, Takano Y, Oikawa T, Shimamura M, Iwaguchi T. Medroxyprogesterone acetate, an anti-cancer and anti-angiogenic steroid, inhibits the plasminogen activator in bovine endothelial cells. *Int J Cancer* 1989; 44: 859-64.
- [162] Nakashio A, Fujita N, Tsuruo T. Topotecan inhibits VEGF- and bFGF-induced vascular endothelial cell migration via downregulation of the PI3K-Akt signaling pathway. *Int J Cancer* 2002; 98: 36-41.
- [163] Presta M, Belleri M, Vacca A, Ribatti D. Anti-angiogenic activity of the purine analog 6-thioguanine. *Leukemia* 2002; 16: 1490-9.

- [164] Basaki Y, Aoyagi K, Chikahisa L, Miyadera K, Hashimoto A, Yonekura K, *et al.* UFT and its metabolites inhibit cancer-induced angiogenesis. *Via a VEGF-related pathway.* *Oncology (Huntingt)* 2000; 14: 68-71.
- [165] Shailubhai K, Dheer S, Picker D, Kaur G, Sausville EA, Jacob GS. Atiprimod is an inhibitor of cancer cell proliferation and angiogenesis. *J Exp Ther Oncol* 2004; 4: 267-79.
- [166] Yamayoshi T, Nagayasu T, Matsumoto K, Abo T, Hishikawa Y, Koji T. Expression of keratinocyte growth factor/fibroblast growth factor-7 and its receptor in human lung cancer: correlation with tumour proliferative activity and patient prognosis. *J Pathol* 2004; 204: 110-8.
- [167] Gnanapragasam VJ, Robinson MC, Marsh C, Robson CN, Hamdy FC, Leung HY. FGF8 isoform b expression in human prostate cancer. *Br J Cancer* 2003; 88: 1432-8.
- [168] Heer R, Douglas D, Mathers M, Robson C, Leung H. Fibroblast growth factor 17 is over-expressed in human prostate cancer. *J Pathol* 2004; 204: 578-86.
- [169] Auguste P, Gursel DB, Lemiere S, Reimers D, Cuevas P, Carceller F, *et al.* Inhibition of fibroblast growth factor/fibroblast growth factor receptor activity in glioma cells impedes tumor growth by both angiogenesis-dependent and -independent mechanisms. *Cancer Res* 2001; 61: 1717-26.
- [170] Polnaszek N, Kwabi-Addo B, Peterson LE, Ozen M, Greenberg NM, Ortega S, *et al.* Fibroblast growth factor 2 promotes tumor progression in an autochthonous mouse model of prostate cancer. *Cancer Res* 2003; 63: 5754-60.
- [171] Hagedom M, Bikfalvi A. Target molecules for anti-angiogenic therapy: from basic research to clinical trials. *Crit Rev Oncol Hematol* 2000; 34: 89-110.
- [172] Ziche M, Donnini S, Morbidelli L. Development of new drugs in angiogenesis. *Curr Drug Targets* 2004; 5: 485-93.
- [173] Patel SR, Jenkins J, Papadopolous N, Burgess MA, Plager C, Gutterman J, *et al.* Pilot study of vitaxin--an angiogenesis inhibitor in patients with advanced leiomyosarcomas. *Cancer* 2001; 92: 1347-8.
- [174] Posey JA, Khazaeli MB, DelGrosso A, Saleh MN, Lin CY, Huse W, *et al.* A pilot trial of Vitaxin, a humanized anti-vitronectin receptor (anti alpha v beta 3) antibody in patients with metastatic cancer. *Cancer Biother Radiopharm* 2001; 16: 125-32.
- [175] Huang S, Bucana CD, Van Arsdall M, Fidler IJ. Stat1 negatively regulates angiogenesis, tumorigenicity and metastasis of tumor cells. *Oncogene* 2002; 21: 2504-12.
- [176] Cassinelli G, Lanzi C, Supino R, Pratesi G, Zuco V, Laccabue D, *et al.* Cellular bases of the antitumor activity of the novel taxane IDN 5109 (BAY59-8862) on hormone-refractory prostate cancer. *Clin Cancer Res* 2002; 8: 2647-54.
- [177] Hotchkiss KA, Ashton AW, Mahmood R, Russell RG, Sparano JA, Schwartz EL. Inhibition of endothelial cell function *in vitro* and angiogenesis *in vivo* by docetaxel (Taxotere): association with impaired repositioning of the microtubule organizing center. *Mol Cancer Ther* 2002; 1: 1191-200.
