

REVIEW ARTICLE

Anti-FGF2 approaches as a strategy to compensate resistance to anti-VEGF therapy: long-pentraxin 3 as a novel antiangiogenic FGF2-antagonist

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ABSTRACT. Angiogenesis, the formation of new blood vessels from the endothelium of the existing vasculature, plays a pivotal role in tumor growth, progression and metastasis. Over the last 30 years, numerous pro- and antiangiogenic molecules, their ligands, and intracellular signaling pathways have been identified, and significant efforts have been undertaken to develop antiangiogenic strategies for cancer therapy. Agents that selectively target vascular endothelial growth factor (VEGF) and its receptors have shown promising activity in clinical trials and have been approved for use in selected cancer indications. However, patients may ultimately develop resistance to these drugs. One proposed mechanism of tumor escape from anti-VEGF therapy is the up-regulation of fibroblast growth factor-2 (FGF2). FGF2 is a pleiotropic, angiogenesis inducer belonging to the family of the heparin-binding FGF growth factors. FGF2 is expressed by numerous tumor types and exerts its proangiogenic activity by interacting with tyrosine kinase receptors, heparan-sulfate proteoglycans, and integrins expressed on the endothelial cell surface. Experimental evidence suggests that targeting FGF2, in addition to VEGF, might provide synergistic effects in the treatment of angiogenesis-related diseases, including cancer. Several FGF2 inhibitors, with different chemical structure and mechanism of action, have been identified. Recent observations have shown the ability of the soluble pattern recognition receptor long-pentraxin-3 (PTX3) to bind FGF2, thus acting as a FGF2 antagonist. PTX3 binds FGF2 with high affinity and specificity. This interaction prevents the binding of FGF2 to its cognate tyrosine kinase receptors, leading to inhibition of the angiogenic activity of the growth factor. Further, preliminary observations support the hypothesis that PTX3 may inhibit FGF2-mediated tumor angiogenesis and growth. The identification of the FGF2-binding domain in the unique N-terminal extension of PTX3 has allowed the design of PTX3-derived synthetic peptides endowed with significant antiangiogenic activity *in vitro* and *in vivo*. These findings may provide the basis for the development of novel antiangiogenic FGF2 antagonists, with potential implications for cancer therapy.

Keywords: angiogenesis, endothelium, FGF2, pentraxins, tumor, antiangiogenic therapy

ANTIANGIOGENESIS AND CANCER

Angiogenesis, the process of new blood vessel formation from pre-existing ones, plays a pivotal role in tumor growth, progression, and metastasis [1]. Angiogenesis is controlled by the balance between proangiogenic and antiangiogenic factors [2]. Strategies to target angiogenesis have been extensively studied, providing substantial data supporting the potential of angiogenic targeting for cancer therapy and prevention [3]. More than a dozen of angiogenic factors and cytokines are overexpressed in tumors [4]. Among these angiogenic factors, the vascular endothelial growth factor (VEGF) family has been a central focus in tumor angiogenesis research. This family is

comprised of five, structurally-related members [including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PlGF)], whose biological functions are mediated by activation of three, structurally-homologous tyrosine kinase receptors: VEGFR1, VEGFR2, and VEGFR3 [4].

Massive efforts have been made to develop antiangiogenic strategies for clinical cancer treatment [5], and the VEGF/VEGFR system has been extensively investigated as a target for antineoplastic therapy [6]. Despite numerous promising results in preclinical models, initial clinical trials provided no convincing evidence of any effective anticancer activity by classical antiangiogenic agents in monotherapy. This led to the development of more suc-

successful combinations of angiogenesis inhibitors with classical cytotoxic chemotherapy and radiotherapy [7-11]. Combined with chemotherapy, antiangiogenesis has proven its clinical efficacy in patients suffering from advanced colorectal cancer, leading to an improved survival time [12]. In 2004, the anti-VEGF mAb bevacizumab (Avastin, Genentech) was finally approved by the Food and Drug Administration as first-line therapy in combination with standard 5-fluorouracil-based chemotherapy in patients with advanced colorectal cancer. Since then, the use of bevacizumab has been extended to other cancers. New agents that selectively target the VEGF/VEGFR system, such as the tyrosine kinase inhibitors sunitinib and sorafenib, have shown promising activity in clinical trials, and have been approved for use in selected cancer indications [13].

