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 differenent 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 thigh 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-allantoid membrane assay quantitatively similar to that elicited by 1.0 μg 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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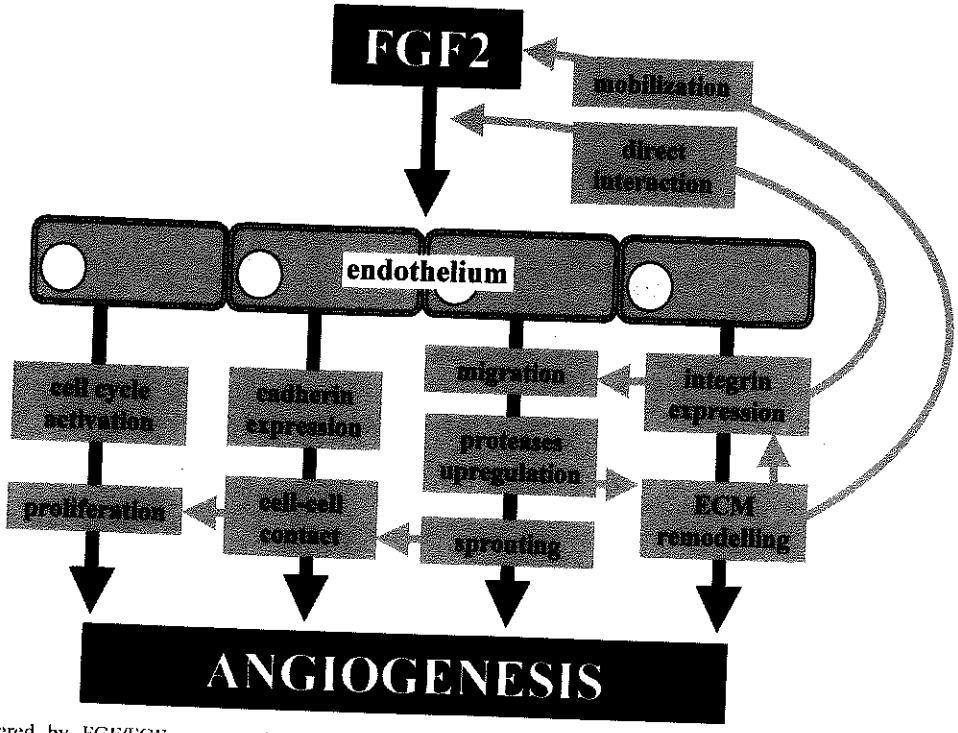

Fig. (1). Events triggered by FGF/FGF receptor interaction in ECs that contribute to the acquisition of the angiogenic phenotype *in vitro* and neovascularization *in vivo*.

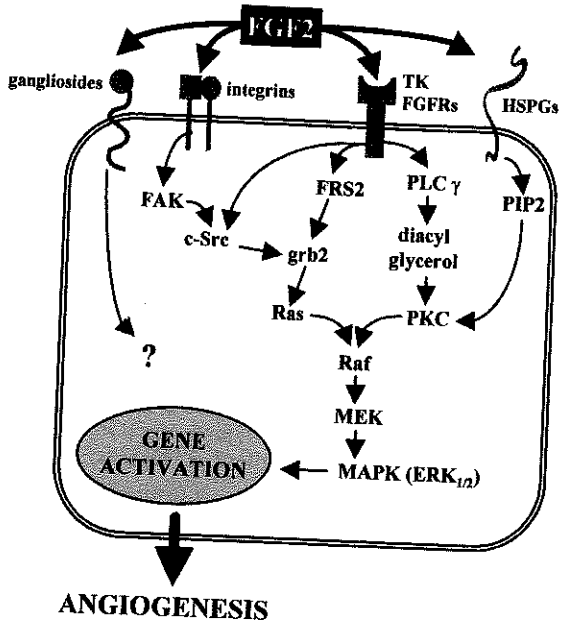

Fig. (2). Signal transduction pathways triggered by the interaction of FGF2 with EC integrins, TK-FGFRs, and HSPGs. Only second messengers converging to the Raf/MEK/MAPK pathway are shown. For more details about the second messengers activated by TK-FGFRs, integrins, and HSPGs see [35], [288], and [289], 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/HS 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 accessi-bility 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 as 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