- [178] Ciardiello F, Caputo R, Bianco R, Damiano V, Fontanini G, Cuccato S, *et al.* Inhibition of growth factor production and angiogenesis in human cancer cells by ZD1839 (Iressa), a selective epidermal growth factor receptor tyrosine kinase inhibitor. *Clin Cancer Res* 2001; 7: 1459-65.
- [179] Ginns LC, Roberts DH, Mark EJ, Bruschi JL, Marler JJ. Pulmonary capillary hemangiomatosis with atypical endotheliomatosis: successful antiangiogenic therapy with doxycycline. *Chest* 2003; 124: 2017-22.
- [180] Dmoszynska A, Bojarska-Junak A, Domanski D, Rolinski J, Hus M, Soroka-Wojtaszko M. Production of proangiogenic cytokines during thalidomide treatment of multiple myeloma. *Leuk Lymphoma* 2002; 43: 401-6.
- [181] Ferretti G, Fabi A, Carlini P, Papaldo P, Cordiali Fei P, Di Cosimo S, *et al.* Zoledronic-acid-induced circulating level modifications of angiogenic factors, metalloproteinases and proinflammatory cytokines in metastatic breast cancer patients. *Oncology* 2005; 69: 35-43.
- [182] Bianco C, Tortora G, Baldassarre G, Caputo R, Fontanini G, Chine S, *et al.* 8-Chloro-cyclic AMP inhibits autocrine and angiogenic growth factor production in human colorectal and breast cancer. *Clin Cancer Res* 1997; 3: 439-48.
- [183] Sasamura H, Takahashi A, Miyao N, Yanase M, Masumori N, Kitamura H, *et al.* Inhibitory effect on expression of angiogenic factors by antiangiogenic agents in renal cell carcinoma. *Br J Cancer* 2002; 86: 768-73.
- [184] Fujimoto J, Hori M, Ichigo S, Hirose R, Sakaguchi H, Tamaya T. Plausible novel therapeutic strategy of uterine endometrial cancer with reduction of basic fibroblast growth factor secretion by progestin and O-(chloroacetyl-carbamoyl) fumagillol (TNP-470; AGM-1470). *Cancer Lett* 1997; 113: 187-94.
- [185] Wesley UV, McGroarty M, Homoyouni A. Dipeptidyl peptidase inhibits malignant phenotype of prostate cancer cells by blocking basic fibroblast growth factor signaling pathway. *Cancer Res* 2005; 65: 1325-34.
- [186] Huang SF, Kim SJ, Lee AT, Karashima T, Bucana C, Kedar D, *et al.* Inhibition of growth and metastasis of orthotopic human prostate cancer in athymic mice by combination therapy with pegylated interferon-alpha-2b and docetaxel. *Cancer Res* 2002; 62: 5720-6.
- [187] Yoshida S, Ono M, Shono T, Izumi H, Ishibashi T, Suzuki H, *et al.* Involvement of interleukin-8, vascular endothelial growth factor, and basic fibroblast growth factor in tumor necrosis factor alpha-dependent angiogenesis. *Mol Cell Biol* 1997; 17: 4015-23.
- [188] Faris M, Ensoli B, Kokot N, Nel AE. Inflammatory cytokines induce the expression of basic fibroblast growth factor (bFGF) isoforms required for the growth of Kaposi's sarcoma and endothelial cells through the activation of AP-1 response elements in the bFGF promoter. *AIDS* 1998; 12: 19-27.
- [189] Khachigian LM. Early growth response-1: blocking angiogenesis by shooting the messenger. *Cell Cycle* 2004; 3: 10-1.
- [190] Maier JA, Morelli D, Menard S, Colnaghi MI, Balsari A. Tumor-necrosis-factor-induced fibroblast growth factor-1 acts as a survival factor in a transformed endothelial cell line. *Am J Pathol* 1996; 149: 945-52.
- [191] Lee HT, Lee JG, Na M, Kay EP. FGF-2 induced by interleukin-1 beta through the action of phosphatidylinositol 3-kinase mediates endothelial mesenchymal transformation in corneal endothelial cells. *J Biol Chem* 2004; 279: 32325-32.
- [192] Albuquerque ML, Akiyama SK, Schnaper HW. Basic fibroblast growth factor release by human coronary artery endothelial cells is enhanced by matrix proteins, 17beta-estradiol, and a PKC signaling pathway. *Exp Cell Res* 1998; 245: 163-9.
- [193] Patel S, Leal AD, Gorski DH. The homeobox gene Gax inhibits angiogenesis through inhibition of nuclear factor-kappaB-dependent endothelial cell gene expression. *Cancer Res* 2005; 65: 1414-24.