ANTIANGIOGENESIS: FACING DRUG RESISTANCE

Even though VEGF plays a central role in switching on a proangiogenic phenotype in most tumors, neoplastic, stromal, and infiltrating cells may produce a plethora of different proangiogenic factors. For example, even though 50% of newly diagnosed breast cancers produced VEGF only, upregulation of other angiogenic factors [including fibroblast growth factor-2 (FGF2), transforming growth factor- β (TGF- β), PIGF, platelet derived-endothelial cell growth factor (PD-ECGF), and pleiotrophin] occurs during tumor progression, recurrence, and metastasis [14, 15]. Thus, VEGF/VEGFR antagonists may not be effective in the treatment of all cancers at all stages. Moreover, when the activity of one angiogenic inducer such as VEGF is suppressed for a long period of time, the expression of other angiogenic factors appears to emerge [16]. The mechanism of this ‘compensatory’ response is complex. For instance, antiangiogenic drugs might favor the growth of a p53 defective tumor cell population less dependent on a blood supply for their survival [17], the selection of tumor cell subclones producing different angiogenic factors, or the upregulation of angiogenesis inducers distinct from VEGF (see [6] for a review).

Thus, even though inhibition of the VEGF/VEGFR2 system markedly disrupts angiogenic switching and initial tumor growth, phenotypic resistance appears to emerge in late-stage lesions, as tumors regrow during treatment, following an initial period of growth suppression. In the clinic, VEGF blockade, while initially effective, is often circumvented with time. This resistance to VEGF/VEGFR2 blockade involves a VEGF-independent reactivation of tumor angiogenesis in the evasion phase. This is associated with hypoxia-mediated induction of other proangiogenic factors. Relevant to this point, experimental evidence indicates that drug resistance to VEGF blockade may occur following reactivation of angiogenesis triggered by the compensatory upregulation of the FGF2/FGF receptor (FGFR) system in experimental tumor models [18] and in cancer patients [19]. The upregulation of this system represents a mechanism of escape from anti-VEGF therapy in cancer treatment.

FGF2 AS A TARGET FOR ANTIANGIOGENIC THERAPY

FGF2 as an angiogenic growth factor

Twenty-three, structurally-related members of the FGF family have been identified [20]. FGFs are pleiotropic factors acting on different cell types, including endothelial cells, following interaction with heparan-sulfate proteoglycans (HSPGs) and tyrosine kinase FGFRs. FGFRs belong to subclass IV of the membrane-spanning receptors, are encoded by four distinct genes, and their structural variability is increased by alternative splicing [21]. FGFR1 [22], and less frequently FGFR2 [23], are expressed by endothelial cells, whereas the expression of FGFR3 or FGFR4 has never been reported in endothelium.

Among the FGF family members, FGF2 represents the prototypic and best characterized proangiogenic factor. FGF2 expression is augmented at sites of chronic inflammation [24-26], after tissue injury [27], and in different types of human cancer [28]. *In vitro*, FGF2 binds all FGFRs, with preferential activation of the alternative spliced IIIc form in FGFRs 1-3 [29]. FGF2/FGFR interaction causes receptor dimerization and autophosphorylation of specific tyrosine residues located in the intra-cytoplasmic tail of the receptor. This, in turn, leads to complex signal transduction pathways and activation of a ‘proangiogenic phenotype’ in endothelium (reviewed in [28]). *In vivo*, FGF2 induces neovascularization in a variety of animal models, including the chick embryo chorioallantoic membrane (CAM) assay, the rodent cornea assay, the subcutaneous Matrigel plug assay in mice, and the zebrafish yolk membrane (ZFYM) assay [28, 30]. FGF2 can exert its effects on endothelial cells *via* a paracrine mode consequent to its release by tumor, stromal, and inflammatory cells and/or by mobilization from the extracellular matrix (ECM). On the other hand, endogenous FGF2 produced by endothelial cells may also play important autocrine, intracrine, or paracrine roles in angiogenesis and in the pathogenesis of vascular lesions, including Kaposi’s sarcoma and hemangiomas (see [31] and references therein).

Angiogenesis and inflammation are closely integrated processes in a number of physiological and pathological conditions, including cancer [32-35]. Inflammation may promote FGF-dependent angiogenesis (reviewed in [36]). Indeed, inflammatory cells can express FGF2. Moreover, inflammatory mediators can activate the endothelium to synthesize and release FGF2 that, in turn, will stimulate angiogenesis by an autocrine mechanism of action. The inflammatory response may also cause cell damage, fluid and plasma protein exudation, and hypoxia, thus resulting in increased FGF2 production and release. Conversely, by interacting with endothelial cells, FGF2 may amplify the inflammatory and angiogenic response. Gene expression profiling of FGF2-stimulated murine microvascular endothelial cells has actually revealed a pro-inflammatory signature characterized by the upregulation of proinflammatory cytokine/chemokines and their receptors, endothelial cell adhesion molecules, and members of the eicosanoid pathway [37]. Accordingly, we have observed that the early recruitment of mononuclear phagocytes

precedes blood vessel formation in FGF2-driven angiogenesis in the s.c. Matrigel plug assay, and that monocytes/macrophages play a functional, non-redundant early role in FGF2-driven angiogenesis [37].