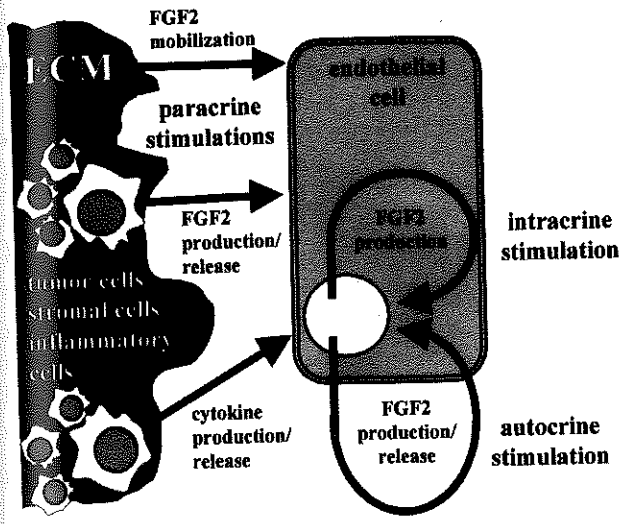

Fig. (3). Different mechanisms of action of FGFs. FGFs released by producing cells or mobilized from ECM activate ECs *via* a paracrine mode of action. Alternatively, cytokines can stimulate ECs to produce FGFs that, in turn, will act at the intracellular level (intracrine stimulation) or in an autocrine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

to nuclear translocation of NF-κB [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 it 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 GM_1 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 GM_1 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 6-0-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 fumonisin B_1, 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

