REVIEW ARTICLE

# Inflammatory cells and chemokines sustain FGF2-induced angiogenesis

Marco Presta, Germán Andrés, Daria Leali, Patrizia Dell'Era, Roberto Ronca

Unit of General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, School of Medicine, University of Brescia, Italy

**Correspondence:** Prof. M. Presta, General Pathology and Immunology, Department of Biomedical Sciences and Biotechnology, Viale Europa 11, 25123 Brescia, Italy
<presta@med.unibs.it>

Accepted for publication April 7, 2009

**ABSTRACT. Angiogenesis and inflammation are closely integrated processes in a number of physiological and pathological conditions, including wound healing, psoriasis, diabetic retinopathy, rheumatoid arthritis, arteriosclerosis, and cancer. Fibroblast growth factor-2 (FGF2) belongs to the family of the heparin-binding FGF growth factors. FGF2 exerts its pro-angiogenic activity by interacting with various endothelial cell surface receptors, including tyrosine kinase receptors, heparan-sulfate proteoglycans, and integrins. Elevated levels of FGF2 have been implicated in the pathogenesis of several diseases characterized by a deregulated angiogenic/inflammatory response. FGF2 induces the expression of a wide repertoire of inflammation-related genes in endothelial cells, including pro-inflammatory cytokines/chemokines and their receptors, endothelial cell adhesion molecules, and components of the prostaglandin pathway. Consistent with this pro-inflammatory signature, *in vivo* evidence points to a non-redundant role for chemokines and infiltrating monocytes/macrophages in FGF2-driven neovascularization. This review will focus on the cross-talk between FGF2 and the inflammatory response in the modulation of blood vessel growth.**

**Keywords:** angiogenesis, endothelium, FGF2, inflammation, chemokines, macrophages

## FGF2 AS AN ANGIOGENIC GROWTH FACTOR

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 [1]. The local, uncontrolled release of angiogenic growth factors and/or alterations in the production of natural angiogenic inhibitors, with a consequent alteration of the angiogenic balance [2], are responsible for the uncontrolled endothelial cell (EC) proliferation that takes place during tumor neovascularization and in angiogenesis-dependent diseases [3].

Angiogenesis is a multi-step process that begins with the degradation of the basement membrane by activated EC that will migrate and proliferate, leading to the formation of solid EC sprouts into the stromal space. Then, vascular loops are formed and capillary tubes develop with formation of tight junctions and deposition of a new basement membrane [4].

Numerous inducers of angiogenesis have been identified, including the members of the vascular endothelial growth factor (VEGF) family, angiopoietins, transforming growth factor-α and -β (TGF-α and -β), platelet-derived growth factor (PDGF), tumor necrosis factor-α (TNF-α), interleukins, chemokines, and members of the fibroblast growth factor (FGF) family.

In 1984, J. Folkman and coworkers discovered a rodent tumor-derived factor able to bind with high affinity to heparin so that it could be purified 200,000-fold by a single passage over a heparin-affinity column. This protein, identified as basic fibroblast growth factor (FGF2), had a molecular weight of 14.8 kD, and stimulated capillary EC proliferation *in vitro* and angiogenesis *in vivo* [5, 6]. FGF2 was then purified from human normal cells/tissues [7] and from their transformed counterparts [8]. Purified FGF2 stimulated DNA synthesis, motility, and protease production (urokinase-type plasminogen activator and metalloproteases) in cultured ECs [8]. Also, FGF2 regulates the expression of cadherins, integrins and various extracellular matrix (ECM) components that contribute to the maturation of the new blood vessels regulating lateral cell-cell and substrate adhesions of ECs [9]. *In vivo*, FGF2 has been shown to induce 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 yolk membrane zebrafish embryo (ZFYM) assay [9, 10].

The single copy, human FGF2 gene encodes four co-expressed isoforms (24, 22.5, 22, and 18 kD). The latter isoform is translationally initiated at a classical AUG codon, while the high molecular weight FGF2 isoforms are colinear $NH_2$-terminal extensions initiated at novel

doi: 10.1684/ecn.2009.0155

CUG codons [11]. At variance with other FGFs, FGF2 isoforms lack a leader sequence for secretion and are released in limited amounts by an alternative secretion pathway, *via* membrane vesicle shedding, or following cell damage [12]. Even though experimental evidence points to significant differences in intracellular and extracellular fate and mechanisms of action of low and high molecular weight FGF2 isoforms [13], both 24 kD and 18 kD isoforms induce neovascularization *in vitro* and *in vivo* [14].

Twenty-three, structurally-related members of the FGF family have been identified [15]. FGFs are pleiotropic factors acting on different cell types, including EC, following interaction with heparan-sulfate proteoglycans (HSPG) and tyrosine kinase FGF receptors (TK-FGFR). Four TK-FGFR have been so far identified, whose structural variability is increased by alternative splicing: FGFR1 (*flg*), FGFR2 (*bek*), FGFR3, and FGFR4. They belong to subclass IV of the membrane-spanning receptors [16] and are encoded by distinct genes. All TK-FGFR bind FGF2, with preferential activation of the alternative spliced IIIc form in FGFR 1-3 [17]. FGFR1 [18], and less frequently FGFR2 [19] are expressed by EC, whereas the expression of FGFR3 or FGFR4 has never been reported in endothelium. FGF2/TK-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 "pro-angiogenic phenotype" in EC (reviewed in [9]). FGF2 can exert its effects on EC via a paracrine mode consequent to its release by tumor and stromal cells and/or by mobilization from the ECM. On the other hand, FGF2 may also play an autocrine role in EC, as suggested by *in vitro* and *in vivo* experimental evidence (see [20] and references therein). Accordingly, FGF2 has been implicated in the pathogenesis of lesions of EC origin, including Kaposi's sarcoma [21] and hemangiomas [22]. To assess the biological consequences of EC activation by endogenous FGF2, we developed a stable mouse aortic EC line transfected with human FGF2 cDNA [20]. FGF2 transfectants show an invasive and morphogenetic behavior *in vitro*. *In vivo*, they are angiogenic, cause the formation of opportunistic vascular tumors in nude mice, and induce hemangiomas in the chick embryo [23]. Accordingly, FGF2 transfection affects the expression of numerous genes implicated in the modulation of the cell cycle, differentiation, cell adhesion, and stress/survival [24]. Some of these genes are similarly modulated *in vitro* and *in vivo* by administration of the recombinant growth factor [24]. Interestingly, experimental evidence points to different functions of FGF2 isoforms in transfected EC [25], possibly related to differences in their subcellular localization and release. Indeed, high molecular weight FGF2 isoforms contain a nuclear localization sequence and are mostly recovered in the nucleus, whereas the 18 kDa FGF2 isoform is mostly cytosolic [26]. The constitutive overexpression of high molecular weight FGF2 isoforms leads to cell immortalization, whereas 18 kDa FGF2 overexpression induces a transformed phenotype [27]. Taken together, these data suggest that endogenous FGF2 produced by EC may play important autocrine, intracrine, or paracrine roles in angiogenesis and in the pathogenesis of vascular lesions.

In contrast with the potent angiogenic response elicited by exogenous FGF2 in different *in vitro* and *in vivo* models, the role of endogenous FGF2 in angiogenesis remains uncertain. Indeed, *fgf2*-knockout mice are morphologically normal [28] and do not show differences in neovascularization following injury [29] or hypoxia [30]. Conversely, transgenic overexpression of FGF2 does not result in spontaneous or inherent vascular defects, even though an amplified angiogenic response can be observed after wounding or subcutaneous implantation of a Matrigel plug [31]. The apparently normal vascularization in $fgf2^{-/-}$ mice as well as in double $fgf2^{-/-}/fgf1^{-/-}$ mice, may reflect the wide redundancy in the FGF family [32] and the contribution to angiogenesis of several other angiogenic growth factors, including VEGF.

## FGF2/VEGF CROSS-TALK

For many years, FGF2 occupied a central stage in the angiogenesis field. Then, the VEGF family of angiogenic growth factors came into the limelight after the discovery of their pivotal role in vasculogenesis and angiogenesis during embryonic development and under numerous physiological and pathological conditions in adults [33]. The VEGF family comprises six members (VEGF-A denoting the originally identified VEGF) that interact differently with three, cell-surface tyrosine kinase VEGFR. To date, VEGF-A/VEGFR-2 interaction appears to play a major role in blood vessel angiogenesis, whereas VEGF-C and VEGF-D are mainly involved in lymphangiogenesis by interacting with VEGFR-3 expressed on lymphatic endothelium [33]. An intimate cross-talk exists among FGF2 and the different members of the VEGF family during angiogenesis, lymphangiogenesis, and vasculogenesis.

