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Fibroblast Growth Factor Signaling in the Vasculature

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Abstract

Despite their discovery as angiogenic factors and mitogens for endothelial cells more than 30 years ago, much remains to be determined about the role of fibroblast growth factors (FGFs) and their receptors in vascular development, homeostasis, and disease. In vitro studies show that members of the FGF family stimulate growth, migration, and sprouting of endothelial cells, and growth, migration, and phenotypic plasticity of vascular smooth muscle cells. Recent studies have revealed important roles for FGFs and their receptors in the regulation of endothelial cell sprouting and vascular homeostasis in vivo. Furthermore, recent work has revealed roles for FGFs in atherosclerosis, vascular calcification, and vascular dysfunction. The large number of FGFs and their receptors expressed in endothelial and vascular smooth muscle cells complicates these studies. In this review, we summarize recent studies in which new and unanticipated roles for FGFs and their receptors in the vasculature have been revealed.

Keywords

Fibroblast growth factor; Endothelial cell; Angiogenesis; Receptor tyrosine kinase; Vascular smooth muscle cell; Atherosclerosis; Vascular calcification

Introduction

The FGF family of ligands is comprised of 18 members (FGF1-FGF10 and FGF16-FGF23) that are divided into 6 subfamilies, 5 paracrine subfamilies, and 1 endocrine subfamily based on sequence similarities and phylogeny [1]. The FGF homologous factors (FHF) formerly known as FGF11-14 do not activate FGF receptors (FGFRs) and therefore are no longer considered members of the FGF family. Several members of the FGF family are expressed in endothelial cells (FGF1, 2, 5, 7, 8, 16, and 18) and vascular smooth muscle cells (FGF1, 2, 5, 8, 16, and 18) [2]. FGFs regulate a diverse array of responses including cell growth, migration, and differentiation by binding to and activating the FGFR family of receptor

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Compliance with Ethics Guidelines

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tyrosine kinases [1]. There are four FGF receptor genes (FGFR1-FGFR4) that encode single pass transmembrane proteins that contain a transmembrane domain; a cytoplasmic tyrosine kinase domain and an extracellular domain comprised of three-immunoglobulin-like (Ig-like) domains. The extracellular Ig-like domains confer ligand-binding specificity through alternative splicing of Ig-loop III generating the IIIc and IIIb isoforms [1, 3, 4]. Endothelial cells (ECs) express the FGFR1IIIc, FGFR2IIIc, and FGFR3IIIc isoforms of FGFRs but not the IIIb isoforms nor FGFR4 [2]. In vascular smooth muscle cells (VMSC), the expression of FGFR iso-forms is similar to that found in ECs [2]. In addition, FGFR5, also known as FGFR4, an FGF-binding receptor without a tyrosine kinase domain is also expressed in ECs [2].

The paracrine FGFs bind to their receptors in a complex that requires heparan sulfate proteoglycans (HSPGs) [1, 5, 6]. The binding of paracrine FGFs to HSPGs limits diffusion of FGFs from their sites of release and protects FGFs from proteolytic degradation [1, 3, 7, 8]. The endocrine FGFs bind HSPGs only weakly, can diffuse from their sites of production, and enter the circulation to act at distant sites. Endocrine FGFs require a co-receptor, Klotho, for binding to and activating FGFRs [9–12]. Interestingly, removal of the HSPG binding capacity of a paracrine FGF can convert it to an endocrine FGF, which then requires Klotho for binding FGFR1 [13•]. Thus, different FGF subfamilies are tightly regulated in their interaction with and activation of FGFRs.

FGFs are known mitogens for endothelial and vascular smooth muscle cells, as well as angiogenic factors in vitro and in vivo [3, 7, 14]. Furthermore, FGF signaling is important to maintaining endothelial barrier function and integrity [14–16]. These properties have lead to clinical trials investigating the efficacy of FGFs in the treatment of ischemic disease. However, recent reports indicate that FGFs may accelerate atherosclerosis and other vascular diseases calling into question their use in treating ischemic diseases [17, 18]. Here, we will review recent findings that show the complexity of FGF signaling in the vasculature and the interplay between FGF and other signaling pathways that regulate angiogenesis and vascular homeostasis.