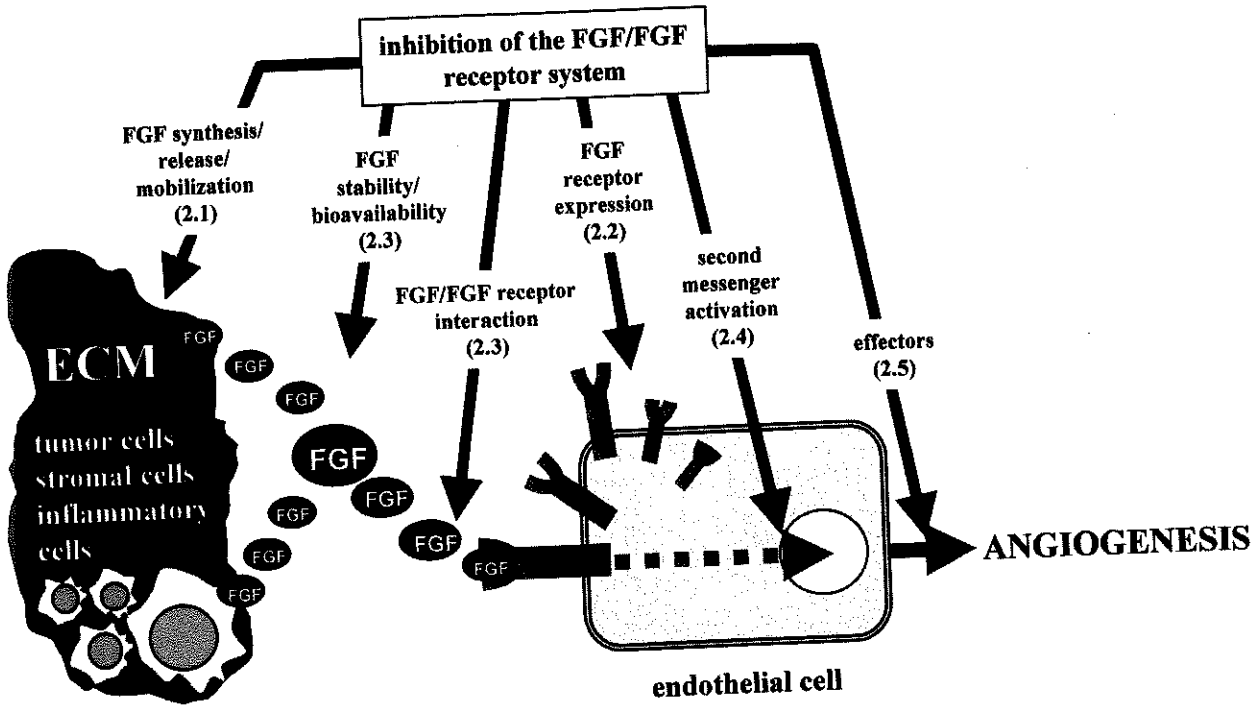

Fig. (4). Anti-FGF strategies for the development of anti-angiogenic therapies. Intracellular and extracellular FGF inhibitors can act on different targets. The numbers in brackets refer to the paragraphs in the text where the different classes of inhibitors are described in more details.

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 NH_2-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-kB 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_2M	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-β1	unknown [14]

(Table 2) Contd....

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)-galacto-oligosaccharides	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 *E. coli*)	proliferation, FGF2-dependent cell-cell interaction, morphogenesis, angiogenesis [99], cell adhesion [57]
suleparoide (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-pepti-domimetics inhibit FGF2-dependent neovascularization and tumori-genesis *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 GM_1 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 inbitors [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 goniodomin-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	angiogensis [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-α2	migration [267]
ONO-RS-082	PLC-α2	migration [267]
rapamycin	$P70^{S6K}$	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 aplidine, 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]. Psammaplin-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 mineralcorticoid 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 cisplatinum 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 α1-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],