There is some experimental evidence that points to the possibility that FGF2 induces neovascularization indirectly by activation of the VEGF/VEGFR system (see [9] and references therein). On the other hand, EC tube formation, stimulated by VEGF in murine embryonic explants, depends on endogenous FGF2 [34]. Also, FGF2 and VEGF may exert a synergistic effect in different angiogenesis models [35-37] even though this may not be the case when the two factors are applied onto the chick embryo CAM [38]. Thus, FGF2 may require the activation of the VEGF/FGFR system for promoting angiogenesis. Conversely, VEGF may require FGF2 in order to exert its angiogenic potential under defined experimental conditions.

Nevertheless, the two growth factors retain distinct biological properties, exerting different biological effects on EC during angiogenesis [39-43]. Indeed, using a genetically-modified experimental tumor model, we have observed that FGF2 and VEGF affect tumor blood vessel maturation and functionality differently, with different consequences for tumor oxygenation and viability [41]. Inhibition of FGF2 production resulted in a signifi-

cant reduction in tumor size and vascularization that was slightly less efficacious than that caused by the inhibition of VEGF production. Inhibition of both systems caused a further, albeit limited decrease in the rate of tumor growth and angiogenesis. However, none of the treatments was able to fully suppress tumor growth in this model. Other growth factors produced during tumor progression may partially rescue tumorigenicity in the absence of sufficient FGF2 and/or VEGF.

Pericytes play a pivotal role in the maturation of newly formed blood vessels. FGF2 causes the recruitment of desmin/NG2-positive pericytes in the chick embryo CAM assay [44] and in the murine subcutaneous Matrigel plug assay [45]. This may be due to a direct effect of FGF2 on mural cells and bone marrow-derived precursors [45] and/or to the ability of FGF2 to induce the expression of platelet-derived growth factor-β and transforming growth factor-β by EC [46]. This, in turn, will affect mural cell behaviour. In this respect, FGF2 and VEGF appear to show a combined effect on the maturation of blood vessels in different experimental settings [47, 48], with important implications for the angiogenic therapy of ischemic diseases [49, 50]. Interestingly, as observed in tumor vessels, VEGF-induced neovessels are characterized by the presence of endothelial fenestrations, interendothelial junctions and incomplete basement membrane, whereas angiogenesis triggered by FGF2 is not accompanied by these endothelial alterations [51]. Accordingly, inhibition of VEGF activity, but not of FGF2 activity, decreases vascular permeability in experimental tumors [41], with possible consequence for interstitial pressure, and drug delivery to the neoplastic tissue.

The lymphatic system drains extravasated fluid, proteins, and immune cells, and transports them back to the venous circulation via the collecting lymphatic vessels and the thoracic duct. In tumors, the development of the lymphatic network may play a critical role in facilitating the metastatic spread of malignant cells. Recent data demonstrate that FGF/VEGF cross-talk may also occur during lymphangiogenesis. FGF2 pellets implanted in mouse cornea, triggers both angiogenesis and lymphangiogenesis, lymphatic vessels being more sensitive than blood vessels to FGF2 [52]. However, the lymphangiogenic activity of FGF2 is mediated by endogenous VEGF-C and VEGF-D upregulation, leading to VEGFR-3 activation [53]. Interestingly, no endothelial fenestration was observed in FGF2, VEGF-A, or VEGF-C-induced lymphatic vessel [54].

The VEGF/VEGFR system is essential for the development of embryonic vasculature [55]. The situation is much less well-defined for the FGF/FGFR system. As stated above, the phenotype of *fgfr* knockout mice is not very informative, even though adenovirus-driven, dominant-negative FGFR1 expression leads to severe, vascular alterations in mouse embryos [56]. Also, FGF2 promotes the proliferation and differentiation of VEGFR-$2^+$ hemangioblast precursors from the mesoderm [57]. In embryoid bodies, embryonic stem cells can differentiate into a variety of cell lineages, including EC [58]. In this model, both VEGF and FGF2 lead to improved angioblast survival, but only VEGF supports the formation of primitive endothelial tubes [59]. Also, in embryoid bodies in which VEGF/VEGFR function is impaired, FGF2 stimulates the formation of EC clusters that fail to develop into primitive vessels. In contrast, VEGF induces the formation of a characteristic vascular plexus also in *fgfr1*$^{-/-}$ embryoid bodies [60].

## FGF2-DEPENDENT ANGIOGENESIS AND INFLAMMATION

Inflammation is the response of a vascularized tissue to sub-lethal injury, designed to destroy or inactivate invading pathogens, remove waste and debris, and permit restoration of normal function, either through resolution or repair. Angiogenesis and inflammation are closely integrated processes in a number of physiological and pathological conditions, including wound healing, psoriasis, diabetic retinopathy, rheumatoid arthritis, arteriosclerosis, and cancer [1, 61, 62].

Inflammation may promote FGF-dependent angiogenesis. Inflammatory cells, including mononuclear phagocytes [63, 64], $CD4^+$ and $CD8^+$ T lymphocytes [65, 66], and mast cells [67] can express FGF2. Moreover, osmotic shock and shear stress induce the release of FGF2 from EC [68, 69]. FGF2 production and release from EC are also triggered by interferon (IFN)-α *plus* IL-2 [70], IL-1β [71], and nitric oxide (NO) [72]. NO is produced by vascular endothelium following stimulation by cytokines, bacterial endotoxins, inflammatory mediators, neuropeptides, and shear stress [73]. Even though FGF2-induced angiogenesis can occur independently of NO synthesis [74], the pro-angiogenic effects exerted by NO and NO-inducing molecules are due, at least in part, to NO-mediated FGF2 upregulation in EC [75]. Similarly, prostaglandin $E_2$-induced angiogenesis is mediated by the activation of EC-surface FGFR1 following mobilization of FGF2 sequestered by the ECM [76]. Thus, 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. EC damage results in increased FGF2 production and release [77]; exudated fibrin(ogen) can bind FGF2 and enhance its biological activity [78, 79]; hypoxia upregulates the production of angiogenic growth factors, including VEGF [80] and FGF2 [63]. Furthermore, hypoxia increases EC responsiveness to FGF2 by promoting HSPG synthesis [81], and also upregulates FGF2 production in vascular pericytes [82]. On the other hand, the soluble pattern recognition receptor pentraxin 3 (PTX3), synthesized locally by EC in response to IL-1β and TNF-α [83], binds FGF2 and acts as a natural angiogenesis inhibitor [84, 85]. Also, the heparin-binding C-X-C chemokine platelet factor 4 (PF4), a well known inhibitor of angiogenesis ([86] and references therein), binds FGF2 [86] and inhibits its interaction with HSPG and FGFR1, internalization and mitogenic activity in EC [86]. Thus, a fine-tuning of the pro-angiogenic activity of FGF2 may occur during inflammation.

Conversely, by interacting with EC, FGF2 may amplify the inflammatory and angiogenic response by inducing

vasoactive effects and the recruitment of an inflammatory infiltrate. Indeed, FGF2 causes vasodilatation of coronary arterioles *via* an increase in NO production [87]. FGF2 can also induce vascular permeability directly [88] and indirectly, by upregulating VEGF and proteases. Transient exposure to FGF2 upregulates the expression of the cell adhesion molecules ICAM-1 and VCAM-1 in EC, increasing polymorphonuclear leukocyte adhesion and transendothelial migration [89].

FGF2-stimulated EC upregulate the synthesis of various chemoattractants, including VEGF, (that may exert a chemotactic activity on monocytes [90]), the angiogenic/monocyte chemotactic protein osteopontin (Opn) [91], monocyte chemoattractant protein-1 [42, 92], and the pro-angiogenic cyclooxygenase-2 (Cox-2) [93]. Moreover, FGF2 exerts a direct chemotactic effect on monocytes (M. Presta, unpublished observations). Finally, in agreement with a possible role of inflammatory cells in FGF2-mediated neovascularization, significant inhibition of the angiogenic response to FGF2 is observed in neutropenic mice [94].

Even though this evidence points to a possible amplification loop of the angiogenic response triggered by FGF2 and mediated by the inflammatory infiltrate, long-lasting exposure to FGF2 down-regulates cytokine-induced ICAM-1, VECAM-1, and E-selectin expression in EC. Consequently, polymorphonuclear leukocyte adhesion and transendothelial migration are reduced [89]. Similarly, monocyte/macrophages adhesion to endothelium, and the chemotactic response to various chemokines are markedly inhibited by long-term stimulation by FGF1 or FGF2, but not by VEGF [95]. Also, FGF2 suppresses transendothelial migration of $CD4^+$ T-lymphocytes [96] and tissue factor expression in EC [97]. These observations suggest that the pro- or anti-inflammatory activity of FGF2 may be contextual and may explain, at least in part, the reduced leukocyte adhesion and transendothelial migration observed in experimental tumors [98] that, nevertheless, are characterized by the presence of pro-angiogenic tumor-associated macrophages [99].