Overview of FGF Signaling Mechanisms

Binding of FGFs to their receptors results in receptor dimerization and transphosphorylation on seven tyrosine residues on the receptor cytoplasmic domain [1, 19••]. These phosphotyrosine residues serve as docking sites for recruitment of signaling molecules such as PLC γ and Grb2. PLC γ was the first FGFR interacting protein identified, binding to phosphorylated Tyr766 of FGFR1 [20, 21]. PLC γ catalyzes the formation of inositol 1,4,5-trisphosphate (IP₃) and diacylglycerol (DAG) from phosphatidylinositol-4,5-bisphosphate [1, 19••]. IP₃ mediates the release of Ca²⁺ from intracellular stores, which in turn activates protein kinase C (PKC) that is recruited to the membrane and binds DAG. PKC phosphorylates and activates multiple downstream pathways including extracellular signal-regulated kinase (ERK) in a Ras-independent manner. Activated PKC isoforms play roles in vascular cell growth and migration.

FGFRs bind the docking protein fibroblast growth factor substrate 2 (FRS2) in a tyrosine-independent manner through the residues in the juxtamembrane domain of FGFR and the PTB domain of FRS2 [22, 23]. Upon activation of FGFR kinase activity, FRS2 becomes phosphorylated on six tyrosine residues, which serve as docking sites for Grb2, SHP2, and Gab1 which enable the activation of the ERK and phosphoinositide 3-kinase (PI3K) pathways [24, 25]. The FRS2-Grb2 complex recruits the guanine nucleotide exchange factor SOS, which activates Ras culminating in the activation of ERK and p38 MAPKs. Activation of the ERK pathway is essential to several biological responses to FGFs including proliferation, migration, and differentiation. Recruitment of Gab1 to the FRS2 complex results in activation of the PI3K-Akt pathway that plays essential roles in cell survival, energy sensing, and several other physiological processes [26]. Evidence suggests that Gab1 does not bind FRS2 directly but rather by binding via one of its SH2 domains to FRS2-bound Grb2. Gab1 is tyrosine phosphorylated by FGFR and binds Grb2 via one of its two SH2 domains [26]. Src family kinases are also important mediators of FGF signaling, and FRS2 is important in the recruitment of Src to FGFR complexes [27]. In addition, Src plays a role in the intracellular trafficking of FGFRs, and signaling intensity and duration [28]. The precise mechanisms by which Src mediates maximal FGFR activation remain to be determined.

FGF signaling is complex and often cell type or cell context specific. In addition to binding to and activating FGFRs, FGFs have been shown to bind other cell surface molecules to initiate signaling cascades. One of these, syndecan-4, is a transmembrane protein with extracellular 2-O and 6-O sulfated heparan sulfate chains that bind FGFs [29, 30]. Syndecan-4 forms a complex with FGFR1 and FGF2 due to heparin-binding domains on both FGF2 and the extracellular domain of FGFR1 [31]. This ternary complex is necessary for FGF-dependent signaling through FGFR1. In addition, the cytoplasmic domain of syndecan-4 is known to bind and activate signaling proteins, mostly through the PIP₂ binding domain and a PDZ domain [7]. The PIP₂ binding domain in complex with PIP₂ binds protein kinase C α (PKC α) to activate it and to promote endothelial cell migration, although the mechanisms remain to be fully elucidated. The PDZ domain of syndecan-4 binds several proteins including synectin, syntenin, CASK/LIN2, and synbindin. Particularly noteworthy is the interaction between syndecan-4 and synectin, which plays a role in regulating Rac1 activation and cell migration [32]. Syndecan-4 has also been shown to play a role in endocytosis of FGFRs [7].

FGFs have been shown to directly bind to the integrin $\alpha\text{v}\beta 3$ promoting EC adhesion and spreading [7, 33, 34]. Blockade of $\alpha\text{v}\beta 3$ on the surface of ECs with antibodies inhibits angiogenesis, although it is not clear whether this involves inhibition of FGF binding or binding of ECM molecules such as fibronectin. FGFRs are able to bind neural cell adhesion molecules (NCAM) in an FGF independent manner through an HAV motif in the extracellular domain [35]. NCAM expression on ECs is variable and dependent upon the vascular bed from which the ECs originate [7]. The interaction of NCAM with FGFR1 results in activation of PLC γ and PKC α ; however, further study is required to determine the significance of these interactions in endothelial cells. N-cadherin has also been shown to interact with FGFR in certain cellular contexts, and N-cadherin is expressed in endothelial cells [7]; however, more study is needed to determine biological significance of this

interaction in endothelial cells. Due to the complexity of FGF signaling and the interaction of FGFRs with several cell surface molecules, much remains to be learned about FGFR regulation and signaling in vascular cells. Table 1 summarizes key components of the FGF signaling pathway, their functions in the vasculature and phenotypes of gene-targeted mice.