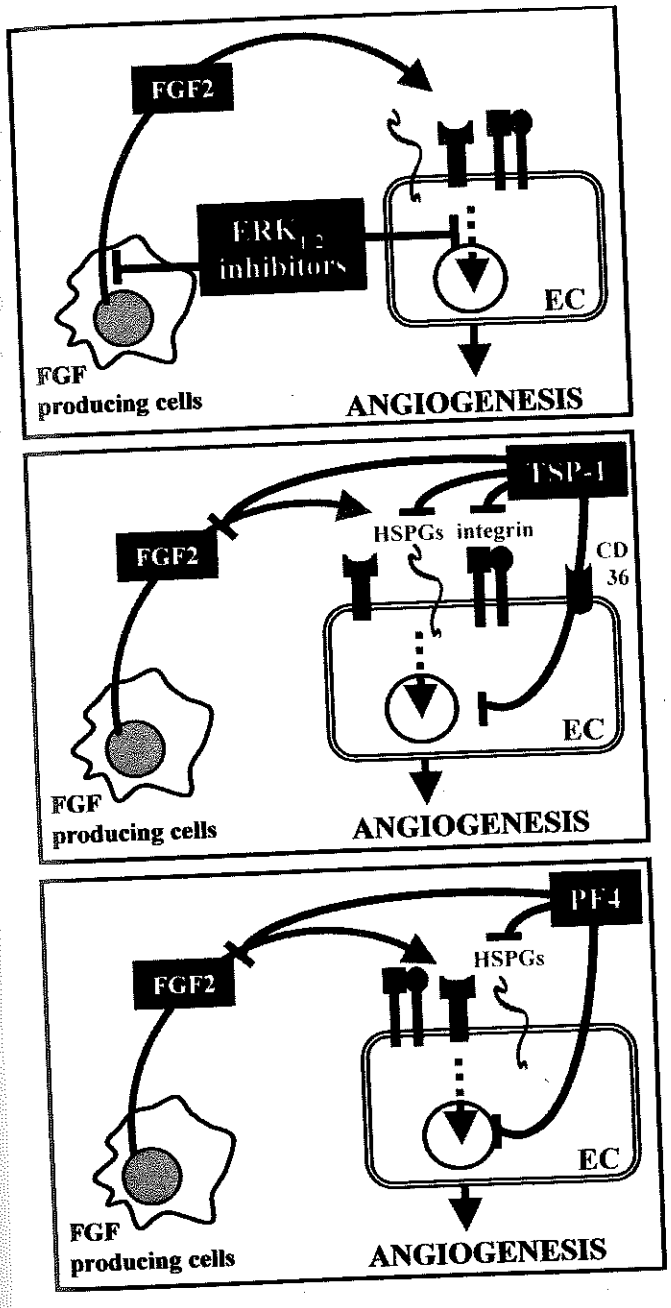

Fig. (5). Multitarget activity of selected FGF inhibitors. Possible mechanisms of action of anti-angiogenic $ERK_{1/2}$ inhibitors, TSP-1, and PF4. See text for further details.

and by the functionally related medroxyprogesterone-acetate that inhibits the release of uPA induced by FGF2 in ECs [161]. The topoisomerase-1 inhibitor topotecan possesses an indirect anti-tumor effect *in vivo* mediated by angiosuppression 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 chemotherapic 6-methylmercaptopurine-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 caspases 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 angiosuppression 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[a]

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-kB [d]	angiogenesis [287]

[a]Inhibition 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 fkB-2A overexpression were used, respectively.

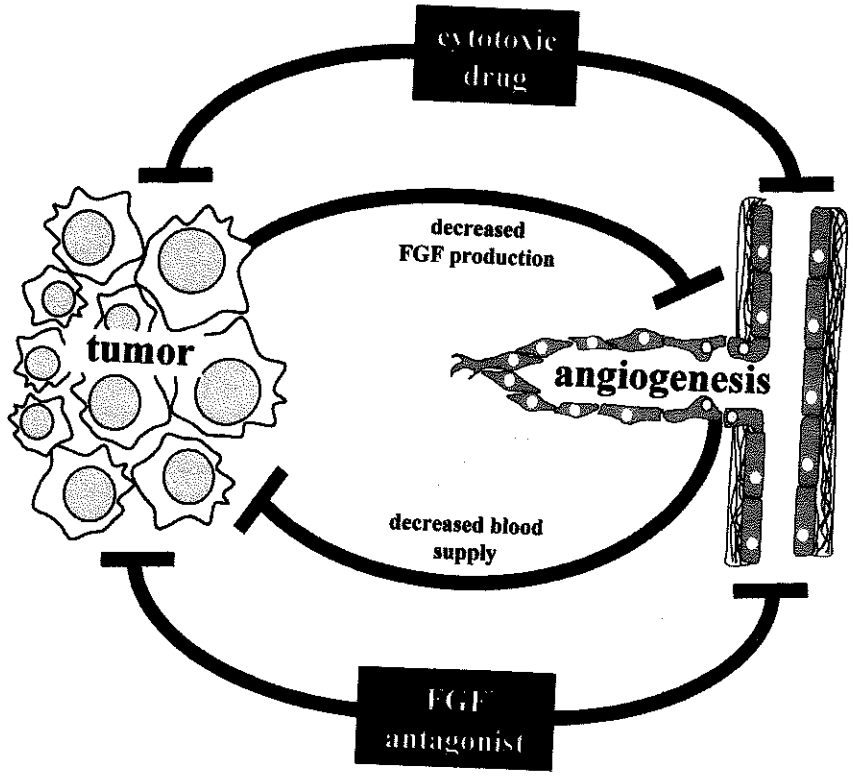

Fig. (6). Multiple effects of FGF antagonists and antineoplastic drugs on tumor growth and neovascularization. FGF antagonists can affect tumor growth indirectly by decreasing blood supply and directly by blocking FGF-dependent tumor cell proliferation. On the other hand, cytotoxic drugs can inhibit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

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