- [194] Hanařusa H, Torii S, Yasunaga T, Nishida E. Sproutyl and Sprout2 provide a control mechanism for the Ras/MAPK signalling pathway. *Nat Cell Biol* 2002; 4: 850-8.
- [195] Kaur G, Belotti D, Burger AM, Fisher-Nielson K, Borsotti P, Riccardi E, *et al.* Antiangiogenic properties of 17-(dimethylaminoethylamino)-17-demethoxygeldanamycin: an orally bioavailable heat shock protein 90 modulator. *Clin Cancer Res* 2004; 10: 4813-21.
- [196] Kroon ME, van Schie ML, van der Vecht B, van Hinsbergh VW, Koolwijk P. Collagen type 1 retards tube formation by human microvascular endothelial cells in a fibrin matrix. *Angiogenesis* 2002; 5: 257-65.
- [197] Staton CA, Brown NJ, Rodgers GR, Corke KP, Tazzyman S, Underwood JC, *et al.* Alphastatin, a 24-amino acid fragment of human fibrinogen, is a potent new inhibitor of activated endothelial cells *in vitro* and *in vivo*. *Blood* 2004; 103: 601-6.
- [198] Dixelius J, Cross M, Matsumoto T, Sasaki T, Timpl R, Claesson-Welsh L. Endostatin regulates endothelial cell adhesion and cytoskeletal organization. *Cancer Res* 2002; 62: 1944-7.
- [199] Dixelius J, Larsson H, Sasaki T, Holmqvist K, Lu L, Engstrom A, *et al.* Endostatin-induced tyrosine kinase signaling through the Shb adaptor protein regulates endothelial cell apoptosis. *Blood* 2000; 95: 3403-11.
- [200] Guan X, Juarez JC, Qi X, Shipulina NV, Shaw DE, Morgan WT, *et al.* Histidine-proline rich glycoprotein (HPRG) binds and transduces anti-angiogenic signals through cell surface tropomyosin on endothelial cells. *Thromb Haemost* 2004; 92: 403-12.
- [201] Ashton AW, Cheng Y, Helisch A, Ware JA. Thromboxane A2 receptor agonists antagonize the proangiogenic effects of fibroblast

- growth factor-2: role of receptor internalization, thrombospondin-1, and alpha(v)beta3. *Circ Res* 2004; 94: 735-42.
- [202] Redlitz A, Daum G, Sage EH. Angiostatin diminishes activation of the mitogen-activated protein kinases ERK-1 and ERK-2 in human dermal microvascular endothelial cells. *J Vasc Res* 1999; 36: 28-34.
- [203] Duenas Z, Torner L, Corbacho AM, Ochoa A, Gutierrez-Ospina G, Lopez-Barrera F, *et al.* Inhibition of rat corneal angiogenesis by 16-kDa prolactin and by endogenous prolactin-like molecules. *Invest Ophthalmol Vis Sci* 1999; 40: 2498-505.
- [204] Kanda S, Mochizuki Y, Miyata Y, Kanetake H, Yamamoto N. Effects of vitamin D(3)-binding protein-derived macrophage activating factor (GcMAF) on angiogenesis. *J Natl Cancer Inst* 2002; 94: 1311-9.
- [205] Baiguera S, Conconi MT, Guidolin D, Mazzocchi G, Malendowicz LK, Parnigotto PP, *et al.* Ghrelin inhibits *in vitro* angiogenic activity of rat brain microvascular endothelial cells. *Int J Mol Med* 2004; 14: 849-54.
- [206] Rikitake Y, Kawashima S, Yamashita T, Ueyama T, Ishido S, Hotta H, *et al.* Lysophosphatidylcholine inhibits endothelial cell migration and proliferation *via* inhibition of the extracellular signal-regulated kinase pathway. *Arterioscler Thromb Vasc Biol* 2000; 20: 1006-12.
- [207] Zhang JC, Donate F, Qi X, Ziats NP, Juarez JC, Mazar AP, *et al.* The antiangiogenic activity of cleaved high molecular weight kininogen is mediated through binding to endothelial cell tropomyosin. *Proc Natl Acad Sci USA* 2002; 99: 12224-9.
- [208] Kim J, Cheon IS, Won YJ, Na HJ, Kim YM, Choe J. IL-4 inhibits cell cycle progression of human umbilical vein endothelial cells by affecting p53, p21(Waf1), cyclin D1, and cyclin E expression. *Mol Cells* 2003; 16: 92-6.
- [209] Sgadari C, Angiolillo AL, Tosato G. Inhibition of angiogenesis by interleukin-12 is mediated by the interferon-inducible protein 10. *Blood* 1996; 87: 3877-82.