FGF Signaling in Endothelial Cells, Angiogenesis, and Neovascularization

The discovery of FGF2 and FGF1 as mitogens for endothelial cells and angiogenic factors both in vitro and in vivo over 30 years ago lead to the discovery of other families of angiogenic factors and their receptors resulting in a better understanding of the mechanisms of both normal and pathological angiogenesis; however, much remains to be determined. Although FGF1 and FGF2 are potent angiogenic factors in vitro and in vivo, mice lacking *Fgf1* show no abnormalities, and *Fgf2* null mice show no phenotypic abnormalities; however, they show some delay in wound healing [36–38]. Mice with null mutations in both *Fgf1* and *Fgf2* showed no defects in angiogenesis, which suggests functional compensation by other ligands of the FGF family. FGF16 and FGF18 are expressed in ECs and in vitro stimulates EC migration but not proliferation [44].

Because of their angiogenic potential in vitro and in vivo assays, clinical trials to treat ischemic disease with FGFs were initiated, but failed to show significant therapeutic benefit [17, 18]. This suggested that more than one growth factor might be necessary to elicit robust formation of new vessels. Indeed, numerous studies show that FGF signaling regulates the vascular endothelial growth factor (VEGF) pathway at more than one level [67]. FGF induces the expression of VEGF in endothelial and stromal cells [14]. In vivo and in vitro experiments using adenoviral vectors encoding dominant negative soluble FGFRs showed that FGF signaling regulates the expression of VEGFR2 in ECs in an ERK-dependent manner [68•]. Systemic administration of soluble dominant negative FGFR1 decreased VEGFR2 expression and impaired post-ischemic neovascularization. Recently, studies with endothelial and hematopoietic-specific deletion of *Fgfr1* and *Fgfr2* revealed that *Fgfr1* and *Fgfr2* are dispensable for normal vascular development; however, loss of endothelial *Fgfr1* and *Fgfr2* results in impaired neovascularization after injury in adult mice [49••]. In addition, studies using a new allosteric FGFR inhibitor, SSR128129E, showed in a zebrafish model of vascular development that FGF signaling regulates angiogenic sprouting of the intersomitic vessels (ISV) [69•]. Administration of SSR128129E at the time of initial sprouting caused vessels to cease elongation in zebrafish embryos. While these data may seem in conflict with one another, these experiments highlight the complexity of FGF signaling in the vasculature. Furthermore, SSR128129E targets all FGFRs, and in these experiments, FGF signaling may have been inhibited in other cell types that may contribute to vessel outgrowth. These data also suggest that FGF signaling lies upstream of VEGF [69•]. The lack of an effect on embryonic vessel formation in endothelial cell-specific *Fgfr1*/*fgfr2* knockout mice suggests the possibility that there may be compensation by FGFR3, which is also expressed in endothelial cells.

Dysregulation of FGF signaling through gene amplification or mutation causes a variety of pathological conditions, thus indicating that FGF signaling must be tightly regulated [19••]. Several feedback inhibitory mechanisms have been identified that dampen FGF signaling,