It must be pointed out that, together with a pro-inflammatory signature, FGF2 also upregulates the expression of a variety of angiogenic growth factors in endothelial cells, including FGF2 itself and VEGF [37]. This suggests that FGF2 is able to activate an autocrine loop of amplification of the angiogenic response that, together with the paracrine activity exerted by endothelium-derived cytokines/chemokines on inflammatory cells, will contribute to the modulation of the neovascularization process triggered by the growth factor.

FGF2 as a target for antiangiogenic strategies

An intimate cross-talk exists among FGF2 and the different members of the VEGF family during angiogenesis [28, 38], and FGF2 is known to elicit its angiogenic effect *via* VEGF-dependent as well as VEGF-independent pathways [39-42]. Thus, the effect of anti-FGF2 and anti-VEGF combinatory treatment might be superior to anti-VEGF treatment alone [43]. Further, as stated above, FGF2 blockade impairs tumor progression in the evasion phase of anti-VEGF therapy. Taken together these observations suggest that targeting FGF2, in addition to VEGF, might result in synergistic effects in the treatment of angiogenesis-related diseases, including cancer. Moreover, certain tumors, including high-grade giant-cell tumors and angioblastomas, produce FGF2 as their predominant angiogenic protein and do not seem to deviate from this. IFN- α has been reported to suppress the production of FGF2 by human cancer cells [44]. Daily, low-dose IFN- α therapy for one to three years is sufficient to return abnormally high levels of FGF2 in the urine of these patients to normal levels. This treatment regimen has produced long-term, complete remissions (up to 10 years) without drug resistance (see [45-47]).

It is interesting to note that in some tumor types (*e.g.* breast and hepatocellular carcinomas), intratumoral levels of FGF2 correlate with the clinical outcome, but not with intratumoral microvessel density [28]. Indeed, the pleiotropic activity of FGF2 may affect both tumor vasculature and tumor parenchyma. Thus, FGF2 might contribute to cancer progression not only by inducing neovascularisation, but also acting directly on tumor cells [48].

The various approaches based on the inhibition of FGF2 have been reviewed extensively elsewhere (see [49] and references therein). Briefly, FGF2 can be neutralized at different levels by: *i*) inhibition of FGF2 production/release; *ii*) inhibition of the expression of the various FGF2 receptors in endothelial cells (including FGFRs, HSPGs, gangliosides); *iii*) engagement by selected antagonists of the various soluble FGF2 receptors (including FGFRs, HSPGs, gangliosides, and integrins); *iv*) sequestration of FGF2 in the extracellular environment; *v*) interruption of the signal transduction pathways triggered by FGF2 in endothelial cells. Since FGF2 induces a complex "pro-angiogenic/pro-inflammatory phenotype" in endothelial cells, the blockage of these processes, mediated by distinct effectors induced/activated by FGF2, may result

in the inhibition of FGF2-dependent angiogenesis [49]. Accordingly, FGF2-mediated angiogenesis is significantly reduced in the CAM assay by the mechanistically distinct anti-inflammatory drugs hydrocortisone and ketoprofen, further implicating inflammatory cells/mediators in FGF2-dependent neovascularization [37]. Moreover, FGF2-induced neovascularization is inhibited by M3 protein [37], a murine gammaherpesvirus 68 protein that binds with high affinity to human and mouse CC, CXC and CX3C chemokines and inhibits their activity [50, 51], with potential therapeutic implications in inflammatory conditions [52].

Interestingly, several ECM and serum components and/or their degradation products affect FGF2-driven angiogenesis. In the search for effective FGF2 antagonists, numerous peptides derived from these natural FGF2-binders, FGFRs, and FGF2 itself have been demonstrated to exert an inhibitory activity on the FGF2/FGFR system (reviewed in [53]).