## FGF2 INDUCES A PRO-INFLAMMATORY SIGNATURE IN ENDOTHELIUM

Taken together, the observations summarized above point to the existence of an intimate cross-talk between inflammatory and angiogenic responses during FGF2-driven neovascularization. In agreement with this hypothesis, gene expression profiling of FGF2-stimulated murine microvascular EC has revealed a pro-inflammatory signature [46] characterized by the upregulation of pro-inflammatory cytokine/chemokines and their receptors, EC adhesion molecules, and members of the eicosanoid pathway *(table 1)*. Indeed, FGF2 upregulates the expression of a number of chemokines involved in the recruitment of different inflammatory cells such as monocytes/macrophages (Ccl2, Ccl7, Cx3cl1, Opn), neutrophils (Cxcl1), NK cells (Cx3cl1) and T lymphocytes (Cxcl16, Cx3cl1). Also, FGF2-induced genes include inflammatory cytokines [IL-6, leukemia inhibitory factor (Lif), Opn] and cytokine receptors [oncostatin M receptor (Osmr), tumor necrosis factor receptor superfamily member 12a (Tnfrsf12a)/Tweak-receptor, interleukin 1 receptor accessory protein (IL1rap)], cell adhesion molecules related to leukocyte recruitment and transendothelial migration [VCAM-1, junctional adhesion molecule 2 (JAM2)], as well as key inflammatory mediators such as the cyclooxygenase Ptgs2/Cox-2 and the prostaglandin $E_2$ receptor Ptger4. Interestingly, FGF2 promotes the early upregulation (one hour after stimulation) for most of the inflammation-related genes examined, indicating that the induction of a pro-inflammatory signature represents an early event in FGF2-driven EC activation [46].

In keeping with the notion that the FGF2-induced inflammatory signature is relevant to the neovascular response triggered by the growth factor, immunofluorescence analysis of subcutaneously-implanted FGF2-containing Matrigel plugs confirms the presence of numerous $CD31^+$ EC, together with a consistent $CD45^+$ leukocyte infiltrate [46]. Characterization of the leukocyte subsets reveals that the inflammatory cell infiltrate consists mainly of $CD11b^+$ monocytes and $F4/80^+$ macrophages *(figure 1)*, whereas only rare $Gr\text{-}1^+$ neutrophils and $CD8^+$ or $CD4^+$ T-lymphocytes are detectable, and no $CD19^+$ B-lymphocytes, $NK1.1^+$ natural killer, or $CD11c^+$ dendritic cells are found. Monocytes/macrophages are active players in pathological angiogenesis, including tumor neovascularization [99-102]. They can produce pro-angiogenic growth factors (*e.g.* VEGF, FGF2, IL-1β, IL-8, TNF-α) and proteases [103, 104]. Also, monocytes may contribute to new blood vessel formation by differentiating into endothelial-like cells [105, 106]. Monocytes/macrophages are frequently associated with proliferating blood vessels [107] and can open passages in existing vasculature to facilitate the development of vascular sprouts [104]. Thus, monocytes/macrophages often precede, temporally and spatially, the formation of new vascular sprouts, altering the microenvironment and promoting subsequent EC migration, proliferation, and vessel formation. Accordingly, we have observed that the early recruitment of mononuclear phagocytes (within two-three days after implantation) precedes blood vessel formation in FGF2-driven angiogenesis in the subcutaneous Matrigel plug assay [46]. Indeed, the capacity of monocytes/macrophages to cooperate with EC precursors during FGF2-driven neovascularization in a long-term Matrigel plug assay has been suggested [108]. In addition, numerous $F4/80^+$ cells were observed surrounding the microvessels within the plug, four weeks after implantation. In this study, the authors hypothesize that monocytes/macrophages may contribute to ECM degradation, thus facilitating the invasion of $Tie2^+$ precursors and blood vessel lumen formation.

Proper migration of leukocyes to chemotactic agonists in inflammatory sites is dependent upon phosphatidylinositol 3-kinase-γ (PI3Kγ) activity [109, 110]. A significant reduction in the $F4/80^+$ cell infiltrate and in $CD31^+$ neovessels is observed when FGF2-embedded Matrigel plugs are implanted in $PI3K\gamma^{-/-}$ mice. Accordingly, macrophage depletion following intraperitoneal pretreatment with clodronate liposomes (Clodrolip) [111, 112] causes a significant reduction in the angiogenic response elicited by FGF2. Thus, monocytes/macrophages play a func-

**Table 1**
Inflammation-related genes upregulated by FGF2 in murine microvascular endothelial cells

| Growth factors, cytokines and chemokines | Symbol | Fold change | Unigene ID |
|---|---|---|---|
| Chemokine (C-C motif) ligand 2 | Ccl2 | 3.5 | Mn.290320 |
| Chemokine (C-C motif) ligand 7 | Ccl7 | 3.1 | Mm.341574 |
| Chemokine (C-X3-C motif) ligand 1 | Cx3cl1 | 2.0 | Mm.103711 |
| Chemokine (C-X-C motif) ligand 1 | Cxcl1 | 2.0 | Mm.21013 |
| Chemokine (C-X-C motif) ligand 16 | Cxcl16 | 2.0 | Mm.301293 |
| Fibroblast growth factor 2 | Fgf2 | 2.3 | Mm.457975 |
| Interleukin 6 | Il6 | 3.8 | Mm.1019 |
| Leukemia inhibitory factor | Lif | 2.3 | Mm.4964 |
| Secreted phosphoprotein 1/osteopontin | Spp1/Opn | 12.2 | Mm.288474 |
| Slit homolog 2 (Drosophila) | Slit2 | 3.0 | Mm.289739 |
| Thrombospondin 1 | Thbs1 | 2.7 | Mm.4159 |
| Transforming growth factor beta 1 | Tgfb1 | 2.2 | Mm.248380 |
| **Membrane receptors and adhesion molecules** | | | |
| CD44 antigen | Cd44 | 6.1 | Mm.423621 |
| Coagulation factor III | F3/Tf | 2.9 | Mm.273188 |
| Endothelial differentiation sphingolipid G-protein-coupled receptor 1 | Edg1 | 4.1 | Mm.982 |
| Interleukin 1 receptor accessory protein | Il1rap | 2.3 | Mm.253424 |
| Junction adhesion molecule 2 | Jam2 | 2.3 | Mm.41758 |
| Oncostatin M receptor | Osmr | 2.2 | Mm.10760 |
| Plasminogen activator, urokinase receptor | Plaur | 3.2 | Mm.1359 |
| Prostaglandin E receptor 4 (subtype EP4) | Ptger4 | 4.9 | Mm.18509 |
| Tumor necrosis factor receptor superfamily, member 12a | Tnfrsf12a | 2.9 | Mm.28518 |
| Vascular cell adhesion molecule 1 | Vcam1 | 3.1 | Mm.76649 |
| **Others** | | | |
| Prostaglandin-endoperoxide synthase 2 | Ptgs2 | 18.5 | Mm.292547 |
| Serine (or cysteine) peptidase inhibitor, clade E, member 1 | Serpine1 | 3.0 | Mm.250422 |
| Tissue inhibitor of metalloproteinase 1 | Timp1 | 2.5 | Mm.8245 |

Murine microvascular EC were stimulated for 10 hours with FGF2, and their transcriptional profile was determined by microarray analysis using Affymetrix MOE4303A genechips (consisting of 22,690 probe sets, corresponding to approximately 15 000 genes) and compared to that of unstimulated cells. Selected FGF2-upregulated genes (fold-change > 2, $p < 0.05$) were classified in terms of their association with the inflammation processes according to the Gene Ontology database and bibliographic searching on PubMed (http://www.ncbi.nlm.nih.gov/) [46]. The official gene name and symbol, the fold-change increase, and the Unigene cluster number are shown.

tional, non-redundant early role in FGF2-driven angiogenesis [46]. It is worth noting that the depletion of monocytes/macrophages also reduces the neovascularization driven by VEGF [113, 114], PIGF [115], and IL-1β [116]. These data indicate that monocytes/macrophages play a pivotal role in the angiogenesis process driven by various angiogenic growth factors, including FGF2. Moreover, other inflammatory cells, such as polymorphonuclear neutrophils and mast cells, may contribute to pathological angiogenesis [103, 117, 118]. In this respect, corneal neovascularization in response to FGF2 is diminished in neutropenic mice [94], indicating that different inflammatory cells may sustain FGF2-induced angiogenesis. Further studies are required to elucidate the possible cross-talk between polymorphonuclear neutrophils and monocytes/macrophages in mediating the activity of angiogenic growth factors, including FGF2.