many of which have been shown to function in vascular cells. Sprouty proteins are feedback inhibitors of receptor tyrosine kinase (RTK) signaling, including FGFRs [70, 71]. The Sprouty family consists of four members (Spry1, Spry2, Spry3, Spry4), all of which are expressed in ECs [2]. Overexpression of Spry1 and Spry2 in EC inhibits FGF- and VEGF-induced ERK activation and proliferation but has no effect on EGF-mediated ERK activation [57]. In addition, Spry1 and Spry2 inhibit in vitro angiogenesis in a Matrigel assay. The inhibition of EC proliferation is due in part to increased expression of the cell cycle inhibitors p21 and p27 and altered cytokine expression profiles [58]. In another study, Spry4 was shown to inhibit FGF- and VEGF-induced EC proliferation and migration in vitro [63]. Injection of a Spry4 adenovirus into mouse embryos disrupted vessel formation in vivo. Overexpression of Spry1 in transgenic mouse embryos results in embryonic lethality due to defects in hematopoiesis and vascular maturation [61]. Gene targeting studies in the mouse show that Spry2 and Spry4 double knockout mice show embryonic lethality by E12.5 due to multiple defects including vascular defects [62]. Spry4 knockout mice are viable and show increased vascular density in a number of tissues. *Spry4*^{-/-} mice were resistant to hindlimb ischemic tissue damage due to accelerated neovascularization [64]. Sprys have also been shown to be important in regulating VSMC proliferation and phenotypic modulation in vitro and in vivo. In a rat carotid artery injury model, treatment of injured vessels with a chimeric protein consisting of the HIV TAT membrane translocation sequence and Spry2 (TAT-hSpry2) inhibited neointima formation and VSMC proliferation for up to 28 days post injury [72]. Furthermore, in vitro studies on VSMC show that Spry1 and Spry4 have different roles in VSMC phenotypic modulation; Spry1 maintains the VSMC contractile phenotype in part by regulating an Akt/FoxO/myocardin pathway [59]. Together, these data show that Spry family members function to maintain proper levels of RTK signaling during vascular development, the vascular response to injury and vascular homeostasis. Whether the observed effects of Sprys on vascular function is attributable to inhibiting FGF signaling or other RTK signaling pathways such as VEGF will be difficult to determine due to similarities in the downstream targets of RTKs in the vasculature. Another feedback inhibitor of FGF signaling, Sef (similar expression to FGF) [73, 74], also known as IL17RD, is expressed in ECs and VSMC [2, 75]. Sef inhibits FGFR signaling by binding to FGFR1 and inhibiting activation of the tyrosine kinase activity [65, 74]. When overexpressed in the endothelial cell, Sef inhibits FGF-induced ERK activation and reduces cell viability [76]. Sef knockout mice do not exhibit an embryonic vascular phenotype; however, postnatal phenotypes include changes in the auditory brainstem and increased cortical bone thickness [66, 77]. The impact on vascular remodeling, adult neovessel formation, and vascular integrity remains to be explored. Together, these recent findings highlight the need for additional studies on FGF signaling mechanisms in vascular development, adult angiogenesis, and neovessel formation, which will inform new therapeutic approaches to ischemic injury.

FGF Signaling in Vascular Homeostasis

While much has been learned about the role of FGF signaling in angiogenesis over the last several years, the importance of FGF signaling to vascular homeostasis and vascular permeability has only recently been investigated. Studies have revealed that FGF signaling

is critical to vascular homeostasis and the maintenance of endothelial cell barrier function [16]. Murakami et al. used a soluble dominant negative FGFR1IIIc ligand trap in vivo to show that disruption of FGF signaling resulted in a loss of endothelial cell-cell adhesion and increased vascular permeability in several vascular beds including lung and heart [15]. Interestingly, soluble FGFR3IIIb had little effect on vascular permeability suggesting that FGF1, FGF9, and FGF20 are not active in maintaining vascular integrity. Because both FGFR1IIIc and FGFR3IIIc had potent effects on vascular permeability, it is likely that FGF2, FGF4, and FGF8 are candidate ligands for maintaining vascular integrity [15]. Further study is required to ascertain the exact FGF ligand/receptor combinations responsible for maintenance of vascular integrity. At a mechanistic level, FGF signaling strengthens VE-cadherin/p120 catenin interactions, which reduces VE-cadherin internalization, thus increasing cell surface VE-cadherin and increasing cell-cell adhesion [15]. Additional studies have revealed that FGF signaling increases VE-cadherin stability by maintaining SHP2 levels which reduced tyrosine phosphorylation of VE-cadherin leading to reduced internalization and increased endothelial cell-cell adhesion [78]. The mechanisms by which FGF signaling stabilizes SHP2 protein levels in endothelial cells remains to be determined. Similar conclusions regarding the requirement for FGF signaling to maintain EC barrier function were reported in studies using a chemical allosteric inhibitor of FGF signaling, SSR128129E, in a zebrafish model system [69•]. Administration of SSR128129E to zebrafish embryos after vessels had become established resulted in a loss of vascular integrity. This was shown to be due in part to reduced VE-cadherin levels, similar to previous studies [15]. However, recent studies using EC-specific deletion of *Fgfr1* and *Fgfr2* in the mouse produced different results. These studies showed that endothelial and hematopoietic-specific deletion of *Fgfr1/2* with either *Flk1-Cre* or *Tie2-Cre* resulted in normal embryonic development, with normal embryonic vascular networks [49••]. As adults, these mice showed normal vascular patterning and density as determined by immunostaining for endothelial cell markers when compared to controls. These mice also maintained normal basal vessel barrier function in contrast to previous studies where FGFR function was inhibited in the vasculature resulting in impaired barrier function [15]. There are several possible explanations for these discrepancies. *Fgfr1/Fgfr2* gene inactivation was efficient (84–87 %) but incomplete and therefore residual signaling may be possible [49••]; there could be compensation for loss of *Fgfr1* and *Fgfr2* by upregulation of FGFR3, which has previously been shown to be expressed in endothelium. Although basal vascular permeability was unaffected in gene-targeted *Fgfr1/Fgfr2* mice, there was a small but significant increase in vascular permeability in response to inflammatory stimuli, suggesting that FGF signaling is dispensable for homeostatic barrier function, but may have a non-homeostatic role [49••]. Interestingly, blood pressure and vascular reactivity were also unaffected by the loss of FGFR1/FGFR2 signaling. Further study will be required to resolve the role of FGF signaling vascular barrier function and to determine the usefulness of targeting the FGF signaling pathway in vascular hemostatic disorders.