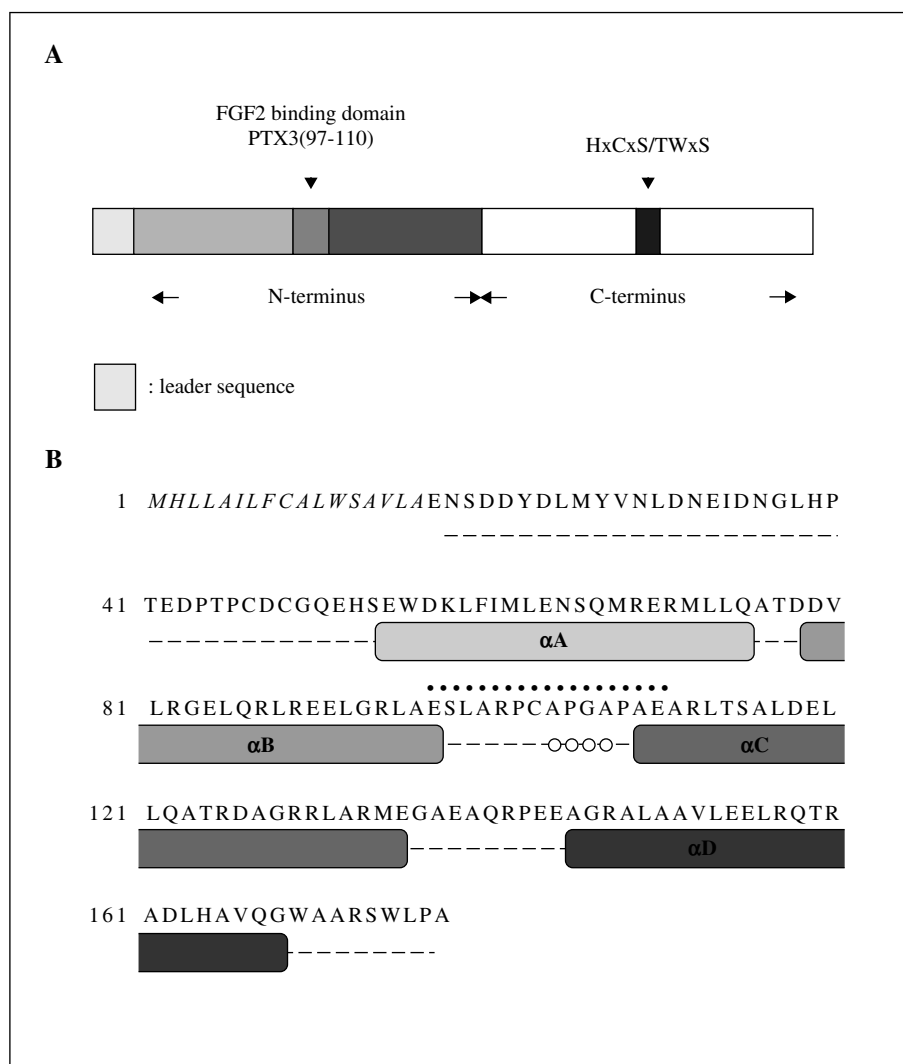
Recent observations from our laboratory have shown the ability of the soluble pattern recognition receptor long-pentraxin-3 (PTX3) [54] to bind FGF2, thus acting as an FGF2 antagonist [55]. Various stimuli (including IL-1, nitric oxide, hypoxia, and cell injury) may induce the co-expression of FGF2 and PTX3 in different pathological settings (reviewed in [56]). Thus, PTX3 produced by various cell types, including inflammatory and endothelial cells, may affect the autocrine and paracrine activity exerted by FGF2 on endothelium. This should allow a fine-tuning of the neovascularization process *via* the production of both angiogenesis inhibitors and stimulators during inflammation, wound healing, atherosclerosis, and neoplasia.

LONG-PENTRAXIN-3

PTX3 as a soluble pattern recognition receptor

Pentraxins are a superfamily of evolutionarily conserved proteins, originally characterized by their cyclic pentameric structure and markers of the acute phase of inflammation [57]. Pentraxins are divided into two subfamilies (short-pentraxins and long-pentraxins), sharing a C-terminal pentraxin domain that contains the HxCxS/TWxS pentraxin signature (where x is any amino acid) [58]. Short-pentraxins are transcriptionally regulated mainly in hepatocytes under the control of a cascade of cytokines, including TNF- α , IL-6 and IL-1 β [59-62]. They are involved in the innate resistance to microbes and scavenging of cellular debris and ECM components [57, 58, 63, 64].