- [210] Angiolillo AL, Sgadari C, Taub DD, Liao F, Farber JM, Maheshwari S, *et al.* Human interferon-inducible protein 10 is a potent inhibitor of angiogenesis *in vivo*. *J Exp Med* 1995; 182: 155-62.
- [211] Kanda S, Mochizuki Y, Nakamura T, Miyata Y, Matsuyama T, Kanetake H. Pigment epithelium-derived factor inhibits fibroblast-growth-factor-2-induced capillary morphogenesis of endothelial cells through Fyn. *J Cell Sci* 2005; 118: 961-70.
- [212] Kaur B, Brat DJ, Devi NS, Van Meir EG. Vasculostatin, a proteolytic fragment of brain angiogenesis inhibitor 1, is an antiangiogenic and antitumorigenic factor. *Oncogene* 2005; 24: 3632-42.
- [213] Pike SE, Yao L, Jones KD, Cherney B, Appella E, Sakaguchi K, *et al.* Vasostatin, a calreticulin fragment, inhibits angiogenesis and suppresses tumor growth. *J Exp Med* 1998; 188: 2349-56.
- [214] Guo YL, Wang S, Colman RW. Kininostatin, an angiogenic inhibitor, inhibits proliferation and induces apoptosis of human endothelial cells. *Arterioscler Thromb Vasc Biol* 2001; 21: 1427-33.
- [215] Lafleur MA, Handsley MM, Knauper V, Murphy G, Edwards DR. Endothelial tubulogenesis within fibrin gels specifically requires the activity of membrane-type-matrix metalloproteinases (MT-MMPs). *J Cell Sci* 2002; 115: 3427-38.
- [216] Norioka K, Mitaka T, Mochizuki Y, Hara M, Kawagoe M, Nakamura H. Interaction of interleukin-1 and interferon-gamma on fibroblast growth factor-induced angiogenesis. *Jpn J Cancer Res* 1994; 85: 522-9.
- [217] Sato N, Nariuchi H, Tsuruoka N, Nishihara T, Beitz JG, Calabresi P, *et al.* Actions of TNF and IFN-gamma on angiogenesis *in vitro*. *J Invest Dermatol* 1990; 95: 85S-89S.
- [218] Grant MB, Caballero S, Millard WJ. Inhibition of IGF-I and b-FGF stimulated growth of human retinal endothelial cells by the somatostatin analogue, octreotide: a potential treatment for ocular neovascularization. *Regul Pept* 1993; 48: 267-78.
- [219] Schulter V, Koolwijk P, Peters E, Frank S, Hrzenjak A, Graier WF, *et al.* Impact of apolipoprotein(a) on *in vitro* angiogenesis. *Arterioscler Thromb Vasc Biol* 2001; 21: 433-8.
- [220] Kessler O, Shraga-Heled N, Lange T, Gutmann-Raviv N, Sabo E, Baruch L, *et al.* Semaphorin-3F is an inhibitor of tumor angiogenesis. *Cancer Res* 2004; 64: 1008-15.
- [221] Foxall C, Wei Z, Schaefer ME, Casabonne M, Fugedi P, Peto C, *et al.* Sulfated malto-oligosaccharides bind to basic FGF, inhibit endothelial cell proliferation, and disrupt endothelial cell tube formation. *J Cell Physiol* 1996; 168: 657-67.
- [222] Kasbauer CW, Paper DH, Franz G. Sulfated beta-(1->4)-galactooligosaccharides and their effect on angiogenesis. *Carbohydr Res* 2001; 330: 427-30.
- [223] Miao HQ, Ornitz DM, Aingorn E, Ben-Sasson SA, Vlodavsky I. Modulation of fibroblast growth factor-2 receptor binding, dimerization, signaling, and angiogenic activity by a synthetic heparin-mimicking polyanionic compound. *J Clin Invest* 1997; 99: 1565-75.
- [224] Hasan J, Shnyder SD, Clamp AR, McGown AT, Bicknell R, Presta M, *et al.* Heparin octasaccharides inhibit angiogenesis *in vivo*. *Clin Cancer Res* 2005; 11: 8172-9.
- [225] Giroux JL, Matou S, Bros A, Tapon-Bretaudiere J, Letourneur D, Fischer AM. Modulation of human endothelial cell proliferation and migration by fucoidan and heparin. *Eur J Cell Biol* 1998; 77: 352-9.