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 EC, including FGF2 itself and VEGF [46]. This suggests that FGF2 is able to activate an autocrine amplification loop of the angiogenic response that, together with the paracrine activity exerted by endothelium-derived cytokines/chemokines on inflammatory cells, might contribute to the modulation of the neovascularization process triggered by the growth factor. As discussed above, an intimate cross-talk exists between FGF2 and VEGF during angiogenesis. Similarlily to FGF2, VEGF is also known to upregulate the expression of inflammation-related genes in endothelium [119-121]. However, the two growth factors appear to activate distinct, even though partially overlapping, gene expression profiles in human EC [42]. In our experiments, the induction of inflammation-related genes by FGF2 represents an early event, most of the genes being upregulated one hour after treatment, whereas a limited upregulation of VEGF expression was observed only eight hours after FGF2 stimulation and onward, reaching a two-fold increase in VEGF mRNA levels at 24 hours. These data appear to rule out the possibility that FGF2 may induce a pro-inflammatory signature in endothelium indirectly *via* VEGF upregulation. Further experiments are required to fully elucidate the complex interplay between FGF2, VEGF and inflammation during angiogenesis.

## FGF2 AS A TARGET FOR ANTI-ANGIOGENIC STRATEGIES

A significant effort has been directed towards the development of anti-angiogenic agents that prevent the growth of new blood vessels, the monoclonal anti-VEGF antibody bevacizumab representing the first FDA approved

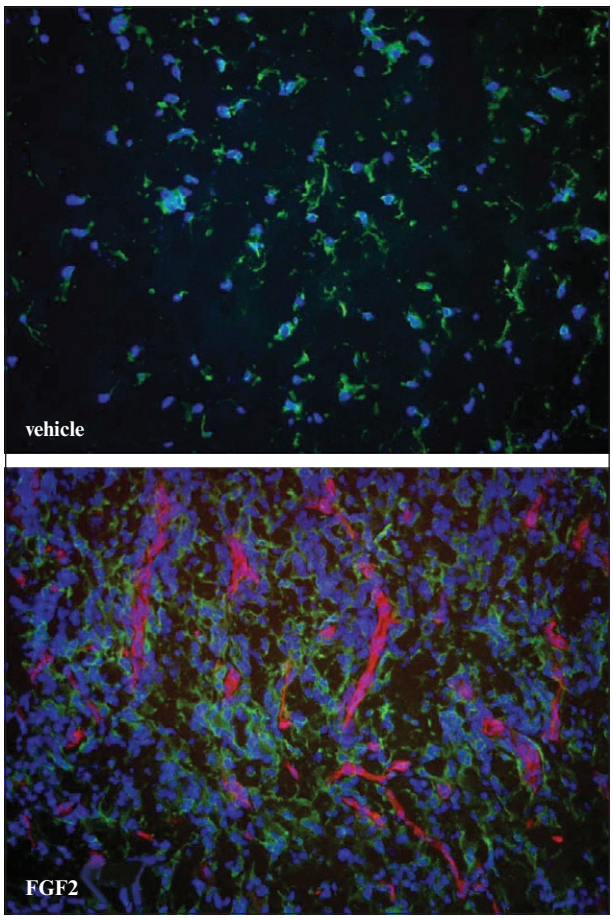


**Figure 1**
Inflammatory cells infiltrate the areas of FGF2-induced neovascularization in a Matrigel plug. Matrigel pellets containing vehicle or FGF2 were implanted subcutaneously in mice and examined after seven days for the presence of $CD31^+$ endothelial cells (in red) and $F4/80^+$ infiltrating macrophages (in green) by double-immunostaining. Nuclei are shown by DAPI counterstaining. Original magnification, x 200.

anti-angiogenic drug [122]. On the other hand, drug resistance to VEGF blockade may occur following reactivation of angiogenesis triggered by compensatory upregulation of the FGF2/TK-FGFR system [123]. Thus, given its potent angiogenic activity, FGF2 may represent the target for the development of novel anti-angiogenic strategies. The various approaches based on the inhibition of FGF2 have been reviewed extensively elsewhere (see [124] 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 EC (including TK-FGFR, HSPG, gangliosides); *iii)* engagement by selected antagonists of the various FGF2 receptors (including TK-FGFR, HSPG, gangliosides, and integrins); *iv)* sequestration of FGF2 in the extracellular environment; *v)* interruption of the signal transduction pathways triggered by FGF2 in EC.

Also, as stated above, FGF2 induces a complex "pro-angiogenic/pro-inflammatory phenotype" in EC. These processes are mediated by distinct effectors induced/activated by FGF2, and their blockage may result in the inhibition of FGF2-dependent angiogenesis [124]. Accordingly, in keeping with the pro-inflammatory signature triggered by FGF2 in EC, we have observed that FGF2-mediated angiogenesis is significantly reduced in the CAM assay by mechanistically distinct steroidal (hydrocortisone) and non-steroidal (ketoprofen) anti-inflammatory drugs, further implicating inflammatory cells/mediators in FGF2-dependent neovascularization [46]. Interestingly, FGF2-induced neovascularization is also inhibited by M3 protein [46], a murine gamma-herpesvirus 68 protein that binds with high affinity to human and mouse CC, CXC and CX3C chemokines and inhibits their activity [125, 126], with potential therapeutic implications in inflammatory conditions [127]. Our findings suggest that M3 protein may represent the basis for the design of novel angiogenesis inhibitors for therapeutic interventions in angiogenesis-dependent pathological conditions, including tumor growth and metastasis.

The bulk of experimental evidence summarized above clearly indicates that the FGF2/TK-FGFR system may represent a target for novel anti-angiogenic strategies in tumors. At present, cancer clinical trials are in progress to assess the safety and efficacy of various compounds with a potential capacity to affect the FGF2/TK-FGFR system at different levels [128, 129]. It must be pointed out however, that the ability to interact with this system may not represent the main rationale that has lead to their testing. This is the case for various heparin derivatives that have been tested in cancer patients because of their anti-thrombotic effect rather than for their potential FGF2-antagonist activity. Similarly, the humanized monoclonal anti-$\alpha_v\beta_3$ antibody vitaxin [130, 131] has been investigated for its ability to affect the cell-adhesive function of this integrin receptor rather than its potential ability to act as a signaling TK-FGFR [132]. Also, numerous cytotoxic drugs can affect the FGF2 activity and angiogenesis [124]. Moreover, the impairment of receptors characterized by a broad spectrum of ligands, such as HSPG or integrins, may result in the simultaneous inhibition of various angiogenic growth factors [133]. Novel strategies aimed at inhibiting multiple targets, including FGF2, may represent an effective approach for the treatment of angiogenesis-dependent diseases, including cancer.

## CONCLUSION

The above observations indicate that FGF2-driven angiogenesis is, at least in part, chemokine-dependent. It is tempting to speculate that chemotactic factors produced by FGF2-stimulated endothelium may recruit mononuclear phagocytes that, in turn, will amplify the angiogenic response by releasing monocyte-derived pro-angiogenic cytokines *(figure 2)*. For instance, Opn, a cytokine endowed with a potent chemoattractant activity for monocytes, is strongly induced by FGF2 in EC [91]. In turn, Opn induces neovascularization [91] by recruiting monocytes/macrophages and promoting the release of the pro-angiogenic cytokine IL-1β [134]. Also, FGF2-induced chemoattractants may play a direct role in neovascularization. Indeed, various chemokines have been shown to function as angiogenic factors by direct interaction with specific chemokine receptors expressed on EC,