Long-pentraxins differ from short-pentraxins by the presence of an unrelated N-terminal domain coupled to the C-terminal domain [65] (*figure 1A*). PTX3 (also named TSG-14) is the prototypic member of the long-pentraxin subfamily [66-68]. PTX3 is produced at extra-hepatic sites of inflammation by several cells, primarily dendritic cells, macrophages, fibroblasts, and activated endothelia [69, 70], as well as by other tissues, including heart and kidney [71-73]. PTX3 is a soluble pattern recognition receptor with unique, non-redundant functions in various

**Figure 1**

PTX3 protein structure. **A**) Schematic representation of PTX3. The FGF2-binding domain located in the N-terminal extension and the eight amino acid-long pentraxin family signature present in the C-terminus are highlighted. **B**) Amino acid sequence (single letter code) and prediction of the secondary structure of the PTX3 N-terminus (residues 1-178). The FGF2-binding domain (residues 97-110) is shown as a dotted line and the signal sequence is in italics. α A, α B, α C, α D = helix regions; O = β -turn.

physiopathological conditions [54]. The biological activity of PTX3 is related to its ability to interact with different ligands (*table 1*) via its N-terminal or C-terminal domain as a consequence of the modular structure of the protein (see [74] for a review). Consensus secondary structure prediction has identified four α -helix regions in the PTX3 N-terminus connected by short loops that span amino acid residues 55-75 (α A), 78-97 (α B), 109-135 (α C), and 144-170 (α D) [75] (*figure 1B*). Recently, the oligomeric assembly of PTX3 has been resolved, and experimental data demonstrate that human PTX3 is mainly composed of octamers covalently linked by in intra- and inter-chain disulfide bonds [76].

Experimental evidence demonstrates that PTX3 may play a role in vascular pathology, including atherosclerosis and restenosis, and has been considered as a marker of vascular damage [74]. Also, PTX3 upregulation is observed in the endothelium from patients affected by systemic sclerosis, a disease characterized by insufficient angiogenesis [77].

Table 1
PTX3 ligands

	Ligand	References
Complement fractions	C1q	[99-103]
	Factor H	[104]
	C4b-binding protein	[105]
Short-pentraxin ligands	histones	[78, 100]
	Isolated microbial components	zymosan [106] KpOmpA [107] viral hemagglutinin [108]
ECM components	inter- α -trypsin inhibitor	[109]
	TSG6	[110]
Eukaryotic cell surfaces/receptors	apoptotic cell extranuclear membrane	[111]
	A-type K(+) channel	[112]
Growth factors	FGF2	[78]
	FGF8	[78]

The biological activity of the soluble pattern recognition receptor PTX3 is related to its ability to interact with different ligands of prokaryotic and eukaryotic origin, via its N-terminal or C-terminal domain, as a consequence of the modular structure of the protein.

PTX3/FGF2 interaction: biochemical characterization

When assessed for the capacity to interact with a variety of extracellular signaling polypeptides, PTX3 was found to bind FGF2 with high specificity [78]. Moreover, PTX3/FGF2 interaction occurs with high affinity, with a K_d value ranging between 3.0×10^{-7} and 3.0×10^{-8} M depending upon the experimental model adopted [75, 78].

In agreement with the inability of short-pentraxins to bind FGF2 [78], the FGF2-binding domain of PTX3 has been located in its N-terminal region [55, 56, 75]. An integrated approach that utilized PTX3-related synthetic peptides, monoclonal antibodies, and surface plasmon resonance analysis has identified the FGF2-binding domain of PTX3 in the (97-110) amino acid sequence within the PTX3 N-terminus [75]. This FGF2-binding domain is predicted to be in an exposed loop region of PTX3 N-terminus (*figure 1*) that comprises the end of the α B helix (Glu⁹⁷), a β -turn on residues Ala¹⁰⁴-Pro¹⁰⁵-Gly¹⁰⁶-Ala¹⁰⁷, and the first two residues of the α C helix (Ala¹⁰⁹-Glu¹¹⁰) [75]. These observations point to a novel, unanticipated function for the N-terminal extension of PTX3.

FGF2 acts on target cells by interacting with high affinity FGFRs and low affinity HSPGs, leading to the formation of HSPG/FGF2/FGFR ternary complex [79, 80]. PTX3 inhibits the formation of this ternary complex [56, 80] (*figure 2*). Furthermore, surface plasmon resonance analysis has shown that PTX3 prevents the interaction of FGF2 with FGFR1 immobilized to a BIAcore sensor chip, but not that with immobilized heparin,

suggesting that PTX3 may interact with the FGFR1-binding domain of FGF2 [56, 81]. Thus, as a consequence of PTX3 interaction, the angiogenic activity of FGF2 is inhibited both *in vitro* and *in vivo*.