- [226] Chabut D, Fischer AM, Collic-Jouault S, Laurendeau I, Matou S, Le Bonniec B, *et al.* Low molecular weight fucoidan and heparin enhance the basic fibroblast growth factor-induced tube formation of endothelial cells through heparan sulfate-dependent alpha6 overexpression. *Mol Pharmacol* 2003; 64: 696-702.
- [227] Gagliardi AR, Taylor MF, Collins DC. Uptake of suramin by human microvascular endothelial cells. *Cancer Lett* 1998; 125: 97-102.
- [228] Takano S, Gately S, Neville ME, Herblin WF, Gross JL, Engelhard H, *et al.* Suramin, an anticancer and angiostatic agent, inhibits endothelial cell binding of basic fibroblast growth factor, migration, proliferation, and induction of urokinase-type plasminogen activator. *Cancer Res* 1994; 54: 2654-60.
- [229] Sofa F, Gualandris A, Belleri M, Giuliani R, Coltrini D, Bastaki M, *et al.* Endothelial cells overexpressing basic fibroblast growth factor (FGF-2) induce vascular tumors in immunodeficient mice. *Angiogenesis* 1997; 1: 102-16.
- [230] Aviezer D, Cotton S, David M, Segev A, Khaselev N, Galili N, *et al.* Porphyrin analogues as novel antagonists of fibroblast growth factor and vascular endothelial growth factor receptor binding that inhibit endothelial cell proliferation, tumor progression, and metastasis. *Cancer Res* 2000; 60: 2973-80.
- [231] Benelli U, Bocci G, Danesi R, Lepri A, Bernardini N, Bianchi F, *et al.* The heparan sulfate sulferoide inhibits rat corneal angiogenesis and *in vitro* neovascularization. *Exp Eye Res* 1998; 67: 133-42.
- [232] Casu B, Guerrini M, Guglieri S, Naggi A, Perez M, Torri G, *et al.* Undersulfated and glycol-split heparins endowed with antiangiogenic activity. *J Med Chem* 2004; 47: 838-48.
- [233] Liekens S, Leali D, Neyts J, Esnouf R, Rusnati M, Dell'Era P, *et al.* Modulation of fibroblast growth factor-2 receptor binding, signaling, and mitogenic activity by heparin-mimicking polysulfonated compounds. *Mol Pharmacol* 1999; 56: 204-13.
- [234] Liekens S, Neyts J, Degreve B, De Clercq E. The sulfonic acid polymers PAMPS [poly(2-acrylamido-2-methyl-1-propanesulfonic acid)] and related analogues are highly potent inhibitors of angiogenesis. *Oncol Res* 1997; 9: 173-81.
- [235] Sakairi N, Kuzuhara H, Okamoto T, Yajima M. Synthesis and biological evaluation of 2-amino-2-deoxy- and 6-amino-6-deoxycyclomaltoheptaose polysulfates as synergists for angiogenesis inhibition. *Bioorg Med Chem* 1996; 4: 2187-92.
- [236] Gagliardi AR, Collins DC. Inhibition of angiogenesis by aurintricarboxylic acid. *Anticancer Res* 1994; 14: 475-9.
- [237] Kitajima I, Unoki K, Maruyama I. Phosphorothioate oligodeoxynucleotides inhibit basic fibroblast growth factor-induced angiogenesis *in vitro* and *in vivo*. *Antisense Nucleic Acid Drug Dev* 1999; 9: 233-9.
- [238] Ziche M, Morbidelli L, Alessandri G, Gullino PM. Angiogenesis can be stimulated or repressed *in vivo* by a change in GM3:GD3 ganglioside ratio. *Lab Invest* 1992; 67: 711-5.
- [239] Vucenic I, Passaniti A, Vitolo MI, Tantivejkul K, Eggleton P, Shamsuddin AM. Anti-angiogenic activity of inositol hexaphosphate (IP6). *Carcinogenesis* 2004; 25: 2115-23.
- [240] Heryanto B, Lipson KE, Rogers PA. Effect of angiogenesis inhibitors on oestrogen-mediated endometrial endothelial cell

- proliferation in the ovariectomized mouse. *Reproduction* 2003; 125: 337-46.
- [241] Wang LL, Li JJ, Zheng ZB, Liu HY, Du GJ, Li S. Antitumor activities of a novel indolin-2-ketone compound, Z24: more potent inhibition on bFGF-induced angiogenesis and bcl-2 over-expressing cancer cells. *Eur J Pharmacol* 2004; 502: 1-10.
- [242] Dimitroff CJ, Klohs W, Sharma A, Pera P, Driscoll D, Veith J, *et al.* Anti-angiogenic activity of selected receptor tyrosine kinase inhibitors, PD166285 and PD173074: implications for combination treatment with photodynamic therapy. *Invest New Drugs* 1999; 17: 121-35.