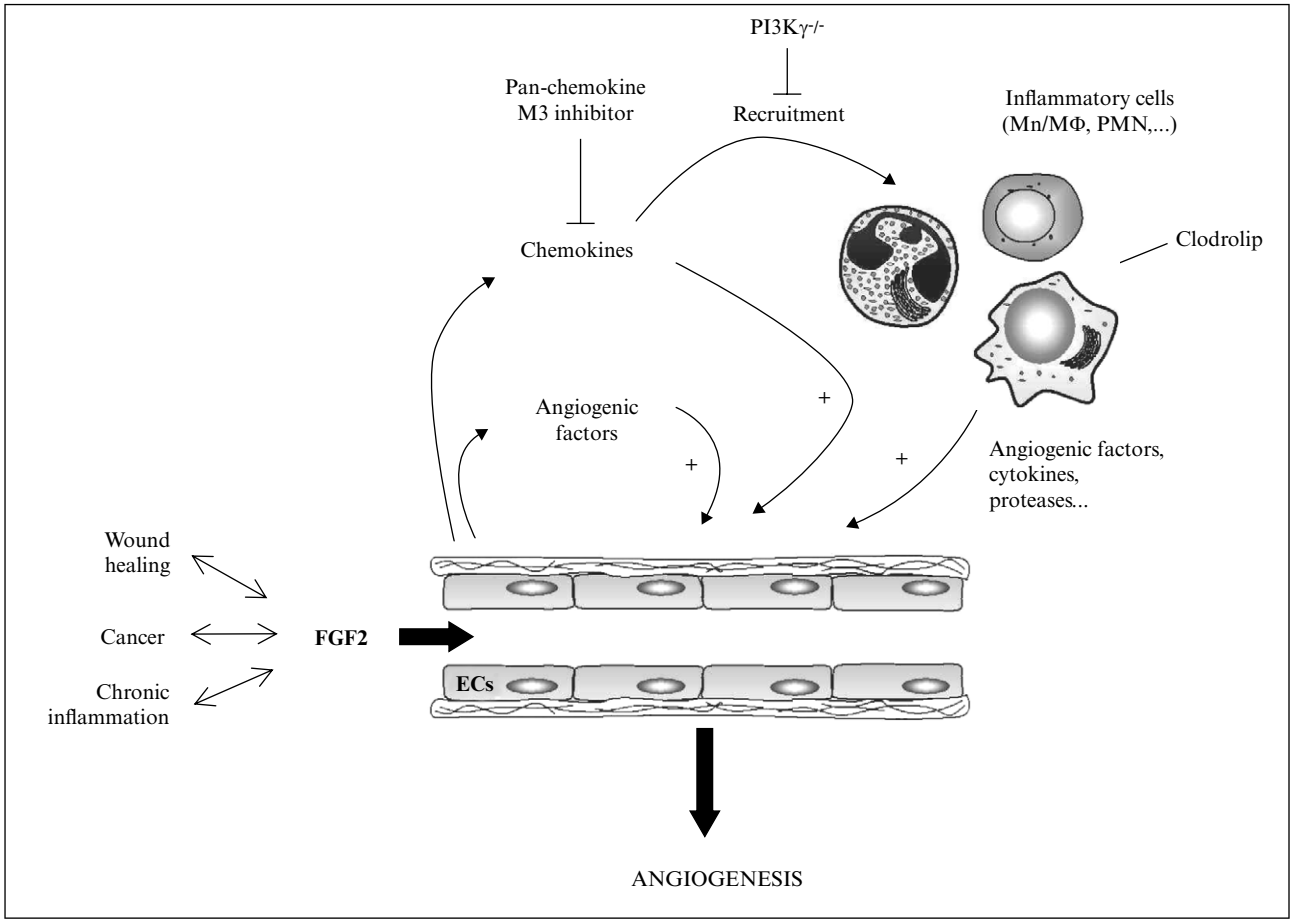


**Figure 2**

Inflammatory cells and chemokines sustain FGF2-induced angiogenesis. Under different pathological conditions, FGF2 interacts with endothelium, triggering a direct angiogenic response. At the same time, chemotactic factors produced by FGF2-stimulated endothelial cells may recruit mononuclear phagocytes that, in turn, will amplify the angiogenic response by releasing pro-angiogenic cytokines and proteases. Also, FGF2 upregulates the production of angiogenic growth factors by endothelial cells (including FGF2 itself), thus activating an autocrine stimulation loop. Thus, inhibition of chemokine activity (by M3 protein), inflammatory cell recruitment (by PI3Kγ knockdown), or macrophage depletion (by Clodrolip treatment) causes a significant inhibition of the angiogenic response elicited by FGF2.

thus promoting proliferation, migration, and capillary tube formation [135, 136]. Among them, the FGF2-induced chemokines Ccl2, Cxcl1, Cxcl16 and Cxc3l1 could act as enhancers of the neovascularization process elicited by the growth factor.

FGF2 expression is increased at sites of chronic inflammation [137-139], after tissue injury [140], and in different types of human cancer [9]. Cell damage, tissue injury and repair, neoplastic transformation, and inflammatory mediators can activate the endothelium, stromal, and parenchymal cells to synthesize and release FGF2 [70-72]. Released FGF2 may contribute to host defense responses by acting as a danger signal molecule, thus activating pro-angiogenic and pro-inflammatory signatures in endothelium that, by acting in concert, will lead to neovessel formation and monocyte/macrophage engagement *(figure 3)*. Indeed, several biological features shown by FGF2 *in vitro* and *in vivo* strongly resemble those shown by the chromosomal high mobility group box-1 (HMGB1) protein (summarized in *figure 3*), a prototypic, damage-associated molecular pattern (DAMP) cytokine [141]. Interestingly, recent observations from our laboratory have shown the capacity of HMGB1 to trigger angiogenesis by interacting with the receptor for advanced glycation end products (RAGE) expressed by EC [142].

In conclusion, chemokines and inflammatory cells are important early mediators of FGF2-driven angiogenesis and play a relevant role in the neovascularization process elicited by the growth factor. Conversely, FGF2 may exert important functions at sites of inflammation and/or tissue injury, not only by inducing neovascularization but also by contributing to the activation of innate immune responses.

***Acknowledgments.*** *We wish to thank Dr. D. Coltrini (University of Brescia) for the images shown in Figure 1. Limitation of space preclude extensive citation of the literature; we apologize to those whose work is not mentioned herein. This work was supported by grants from the Istituto Superiore di Sanità (Oncotechnological Program), Ministero dell'Istruzione, Università e Ricerca (Centro di Eccellenza per l'Innovazione Diagnostica e Terapeutica, Cofin projects), Associazione Italiana per la Ricerca sul Cancro, Fondazione Berlucchi, and Fondazione Cariplo (Grant 2008-2264 and NOBEL Project) to MP.*

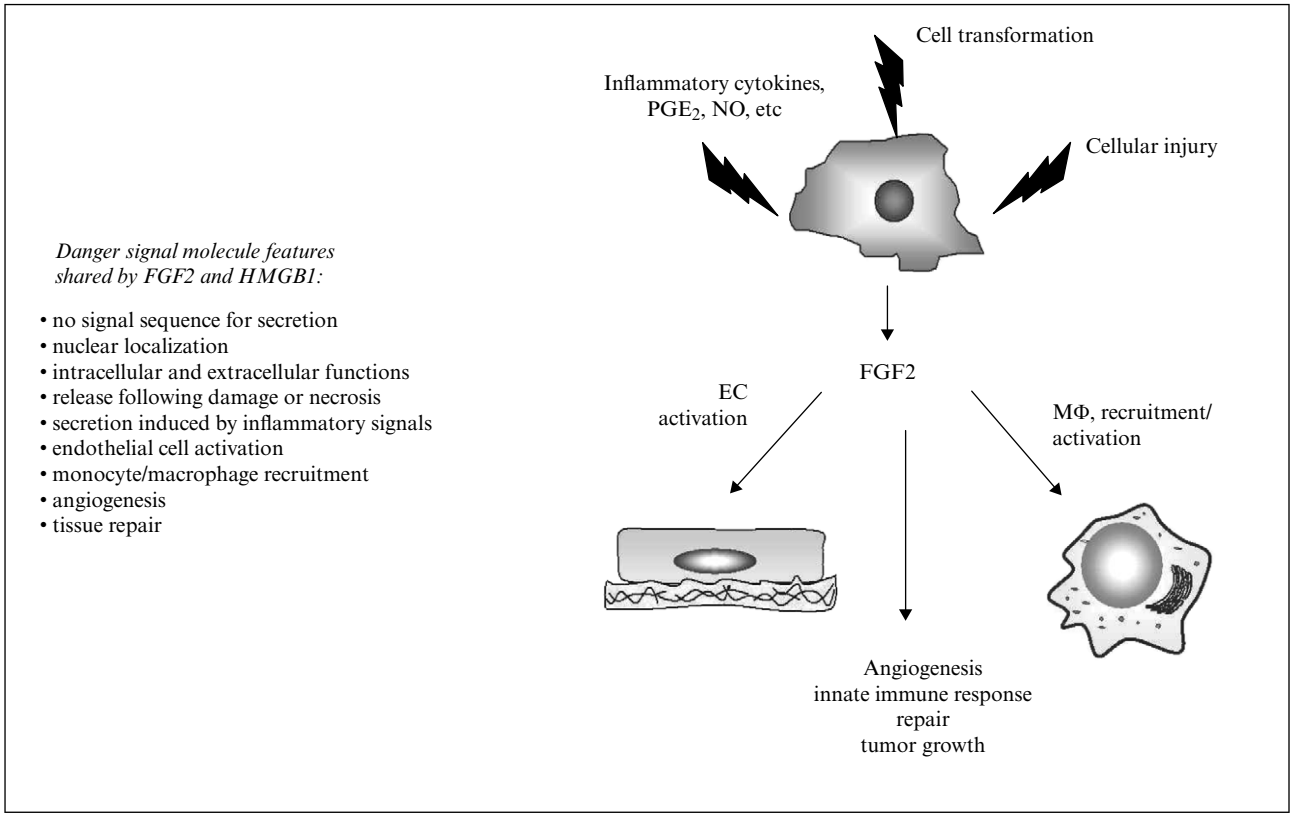


**Figure 3**
FGF2 as a danger signal molecule in pathological contexts. Cell damage, tissue injury and repair, neoplastic transformation, and inflammatory mediators can trigger the synthesize/release of FGF2. Released FGF2 may contribute to host defense responses by acting as a danger signal molecule, thus activating pro-angiogenic and pro-inflammatory signatures in endothelium that, by acting in concert, will lead to neovessel formation and monocyte/macrophage engagement. Accordingly, FGF2 shares various biological features with the prototypic damage-associated molecular pattern cytokine HMGB1.