PTX3/FGF2 interaction: biological consequences

PTX3 interaction inhibits the mitogenic activity exerted by FGF2 on endothelial cells *in vitro*, without affecting cell proliferation triggered by various mitogens (including serum, diacylglycerol, epidermal growth factor, phorbol ester, or VEGF) [78]. Also, in keeping with its ability to interact differently with the various members of the FGF family, PTX3 does not affect the mitogenic activity of FGF4, whereas it exerts an antagonist effect on FGF8 activity [78].

In keeping with the *in vitro* observations, several experimental data demonstrate the ability of PTX3 to inhibit FGF2-driven neovascularization in different animal models. When implanted on the top of an eight day-old chick embryo CAM, PTX3 causes a significant inhibition of FGF2-induced angiogenesis, whereas it does not affect basal physiological vascularization of this embryonic *adnexum* [81]. Similarly, PTX3 exerts a significant inhibitory activity when co-injected with FGF2 in a ZFYM assay performed on the zebrafish embryo yolk membrane [82]. Moreover, PTX3 is able to inhibit the angiogenic activity exerted by FGF2 in a murine Matrigel plug assay (*figure 3A*). Similar results are obtained in this assay when PTX3 protein is replaced by PTX3-EcoPack2-293 packaging cells, producing a PTX3-harboring retrovirus (*figure 3A*). Accordingly, double

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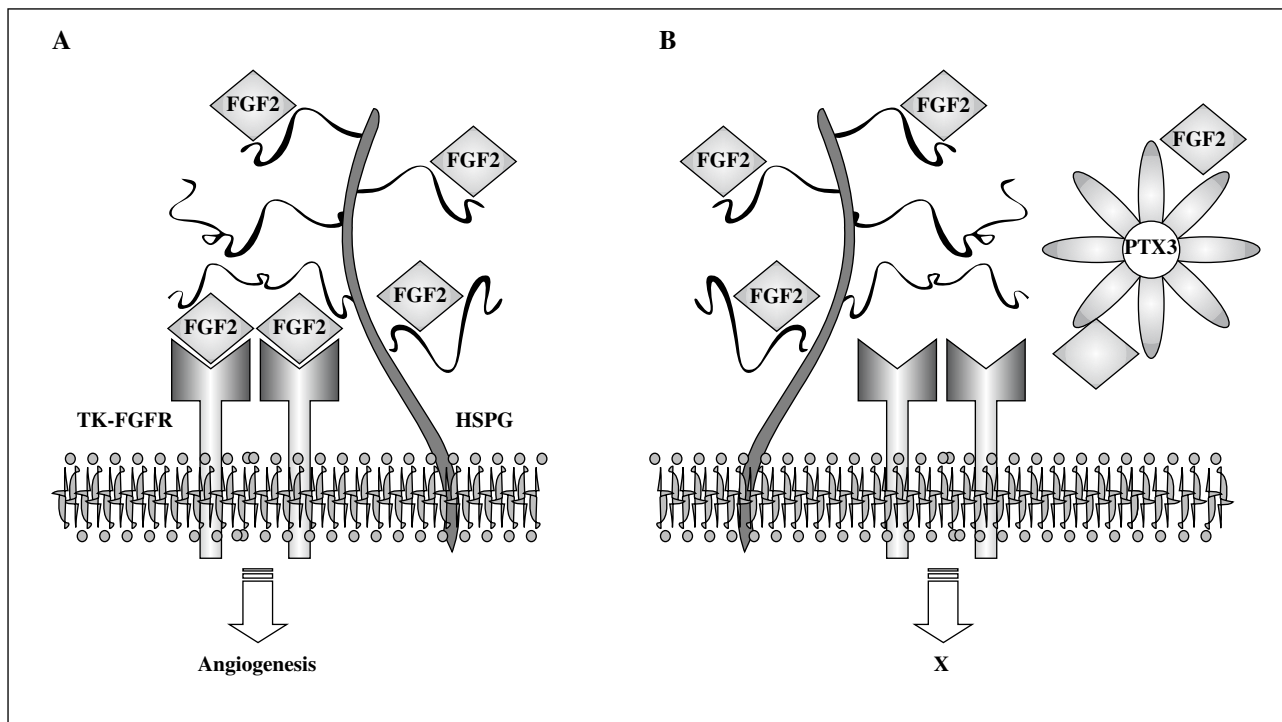


Figure 2

Effect of PTX3 on the formation of the HSPG/FGF2/FGFR ternary complex. **A)** FGF2 triggers an angiogenic response in endothelial cells by interacting with cell surface high affinity tyrosine kinase FGFRs and low affinity HSPGs, leading to the formation of HSPG/FGF2/FGFR ternary complexes. **B)** PTX3 binds FGF2 by interacting with its receptor-binding region, thus preventing HSPG/FGF2/FGFR complex formation and hampering the angiogenic activity of the growth factor.