- [243] Beebe JS, Jani JP, Knauth E, Goodwin P, Higdon C, Rossi AM, *et al.* Pharmacological characterization of CP-547,632, a novel vascular endothelial growth factor receptor-2 tyrosine kinase inhibitor for cancer therapy. *Cancer Res* 2003; 63: 7301-9.
- [244] Alavi A, Hood JD, Frausto R, Stupack DG, Cheresch DA. Role of Raf in vascular protection from distinct apoptotic stimuli. *Science* 2003; 301: 94-6.
- [245] Giuhani R, Bastaki M, Coltrini D, Presta M. Role of endothelial cell extracellular signal-regulated kinase1/2 in urokinase-type plasminogen activator upregulation and *in vitro* angiogenesis by fibroblast growth factor-2. *J Cell Sci* 1999; 112 (Pt 15): 2597-606.
- [246] Pintucci G, Yu PJ, Sharony R, Baumann FG, Saponara F, Frasca A, *et al.* Induction of stromelysin-1 (MMP-3) by fibroblast growth factor-2 (FGF-2) in FGF-2/- microvascular endothelial cells requires prolonged activation of extracellular signal-regulated kinases-1 and -2 (ERK-1/2). *J Cell Biochem* 2003; 90: 1015-25.
- [247] Naik MU, Vuppalachandi D, Naik UP. Essential role of junctional adhesion molecule-1 in basic fibroblast growth factor-induced endothelial cell migration. *Arterioscler Thromb Vasc Biol* 2003; 23: 2165-71.
- [248] Bhagwat SV, Petrovic N, Okamoto Y, Shapiro LH. The angiogenic regulator CD13/APN is a transcriptional target of Ras signaling pathways in endothelial morphogenesis. *Blood* 2003; 101: 1818-26.
- [249] Kuzuya M, Satake S, Ramos MA, Kanda S, Koike T, Yoshino K, *et al.* Induction of apoptotic cell death in vascular endothelial cells cultured in three-dimensional collagen lattice. *Exp Cell Res* 1999; 248: 498-508.
- [250] Santiago FS, Lowe HC, Day FL, Chesterman CN, Khachigian LM. Early growth response factor-1 induction by injury is triggered by release and paracrine activation by fibroblast growth factor-2. *Am J Pathol* 1999; 154: 937-44.
- [251] Hata Y, Rook SL, Aiello LP. Basic fibroblast growth factor induces expression of VEGF receptor KDR through a protein kinase C and p44/p42 mitogen-activated protein kinase-dependent pathway. *Diabetes* 1999; 48: 1145-55.
- [252] Langford D, Hurford R, Hashimoto M, Digicaylioglu M, Masliah E. Signalling crosstalk in FGF2-mediated protection of endothelial cells from HIV-gp120. *BMC Neurosci* 2005; 6: 8.
- [253] Zubilewicz A, Hecquet C, Jeanny JC, Soubrane G, Courtois Y, Mascarelli F. Two distinct signalling pathways are involved in FGF2-stimulated proliferation of choriocapillary endothelial cells: a comparative study with VEGF. *Oncogene* 2001; 20: 1403-13.
- [254] Tanaka K, Abe M, Sato Y. Roles of extracellular signal-regulated kinase 1/2 and p38 mitogen-activated protein kinase in the signal transduction of basic fibroblast growth factor in endothelial cells during angiogenesis. *Jpn J Cancer Res* 1999; 90: 647-54.
- [255] Lu L, Holmqvist K, Cross M, Welsh M. Role of the Src homology 2 domain-containing protein Shb in murine brain endothelial cell proliferation and differentiation. *Cell Growth Differ* 2002; 13: 141-8.
- [256] Kay EP, Park SY, Ko MK, Lee SC. Fibroblast growth factor 2 uses PLC-gamma1 for cell proliferation and PI3-kinase for alteration of cell shape and cell proliferation in corneal endothelial cells. *Mol Vis* 1998; 4: 22.
- [257] Kent KC, Mii S, Harrington EO, Chang JD, Mallette S, Ware JA. Requirement for protein kinase C activation in basic fibroblast growth factor-induced human endothelial cell proliferation. *Circ Res* 1995; 77: 231-8.
- [258] Presta M, Tiberio L, Rusnati M, Dell'Era P, Ragnotti G. Basic fibroblast growth factor requires a long-lasting activation of protein kinase C to induce cell proliferation in transformed fetal bovine aortic endothelial cells. *Cell Regul* 1991; 2: 719-26.