## REFERENCES


1. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature* 2000; 407: 249.
2. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; 86: 353.
3. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; 1: 27.
4. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *NatMed* 2000; 6: 389.
5. Shing Y, Folkman J, Sullivan R, *et al.* Heparin affinity: purification of a tumor-derived capillary endothelial cell growth factor. *Science* 1984; 223: 1296.
6. Shing Y, Folkman J, Haudenschild C, *et al.* Angiogenesis is stimulated by a tumor-derived endothelial cell growth factor. *JCell Biochem* 1985; 29: 275.
7. Bohlen P, Baird A, Esch F, *et al.* Isolation and partial molecular characterization of pituitary fibroblast growth factor. *Proc Natl Acad Sci USA* 1984; 81: 5364.
8. Presta M, Moscatelli D, Joseph-Silverstein J, *et al.* Purification from a human hepatoma cell line of a basic fibroblast growth factor-like molecule that stimulates capillary endothelial cell plasminogen activator production, DNA synthesis, and migration. *Mol Cell Biol* 1986; 6: 4060.
9. Presta M, Dell'Era P, Mitola S, *et al.* Fibroblast growth factor/ fibroblast growth factor receptor system in angiogenesis. *Cytokine Growth Factor Rev* 2005; 16: 159.
10. Nicoli S, De Sena G, Presta M. Fibroblast Growth Factor 2-induced angiogenesis in zebrafish: the zebrafish yolk membrane (ZFYM) angiogenesis assay. *J Cell Mol Med* 2008 [Epub ahead of print].
11. Florkiewicz RZ, Sommer A. Human basic fibroblast growth factor gene encodes four polypeptides: three initiate translation from non-AUG codons. *Proc Natl Acad Sci USA* 1989; 86: 3978.
12. Taverna S, Ghersi G, Ginestra A, *et al.* Shedding of membrane vesicles mediates fibroblast growth factor-2 release from cells. *J Biol Chem* 2003; 278: 51911.
13. Bikfalvi A, Savona C, Perollet C, *et al.* New insights in the biology of fibroblast growth factor-2. *Angiogenesis* 1998; 1: 155.
14. Gualandris A, Urbinati C, Rusnati M, *et al.* Interaction of high-molecular-weight basic fibroblast growth factor with endothelium: biological activity and intracellular fate of human recombinant M(r) 24,000 bFGF. *J Cell Physiol* 1994; 161: 149.
15. Itoh N, Ornitz DM. Evolution of the Fgf and Fgfr gene families. *Trends Genet* 2004; 20: 563.
16. Fantl WJ, Escobedo JA, Martin GA, *et al.* Distinct phosphotyrosines on a growth factor receptor bind to specific molecules that mediate different signaling pathways. *Cell* 1992; 69: 413.
17. Ornitz DM, Xu J, Colvin JS, *et al.* Receptor specificity of the fibroblast growth factor family. *J Biol Chem* 1996; 271: 15292.
18. Javerzat S, Auguste P, Bikfalvi A. The role of fibroblast growth factors in vascular development. *Trends Mol Med* 2002; 8: 483.

19. Dell'Era P, Belleri M, Stabile H, *et al.* Paracrine and autocrine effects of fibroblast growth factor-4 in endothelial cells. *Oncogene* 2001; 20: 2655.

20. Gualandris A, Rusnati M, Belleri M, *et al.* Basic fibroblast growth factor overexpression in endothelial cells: an autocrine mechanism for angiogenesis and angioproliferative diseases. *Cell Growth Differ* 1996; 7: 147.

21. Ensoli B, Gendelman R, Markham P, *et al.* Synergy between basic fibroblast growth factor and HIV-1 Tat protein in induction of Kaposi's sarcoma. *Nature* 1994; 371: 674.

22. Takahashi K, Mulliken JB, Kozakewich HP, *et al.* Cellular markers that distinguish the phases of hemangioma during infancy and childhood. *J Clin Invest* 1994; 93: 2357.

23. Ribatti D, Gualandris A, Belleri M, *et al.* Alterations of blood vessel development by endothelial cells overexpressing fibroblast growth factor-2. *J Pathol* 1999; 189: 590.

24. Dell'Era P, Coco L, Ronca R, *et al.* Gene expression profile in fibroblast growth factor 2-transformed endothelial cells. *Oncogene* 2002; 21: 2433.

25. Bikfalvi A, Klein S, Pintucci G, *et al.* 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.

26. Florkiewicz RZ, Baird A, Gonzalez AM. Multiple forms of bFGF: differential nuclear and cell surface localization. *Growth Factors* 1991; 4: 265.

27. Quarto N, Talarico D, Florkiewicz R, *et al.* Selective expression of high molecular weight basic fibroblast growth factor confers a unique phenotype to NIH 3T3 cells. *Cell Regul* 1991; 2: 699.

28. Zhou M, Sutliff RL, Paul RJ, *et al.* Fibroblast growth factor 2 control of vascular tone. *Nat Med* 1998; 4: 201.

29. Tobe T, Ortega S, Luna JD, *et al.* Targeted disruption of the FGF2 gene does not prevent choroidal neovascularization in a murine model. *Am J Pathol* 1998; 153: 1641.

30. Ozaki H, Okamoto N, Ortega S, *et al.* Basic fibroblast growth factor is neither necessary nor sufficient for the development of retinal neovascularization. *Am J Pathol* 1998; 153: 757.

31. Fulgham DL, Widhalm SR, Martin S, *et al.* FGF-2 dependent angiogenesis is a latent phenotype in basic fibroblast growth factor transgenic mice. *Endothelium* 1999; 6: 185.

32. Miller DL, Ortega S, Bashayan O, *et al.* Compensation by fibroblast growth factor 1 (FGF1) does not account for the mild phenotypic defects observed in FGF2 null mice. *Mol Cell Biol* 2000; 20: 2260.

33. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003; 9: 669.

34. Tomanek RJ, Sandra A, Zheng W, *et al.* Vascular endothelial growth factor and basic fibroblast growth factor differentially modulate early postnatal coronary angiogenesis. *Circ Res* 2001; 88: 1135.

35. Xue L, Greisler HP. Angiogenic effect of fibroblast growth factor-1 and vascular endothelial growth factor and their synergism in a novel *in vitro* quantitative fibrin-based 3-dimensional angiogenesis system. *Surgery* 2002; 132: 259.

36. Castellon R, Hamdi HK, Sacerio I, *et al.* Effects of angiogenic growth factor combinations on retinal endothelial cells. *Exp Eye Res* 2002; 74: 523.

37. Pepper MS, Mandriota SJ. Regulation of vascular endothelial growth factor receptor-2 (Flk-1) expression in vascular endothelial cells. *Exp Cell Res* 1998; 241: 414.

38. Nico B, de Falco G, Vacca A, *et al.* In vivo absence of synergism between fibroblast growth factor-2 and vascular endothelial growth factor. *J Hematother Stem Cell Res* 2001; 10: 905.

39. Liekens S, Neyts J, De Clercq E, *et al.* Inhibition of fibroblast growth factor-2-induced vascular tumor formation by the acyclic nucleoside phosphonate cidofovir. *Cancer Res* 2001; 61: 5057.

40. Parsons-Wingerter P, Elliott KE, Clark JI, *et al.* Fibroblast growth factor-2 selectively stimulates angiogenesis of small vessels in arterial tree. *Arterioscler Thromb Vasc Biol* 2000; 20: 1250.

41. Giavazzi R, Sennino B, Coltrini D, *et al.* Distinct role of fibroblast growth factor-2 and vascular endothelial growth factor on tumor growth and angiogenesis. *Am J Pathol* 2003; 162: 1913.

42. Jih YJ, Lien WH, Tsai WC, *et al.* Distinct regulation of genes by bFGF and VEGF-A in endothelial cells. *Angiogenesis* 2001; 4: 313.

43. Ho M, Yang E, Matcuk G, *et al.* Identification of endothelial cell genes by combined database mining and microarray analysis. *Physiol Genomics* 2003; 13: 249.

44. Nico B, Ennas MG, Crivellato E, *et al.* Desmin-positive pericytes in the chick embryo chorioallantoic membrane in response to fibroblast growth factor-2. *Microvasc Res* 2004; 68: 13.

45. Tigges U, Hyer EG, Scharf J, *et al.* FGF2-dependent neovascularization of subcutaneous Matrigel plugs is initiated by bone marrow-derived pericytes and macrophages. *Development* 2008; 135: 523.