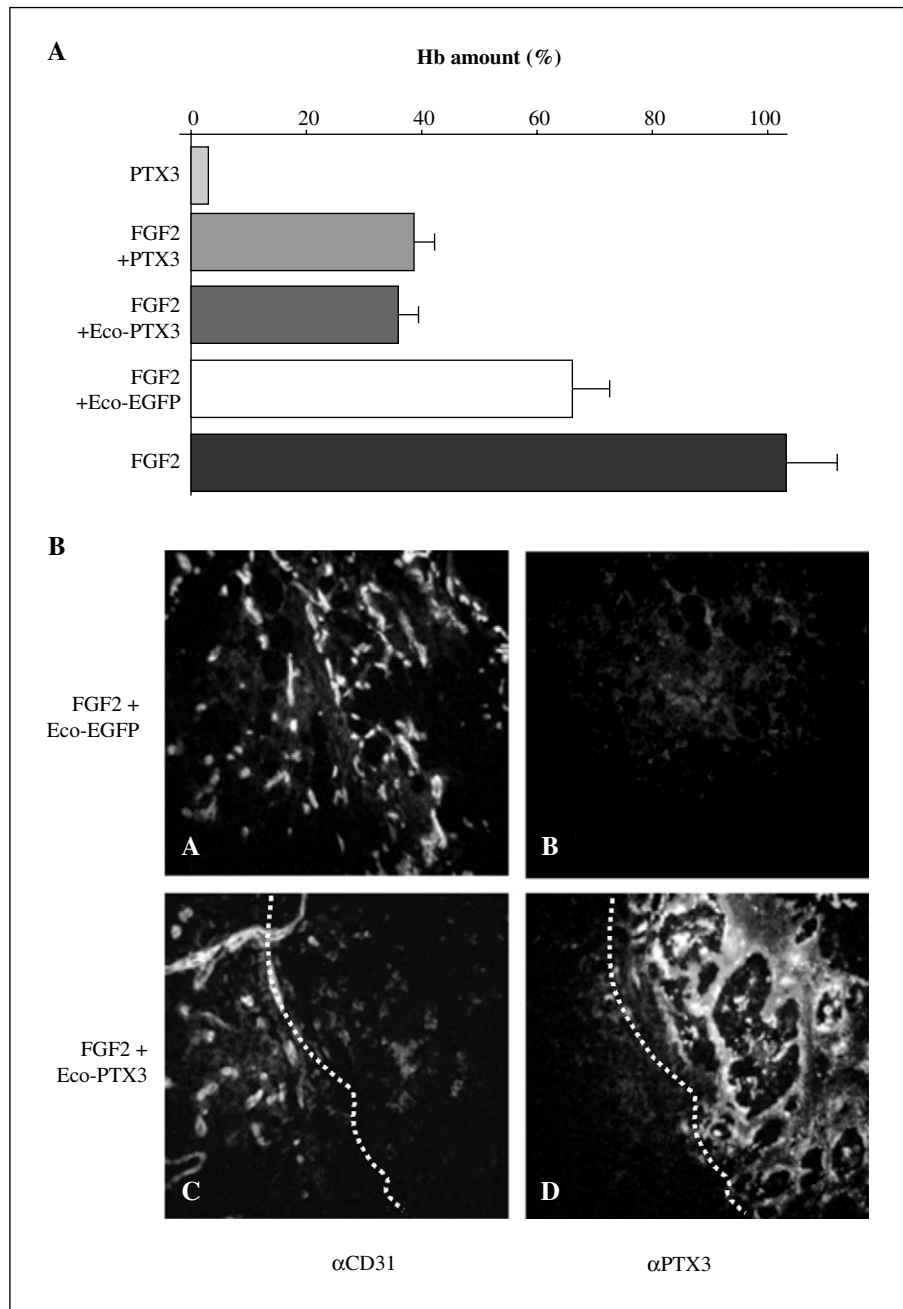


Figure 3

Purified and retroviral transduced PTX3 inhibits the angiogenic activity of FGF2 *in vivo*. Three-week-old C57BL6/N female mice (10 animals per group) were injected s.c. with 400 μ L of Matrigel containing vehicle, FGF2 (500 ng), purified PTX3 (5 μ g) or both, with FGF2 in combination with 1×10^6 PTX3-EcoPack2-293 packaging cells producing a PTX3-harboring retrovirus (Eco-PTX3), or with control 10^6 EGFP-EcoPack2-293 packaging cells (Eco-EGFP). After seven days, mice were sacrificed and Matrigel plugs were collected. **A**) Vascularization of the Matrigel plugs was assessed by measuring their hemoglobin (Hb) content using a colorimetric assay. Both purified and retroviral transduced PTX3 inhibit the angiogenic activity of FGF2 in this assay. **B**) Double immunostaining of the Matrigel plugs with anti-CD31 (**a, c**) and anti-PTX3 (**b, d**) antibodies shows that PTX3 overexpressing areas in “FGF2+Eco-PTX3” plugs (**d**) are devoid of CD31⁺ vessels (**c**). At variance, a homogeneous CD31⁺ network is observed in control “FGF2+Eco-EGFP” plugs (**a**) that do not express PTX3 (**b**).

immunostaining of these Matrigel plugs confirms the paucity of CD31⁺ neovascularization in the areas of PTX3 expression (*figure 3B*).

Preliminary data support the hypothesis that PTX3 may also inhibit FGF2-driven tumor angiogenesis and growth. Tumorigenic, FGF2-overexpressing mouse aortic endothelial FGF2-*T*-MAE cells are characterized by the capacity to generate opportunistic vascular lesions in nude mice [83]. When FGF2-*T*-MAE cells were stably

transfected with an expression vector harboring the full-length human PTX3 cDNA, these lesions showed a reduced rate of growth when compared to tumors originated by parental or control, enhanced green fluorescent protein (EGFP)-transduced cells [78]. Similarly, PTX3 protein caused a significant inhibition of the angiogenic response elicited by FGF2-*T*-MAE cells in a zebrafish/tumor xenograft model, in which injection of mammalian tumor cells into the perivitelline space induces the formation

of tumor-driven neovessels sprouting from the sub-intestinal plexus of the embryo [84]. In keeping with these observations, preliminary results obtained in our laboratories have shown that *in vivo* retroviral delivery of PTX3 inhibits the growth of FGF2-overexpressing human endometrial adenocarcinoma Tet-FGF2 cells [85] when co-injected in nude mice with PTX3-EcoPack2-293 packaging cells (M. Presta and A. Albini, unpublished observations). Also, PTX3 overexpression in human breast cancer cell lines reduces their angiogenic potential and tumorigenic activity [86].