- [259] Haimovitz-Friedman A, Balaban N, McLoughlin M, Ehleiter D, Michaelli J, Vladavsky I, *et al.* Protein kinase C mediates basic fibroblast growth factor protection of endothelial cells against radiation-induced apoptosis. *Cancer Res* 1994; 54: 2591-7.
- [260] Takano S, Gately S, Jiang JB, Brem S. A diaminoanthraquinone inhibitor of angiogenesis. *J Pharmacol Exp Ther* 1994; 271: 1027-33.
- [261] Hu DE, Fan TP. Protein kinase C inhibitor calphostin C prevents cytokine-induced angiogenesis in the rat. *Inflammation* 1995; 19: 39-54.
- [262] Klint P, Kanda S, Kloog Y, Claesson-Welsh L. Contribution of Src and Ras pathways in FGF-2 induced endothelial cell differentiation. *Oncogene* 1999; 18: 3354-64.
- [263] van Hinsbergh VW, Vermeer M, Koolwijk P, Grimbergen J, Kooistra T. Genistein reduces tumor necrosis factor alpha-induced plasminogen activator inhibitor-1 transcription but not urokinase expression in human endothelial cells. *Blood* 1994; 84: 2984-91.
- [264] Shono T, Kanetake H, Kanda S. The role of mitogen-activated protein kinase activation within focal adhesions in chemotaxis toward FGF-2 by murine brain capillary endothelial cells. *Exp Cell Res* 2001; 264: 275-83.
- [265] Kilarski WW, Jura N, Gerwins P. Inactivation of Src family kinases inhibits angiogenesis *in vivo*: implications for a mechanism involving organization of the actin cytoskeleton. *Exp Cell Res* 2003; 291: 70-82.
- [266] Lee HT, Kim TY, Kay EP. Cdk4 and p27Kip1 play a role in PLC-gamma1-mediated mitogenic signaling pathway of 18 kDa FGF-2 in corneal endothelial cells. *Mol Vis* 2002; 8: 17-25.
- [267] Sa G, Fox PL. Basic fibroblast growth factor-stimulated endothelial cell movement is mediated by a pertussis toxin-sensitive pathway regulating phospholipase A2 activity. *J Biol Chem* 1994; 269: 3219-25.
- [268] Anwar KN, Fazal F, Malik AB, Rahman A. RhoA/Rho-associated kinase pathway selectively regulates thrombin-induced intercellular adhesion molecule-1 expression in endothelial cells via activation of I kappa B kinase beta and phosphorylation of RelA/p65. *J Immunol* 2004; 173: 6965-72.
- [269] Soriano JV, Liu N, Gao Y, Yao ZJ, Ishibashi T, Underhill C, *et al.* Inhibition of angiogenesis by growth factor receptor bound protein 2-Src homology 2 domain bound antagonists. *Mol Cancer Ther* 2004; 3: 1289-99.
- [270] D'Angelo G, Lee H, Weiner RI. cAMP-dependent protein kinase inhibits the mitogenic action of vascular endothelial growth factor and fibroblast growth factor in capillary endothelial cells by blocking Raf activation. *J Cell Biochem* 1997; 67: 353-66.
- [271] Forough R, Weylie B, Patel C, Ambrus S, Singh US, Zhu J. Role of AKT/PKB signaling in fibroblast growth factor-1 (FGF-1)-induced angiogenesis in the chicken chorioallantoic membrane (CAM). *J Cell Biochem* 2005; 94: 109-16.
- [272] Hoffmann S, He S, Jin ML, Masiero L, Wiedemann P, Ryan SJ, *et al.* Carboxyamido-triazole modulates retinal pigment epithelial and choroidal endothelial cell attachment, migration, proliferation, and MMP-2 secretion of choroidal endothelial cells. *Curr Eye Res* 2005; 30: 103-13.
- [273] Wijelath ES, Carlsen B, Cole T, Chen J, Kothari S, Hammond WP. Oncostatin M induces basic fibroblast growth factor expression in endothelial cells and promotes endothelial cell proliferation, migration and spindle morphology. *J Cell Sci* 1997; 110 (Pt 7): 871-9.
- [274] Danesi R, Del Bianchi S, Soldani P, Campagni A, La Rocca RV, Myers CE, *et al.* Suramin inhibits bFGF-induced endothelial cell proliferation and angiogenesis in the chick chorioallantoic membrane. *Br J Cancer* 1993; 68: 932-8.