46. Andres G, Leali D, Mitola S, *et al.* A Pro-inflammatory Signature Mediates FGF2-induced Angiogenesis. *J Cell Mol Med* 2008 Epub ahead of print.

47. Rophael JA, Craft RO, Palmer JA, *et al.* Angiogenic growth factor synergism in a murine tissue engineering model of angiogenesis and adipogenesis. *Am J Pathol* 2007; 171: 2048.

48. Nillesen ST, Geutjes PJ, Wismans R, *et al.* Increased angiogenesis and blood vessel maturation in acellular collagen-heparin scaffolds containing both FGF2 and VEGF. *Biomaterials* 2007; 28: 1123.

49. Asahara T, Bauters C, Zheng LP, *et al.* Synergistic effect of vascular endothelial growth factor and basic fibroblast growth factor on angiogenesis in vivo. *Circulation* 1995; 92: II365.

50. Kano MR, Morishita Y, Iwata C, *et al.* VEGF-A and FGF-2 synergistically promote neoangiogenesis through enhancement of endogenous PDGF-B-PDGFRbeta signaling. *J Cell Sci* 2005; 118: 3759.

51. Ribatti D, Nico B, Morbidelli L, *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.

52. Chang L, Kaipainen A, Folkman J. Lymphangiogenesis new mechanisms. *Ann N Y Acad Sci* 2002; 979: 111.

53. Chang LK, Garcia-Cardena G, Farnebo F, *et al.* Dose-dependent response of FGF-2 for lymphangiogenesis. *Proc Natl Acad Sci USA* 2004; 101: 11658.

54. Cao R, Eriksson A, Kubo H, *et al.* Comparative evaluation of FGF-2-, VEGF-A-, and VEGF-C-induced angiogenesis, lymphangiogenesis, vascular fenestrations, and permeability. *Circ Res* 2004; 94: 664.

55. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2000; 6: 389.

56. 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.

57. Poole TJ, Finkelstein EB, Cox CM. The role of FGF and VEGF in angioblast induction and migration during vascular development. *Dev Dyn* 2001; 220: 1.

58. Desbaillets I, Ziegler U, Groscurth P, *et al.* Embryoid bodies: an in vitro model of mouse embryogenesis. *Exp Physiol* 2000; 85: 645.

59. Kazemi S, Wenzel D, Kolossov E, *et al.* Differential role of bFGF and VEGF for vasculogenesis. *Cell Physiol Biochem* 2002; 12: 55.

60. Magnusson P, Rolny C, Jakobsson L, *et al.* Deregulation of Flk-1/vascular endothelial growth factor receptor-2 in fibroblast growth factor receptor-1-deficient vascular stem cell development. *J Cell Sci* 2004; 117: 1513.

61. Jackson JR, Seed MP, Kircher CH, *et al.* The codependence of angiogenesis and chronic inflammation. *Faseb J* 1997; 11: 457.

62. Schmid MC, Varner JA. Myeloid cell trafficking and tumor angiogenesis. *Cancer Lett* 2007; 250: 1.

63. Kuwabara K, Ogawa S, Matsumoto M, *et al.* Hypoxia-mediated induction of acidic/basic fibroblast growth factor and platelet-derived growth factor in mononuclear phagocytes stimulates growth of hypoxic endothelial cells. *Proc Natl Acad Sci USA* 1995; 92: 4606.

64. Baird A, Mormede P, Bohlen P. Immunoreactive fibroblast growth factor in cells of peritoneal exudate suggests its identity with macrophage-derived growth factor. *Biochem Biophys Res Commun* 1985; 126: 358.

65. Blotnick S, Peoples GE, Freeman MR, *et al.* T lymphocytes synthesize and export heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor, mitogens for vascular cells and fibroblasts: differential production and release by CD4+ and CD8+ T cells. *Proc Natl Acad Sci USA* 1994; 91: 2890.

66. Peoples GE, Blotnick S, Takahashi K, *et al.* T lymphocytes that infiltrate tumors and atherosclerotic plaques produce heparin-binding epidermal growth factor-like growth factor and basic fibroblast growth factor: a potential pathologic role. *Proc Natl Acad Sci USA* 1995; 92: 6547.

67. Ribatti D, Crivellato E, Candussio L, *et al.* Angiogenic activity of rat mast cells in the chick embryo chorioallantoic membrane is down-regulated by treatment with recombinant human alpha-2a interferon and partly mediated by fibroblast growth factor-2. *Haematologica* 2002; 87: 465.

68. Gloe T, Sohn HY, Meininger GA, *et al.* Shear stress-induced release of basic fibroblast growth factor from endothelial cells is mediated by matrix interaction via integrin alpha(v)beta3. *J Biol Chem* 2002; 277: 23453.

69. Hartnett ME, Garcia CM, D'Amore PA. Release of bFGF, an endothelial cell survival factor, by osmotic shock. *Invest Ophthalmol Vis Sci* 1999; 40: 2945.

70. Cozzolino F, Torcia M, Lucibello M, *et al.* Interferon-alpha and interleukin 2 synergistically enhance basic fibroblast growth factor synthesis and induce release, promoting endothelial cell growth. *J Clin Invest* 1993; 91: 2504.

71. Lee HT, Lee JG, Na M, *et al.* 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.

72. Walford G, Loscalzo J. Nitric oxide in vascular biology. *J Thromb Haemost* 2003; 1: 2112.

73. Morbidelli L, Donnini S, Ziche M. Role of nitric oxide in the modulation of angiogenesis. *Curr Pharm Des* 2003; 9: 521.

74. Ziche M, Morbidelli L, Choudhuri R, *et al.* Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 1997; 99: 2625.

75. Ziche M, Parenti A, Ledda F, *et al.* Nitric oxide promotes proliferation and plasminogen activator production by coronary venular endothelium through endogenous bFGF. *Circ Res* 1997; 80: 845.

76. Finetti F, Solito R, Morbidelli L, *et al.* Prostaglandin E2 regulates angiogenesis via activation of fibroblast growth factor receptor-1. *J Biol Chem* 2008; 283: 2139.

77. Gajdusek CM, Carbon S. Injury-induced release of basic fibroblast growth factor from bovine aortic endothelium. *J Cell Physiol* 1989; 139: 570.

78. Sahni A, Altland OD, Francis CW. FGF-2 but not FGF-1 binds fibrin and supports prolonged endothelial cell growth. *J Thromb Haemost* 2003; 1: 1304.

79. 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.

80. Pugh CW. Oxygen sensing in cancer. *Ann Med* 2003; 35: 380.

81. Li J, Shworak NW, Simons M. Increased responsiveness of hypoxic endothelial cells to FGF2 is mediated by HIF-1alpha-dependent regulation of enzymes involved in synthesis of heparan sulfate FGF2-binding sites. *J Cell Sci* 2002; 115: 1951.

82. Wang L, Xiong M, Che D, *et al.* The effect of hypoxia on expression of basic fibroblast growth factor in pulmonary vascular pericytes. *J Tongji Med Univ* 2000; 20: 265.

83. Breviario F, d'Aniello EM, Golay J, *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.

84. Rusnati M, Camozzi M, Moroni E, *et al.* Selective recognition of fibroblast growth factor-2 by the long pentraxin PTX3 inhibits angiogenesis. *Blood* 2004; 104: 92.

85. Presta M, Camozzi M, Salvatori G, *et al.* Role of the soluble pattern recognition receptor PTX3 in vascular biology. *J Cell Mol Med* 2007; 11: 723.

86. Perollet C, Han ZC, Savona C, *et al.* Platelet factor 4 modulates fibroblast growth factor 2 (FGF-2) activity and inhibits FGF-2 dimerization. *Blood* 1998; 91: 3289.

87. Tiefenbacher CP, Chilian WM. Basic fibroblast growth factor and heparin influence coronary arteriolar tone by causing endothelium-dependent dilation. *Cardiovasc Res* 1997; 34: 411.

88. Reuss B, Dono R, Unsicker K. Functions of fibroblast growth factor (FGF)-2 and FGF-5 in astroglial differentiation and blood-brain barrier permeability: evidence from mouse mutants. *J Neurosci* 2003; 23: 6404.

89. Zhang H, Issekutz AC. Growth factor regulation of neutrophil-endothelial cell interactions. *J Leukoc Biol* 2001; 70: 225.

90. Barleon B, Sozzani S, Zhou D, *et al.* Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood* 1996; 87: 3336.

91. Leali D, Dell'Era P, Stabile H, *et al.* Osteopontin (Eta-1) and fibroblast growth factor-2 cross-talk in angiogenesis. *J Immunol* 2003; 171: 1085.

92. Wempe F, Lindner V, Augustin HG. Basic fibroblast growth factor (bFGF) regulates the expression of the CC chemokine monocyte chemoattractant protein-1 (MCP-1) in autocrine-activated endothelial cells. *Arterioscler Thromb Vasc Biol* 1997; 17: 2471.