Taken together, these results raise the possibility that PTX3 may inhibit tumor growth and angiogenesis driven by FGF2. Relevant to this point, upregulation of PTX3 expression has been observed in human soft tissue liposarcoma [87]. It must be pointed out that PTX3 interacts with and inhibits the biological activity of FGF2 at doses comparable to those measured in the blood of patients affected by inflammatory diseases [88, 89]. Moreover, due to its capacity to accumulate in the ECM, the local concentration of PTX3 should be significantly higher than that measured in the blood stream, supporting the possibility that PTX3/FGF2 interaction may indeed occur and be biologically relevant *in vivo*.

CONCLUSION

Given the key importance of VEGF and its receptors in angiogenesis, hopes were raised that blocking this pathway would eradicate tumor vasculature and heal cancer. Indeed, the monoclonal anti-VEGF antibody bevacizumab [12, 90] and the second-generation multitarget receptor tyrosine kinase inhibitors sunitinib [91, 92] and sorafenib [93, 94] have shown clinical benefits in cancer patients. However, clinical experience has also revealed that VEGF-targeted therapy often provides a limited improvement of the overall survival of cancer patients, without offering enduring cure [95] and potentially promoting tumor invasiveness and metastasis [96-98]. Tumor evasion from anti-VEGF therapy highlights the need for new antiangiogenic drugs directed against different angiogenic factors. When the VEGF pathway is blocked by an antiangiogenic drug, FGF2 upregulation is able to compensate for its absence and allows tumor vascularization and regrowth. The pattern recognition receptor PTX3 acts as a natural inhibitor of the autocrine and paracrine activity exerted by FGF2 on endothelial cells. Preliminary observations in animal models indicate that PTX3 overexpression may affect tumor growth *via* angiogenesis-dependent and -independent mechanisms of action. Further experiments are required to clarify the impact of PTX3 on tumor progression and to explore the possibility of designing PTX3-derived, anti-neoplastic and/or antiangiogenic agents. Importantly, the recent identification of the PTX3-derived acetylated ARPCA pentapeptide [55] as a short, FGF2-binding peptide able to interfere with FGF2/FGFR1 interaction and to exert a significant FGF2-antagonist activity *in vitro* and *in vivo*, may provide the basis for the design of novel, PTX3-derived peptidomimetic FGF2 antagonists.

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