- [275] Cross MJ, Hodgkin MN, Roberts S, Landgren E, Wakelam MJ, Claesson-Welsh L. Tyrosine 766 in the fibroblast growth factor receptor-1 is required for FGF-stimulation of phospholipase C, phospholipase D, phospholipase A(2), phosphoinositide 3-kinase and cytoskeletal reorganisation in porcine aortic endothelial cells. *J Cell Sci* 2000; 113 (Pt 4): 643-51.
- [276] Lee SH, Schloss DJ, Swain JL. Maintenance of vascular integrity in the embryo requires signaling through the fibroblast growth factor receptor. *J Biol Chem* 2000; 275: 33679-87.
- [277] Hood JD, Frausto R, Kiosses WB, Schwartz MA, Cheresch DA. Differential alpha integrin-mediated Ras-ERK signaling during two pathways of angiogenesis. *J Cell Biol* 2003; 162: 933-43.
- [278] Mettouchi A, Klein S, Guo W, Lopez-Lago M, Lemichez E, Westwick JK, *et al.* Integrin-specific activation of Rac controls

- progression through the G(1) phase of the cell cycle. *Mol Cell* 2001; 8: 115-27.
- [279] Cross MJ, Lu L, Magnusson P, Nyqvist D, Holmqvist K, Welsh M, *et al.* The Shb adaptor protein binds to tyrosine 766 in the FGFR-1 and regulates the Ras/MEK/MAPK pathway via FRS2 phosphorylation in endothelial cells. *Mol Biol Cell* 2002; 13: 2881-93.
- [280] Murakami M, Horowitz A, Tang S, Ware JA, Simons M. Protein kinase C (PKC) delta regulates PKCalpha activity in a Syndecan-4-dependent manner. *J Biol Chem* 2002; 277: 20367-71.
- [281] Kanda S, Lerner EC, Tsuda S, Shono T, Kanetake H, Smithgall TE. The nonreceptor protein-tyrosine kinase c-Fes is involved in fibroblast growth factor-2-induced chemotaxis of murine brain capillary endothelial cells. *J Biol Chem* 2000; 275: 10105-11.
- [282] Tan J, Hallahan DE. Growth factor-independent activation of protein kinase B contributes to the inherent resistance of vascular endothelium to radiation-induced apoptotic response. *Cancer Res* 2003; 63: 7663-7.
- [283] Gu Q, Wang D, Wang X, Peng R, Liu J, Jiang T, *et al.* Basic fibroblast growth factor inhibits radiation-induced apoptosis of HUVECs. I. The PI3K/AKT pathway and induction of phosphorylation of BAD. *Radiat Res* 2004; 161: 692-702.
- [284] Kanda S, Miyata Y, Kanetake H. Fibroblast growth factor-2-mediated capillary morphogenesis of endothelial cells requires signals via Flt-1/vascular endothelial growth factor receptor-1: possible involvement of c-Akt. *J Biol Chem* 2004; 279: 4007-16.
- [285] Tsuda S, Ohtsuru A, Yamashita S, Kanetake H, Kanda S. Role of c-Fyn in FGF-2-mediated tube-like structure formation by murine brain capillary endothelial cells. *Biochem Biophys Res Commun* 2002; 290: 1354-60.
- [286] Pourtier-Manzanedo A, Vercamer C, Van Belle E, Mattot V, Mouquet F, Vandebunder B. Expression of an Ets-1 dominant-negative mutant perturbs normal and tumor angiogenesis in a mouse ear model. *Oncogene* 2003; 22: 1795-806.
- [287] Klein S, de Fougères AR, Blaikie P, Khan L, Pepe A, Green CD, *et al.* Alpha 5 beta 1 integrin activates an NF-kappa B-dependent program of gene expression important for angiogenesis and inflammation. *Mol Cell Biol* 2002; 22: 5912-22.
- [288] Guo W, Giancotti FG. Integrin signalling during tumour progression. *Nat Rev Mol Cell Biol* 2004; 5: 816-26.
- [289] Simons M, Horowitz A. Syndecan-4-mediated signalling. *Cell Signal* 2001; 13: 855-62.
- [290] Benelli R, Lorusso G, Albin A, Noonan DM. Cytokines and chemokines as regulators of angiogenesis in health and disease. *Curr Pharm Des* 2006; 12(24): 3101-15.
- [291] Furness MS, Robinson TP, Ehlers T, Hubbard RB 4th, Arbiser JL, Goldsmith DJ, *et al.* Antiangiogenic agents: studies on fumagillin and curcumin analogs. *Curr Pharm Des* 2005; 11(3): 357-73.
- [292] Verhoef C, de Wilt JH, Verheul HM. Angiogenesis inhibitors: perspectives for medical, surgical and radiation oncology. *Curr Pharm Des* 2006; 12(21): 2623-30.