93. Kage K, Fujita N, Oh-hara T, *et al.* Basic fibroblast growth factor induces cyclooxygenase-2 expression in endothelial cells derived from bone. *Biochem Biophys Res Commun* 1999; 254: 259.

94. Shaw JP, Chuang N, Yee H, *et al.* Polymorphonuclear neutrophils promote rFGF-2-induced angiogenesis in vivo. *J Surg Res* 2003; 109: 37.

95. Zhang H, Issekutz AC. Down-modulation of monocyte transendothelial migration and endothelial adhesion molecule expression by fibroblast growth factor: reversal by the anti-angiogenic agent SU6668. *Am J Pathol* 2002; 160: 2219.

96. Kitayama J, Nagawa H, Yasuhara H, *et al.* Suppressive effect of basic fibroblast growth factor on transendothelial emigration of CD4(+) T-lymphocyte. *Cancer Res* 1994; 54: 4729.

97. Pendurthi UR, Williams JT, Rao LV. Acidic and basic fibroblast growth factors suppress transcriptional activation of tissue factor and other inflammatory genes in endothelial cells. *Arterioscler Thromb Vasc Biol* 1997; 17: 940.

98. Griffioen AW, Damen CA, Blijham GH, *et al.* Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. *Blood* 1996; 88: 667.

99. Mantovani A, Allavena P, Sica A. Tumour-associated macrophages as a prototypic type II polarised phagocyte population: role in tumour progression. *Eur J Cancer* 2004; 40: 1660.

100. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 2004; 4: 71.

101. Porta C, Subhra Kumar B, Larghi P, *et al.* Tumor promotion by tumor-associated macrophages. *Adv Exp Med Biol* 2007; 604: 67.

102. Dirkx AE, Oude Egbrink MG, Wagstaff J, *et al.* Monocyte/macrophage infiltration in tumors: modulators of angiogenesis. *J Leukoc Biol* 2006; 80: 1183.

103. Sunderkotter C, Steinbrink K, Goebeler M, *et al.* Macrophages and angiogenesis. *J Leukoc Biol* 1994; 55: 410.

104. Moldovan NI, Goldschmidt-Clermont PJ, Parker-Thornburg J, *et al.* Contribution of monocytes/macrophages to compensatory neovascularization: the drilling of metalloelastase-positive tunnels in ischemic myocardium. *Circ Res* 2000; 87: 378.

105. Rehman J, Li J, Orschell CM, *et al.* Peripheral blood "endothelial progenitor cells" are derived from monocyte/macrophages and secrete angiogenic growth factors. *Circulation* 2003; 107: 1164.

106. Schmeisser A, Garlichs CD, Zhang H, *et al.* Monocytes coexpress endothelial and macrophagocytic lineage markers and form cord-like structures in Matrigel under angiogenic conditions. *Cardiovasc Res* 2001; 49: 671.

107. Arras M, Ito WD, Scholz D, *et al.* Monocyte activation in angiogenesis and collateral growth in the rabbit hindlimb. *J Clin Invest* 1998; 101: 40.

108. Anghelina M, Krishnan P, Moldovan L, *et al.* Monocytes/macrophages cooperate with progenitor cells during neovascularization and tissue repair: conversion of cell columns into fibrovascular bundles. *Am J Pathol* 2006; 168: 529.

109. Jones GE, Prigmore E, Calvez R, *et al.* Requirement for PI 3-kinase gamma in macrophage migration to MCP-1 and CSF-1. *Exp Cell Res* 2003; 290: 120.

110. Hirsch E, Katanaev VL, Garlanda C, *et al.* Central role for G protein-coupled phosphoinositide 3-kinase gamma in inflammation. *Science* 2000; 287: 1049.

111. Van Rooijen N, Sanders A. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applications. *J Immunol Methods* 1994; 174: 83.

112. Sakurai E, Anand A, Ambati BK, *et al.* Macrophage depletion inhibits experimental choroidal neovascularization. *Invest Ophthalmol Vis Sci* 2003; 44: 3578.

113. Cursiefen C, Chen L, Borges LP, *et al.* VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004; 113: 1040.

114. Ishida S, Usui T, Yamashiro K, *et al.* VEGF164-mediated inflammation is required for pathological, but not physiological, ischemia-induced retinal neovascularization. *J Exp Med* 2003; 198: 483.

115. Pipp F, Heil M, Issbrucker K, *et al.* VEGFR-1-selective VEGF homologue PlGF is arteriogenic: evidence for a monocyte-mediated mechanism. *Circ Res* 2003; 92: 378.

116. Nakao S, Kuwano T, Tsutsumi-Miyahara C, *et al.* Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1 beta-induced neovascularization and tumor growth. *J Clin Invest* 2005; 115: 2979.

117. Coussens LM, Raymond WW, Bergers G, *et al.* Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev* 1999; 13: 1382.

118. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci USA* 2006; 103: 12493.

119. Reinders ME, Sho M, Izawa A, *et al.* Proinflammatory functions of vascular endothelial growth factor in alloimmunity. *J Clin Invest* 2003; 112: 1655.

120. Kim I, Moon SO, Kim SH, *et al.* Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem* 2001; 276: 7614.

121. Abe M, Sato Y. cDNA microarray analysis of the gene expression profile of VEGF-activated human umbilical vein endothelial cells. *Angiogenesis* 2001; 4: 289.

122. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. *Endocr Rev* 2004; 25: 581.

123. Casanovas O, Hicklin DJ, Bergers G, *et al.* Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. *Cancer Cell* 2005; 8: 299.

124. Rusnati M, Presta M. Fibroblast growth factors/fibroblast growth factor receptors as targets for the development of anti-angiogenesis strategies. *current pharmacological design* 2006; in press:

125. van Berkel V, Barrett J, Tiffany HL, *et al.* Identification of a gammaherpesvirus selective chemokine binding protein that inhibits chemokine action. *J Virol* 2000; 74: 6741.

126. Parry CM, Simas JP, Smith VP, *et al.* A broad spectrum secreted chemokine binding protein encoded by a herpesvirus. *J Exp Med* 2000; 191: 573.

127. Fallon PG, Alcami A. Pathogen-derived immunomodulatory molecules: future immunotherapeutics? *Trends Immunol* 2006; 27: 470.

128. Hagedorn M, Bikfalvi A. Target molecules for anti-angiogenic therapy: from basic research to clinical trials. *Crit Rev Oncol Hematol* 2000; 34: 89.

129. Ziche M, Donnini S, Morbidelli L. Development of new drugs in angiogenesis. *Curr Drug Targets* 2004; 5: 485.

130. Patel SR, Jenkins J, Papadopolous N, *et al.* Pilot study of vitaxin--an angiogenesis inhibitor-in patients with advanced leiomyosarcomas. *Cancer* 2001; 92: 1347.

131. Posey JA, Khazaeli MB, DelGrosso A, *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.

132. Tanghetti E, Ria R, Dell'Era P, *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.

133. Rusnati M, Presta M. Extracellular angiogenic growth factor interactions: an angiogenesis interactome survey. *Endothelium* 2006; 13: 93.

134. Naldini A, Leali D, Pucci A, *et al.* Cutting edge: IL-1beta mediates the proangiogenic activity of osteopontin-activated human monocytes. *J Immunol* 2006; 177: 4267.

135. Bernardini G, Ribatti D, Spinetti G, *et al.* Analysis of the role of chemokines in angiogenesis. *J Immunol Methods* 2003; 273: 83.

136. Romagnani P, Lasagni L, Annunziato F, *et al.* CXC chemokines: the regulatory link between inflammation and angiogenesis. *Trends Immunol* 2004; 25: 201.

137. Redington AE, Roche WR, Madden J, *et al.* Basic fibroblast growth factor in asthma: measurement in bronchoalveolar lavage fluid basally and following allergen challenge. *J Allergy Clin Immunol* 2001; 107: 384.

138. Kanazawa S, Tsunoda T, Onuma E, *et al.* VEGF, basic-FGF, and TGF-beta in Crohn's disease and ulcerative colitis: a novel mechanism of chronic intestinal inflammation. *Am J Gastroenterol* 2001; 96: 822.

139. Yamashita A, Yonemitsu Y, Okano S, *et al.* Fibroblast growth factor-2 determines severity of joint disease in adjuvant-induced arthritis in rats. *J Immunol* 2002; 168: 450.

140. Gibran NS, Isik FF, Heimbach DM, *et al.* Basic fibroblast growth factor in the early human burn wound. *J Surg Res* 1994; 56: 226.

141. Klune JR, Dhupar R, Cardinal J, *et al.* HMGB1: endogenous danger signaling. *Mol Med* 2008; 14: 476.

142. Mitola S, Belleri M, Urbinati C, *et al.* Cutting edge: extracellular high mobility group box-1 protein is a proangiogenic cytokine. *J Immunol* 2006; 176: 12.