FGF Signaling in Atherosclerosis

Atherosclerosis is an inflammatory process involving VSMC and macrophages that is initiated as a result of EC dysfunction. Under normal physiological conditions, ECs produce

nitric oxide, which has anti-inflammatory properties and suppresses the development of atherosclerosis. Endothelial dysfunction caused by hypercholesterolemia and other cardiovascular stressors results in a condition where inflammatory cytokines and endothelial adhesion molecules attract inflammatory cells such as monocytes to the subendothelial space and activate the migration and proliferation of VSMC leading to the development of atherosclerosis [79]. Although the roles of FGFs have been studied intensively in angiogenesis and ischemic disease, much less is known about FGFs in the development of atherosclerosis. FGFs produced by VSMC and macrophages are potent mitogens and chemotactic factors for VSMC. FGFs and their receptors are expressed in atherosclerotic plaques in humans and in atherosclerotic lesions of ApoE-deficient mice [80]. Anti-FGF2 antibodies or soluble dominant negative FGFR1 inhibit injury-induced proliferation of VSMC in vivo [81, 82]. Because it was recognized that most coronary events are the result of rupture of the fibrous cap of atherosclerotic plaques and not due to narrowing of vessel lumen as a result of VSMC proliferation per se, inhibiting FGF signaling to combat atherosclerosis fell out of favor. However, a recent study showed that inhibiting FGFR signaling with the RTK inhibitor SU5402 in ApoE^{-/-} mice fed a high-fat diet inhibited neointimal thickening by almost 85 % [83]. Detailed analyses attributed most the effect of SU5402 to inhibition of FGFR1, although other FGFRs are expressed in the vasculature. One caveat to these studies is that SU5402 also inhibits VEGFR2, and thus effects on other cells such as endothelium cannot be ruled out [83]. In a later study, transgenic mice with endothelial cell-specific expression of a constitutively active FGFR2 were generated on an ApoE-deficient background and fed a high-fat diet [84•]. These mice had a significant increase in atherosclerotic lesion area relative to ApoE deficiency alone. Endothelial cells overexpressing FGFR2 expressed higher levels of the cell cycle inhibitor p21^{Cip1}, VCAM-1, and ICAM-1 compared to ApoE^{-/-} alone. Mechanistically, elevated levels of p21^{Cip1} lead to increased expression of PDGF-B in endothelium resulting in paracrine stimulation of VSMC growth [84•]. Thus, FGF signaling in endothelium exacerbates development of atherosclerosis through increased endothelial cell dysfunction thus suggesting that caution should be used in the use of FGF therapeutically for ischemic disease in the context of other vascular pathologies. To further elucidate the role of FGF signaling in atherosclerosis, a highly selective inhibitor of FGF signaling, SSR128129E, was tested in atherosclerotic and restenotic mouse models [85]. SSR128129E decreased neointima proliferation in both models, suggesting that targeting FGF signaling with this orally bioavailable compound may have therapeutic value in the treatment of atherosclerosis. Other studies suggest that reduced FGF signaling in the endothelium results in reduced let-7 miRNA levels which results in increased TGFβ signaling and the induction of endothelial-mesenchymal transition (Endo-MT) and endothelial dysfunction [86••]. Endo-MT due to disrupted FGF signaling in endothelium promoted neointima formation in a mouse model of transplant arteriopathy. These studies are supported by the recent finding that endothelial-specific deletion of FGFR1 resulted in increased TGFβ signaling and Endo-MT [87•]. Furthermore, human diseased arteries showed reduced FGFR1 expression in association with Endo-MT. Together, these studies suggest that normal vascular homeostasis requires a fine tuning of FGF signaling and that too much or too little FGF signaling results in endothelial dysfunction and cardiovascular disease.

In addition, these studies do not resolve whether FGF signaling in a specific cell type is the driver of atherosclerosis or whether FGF mediates complex interdependent regulatory signals between different cell types in atherosclerotic and restenotic lesions [85]. Further study with cell type-specific transgenic and gene-targeting strategies will be necessary to resolve this question.

FGF Signaling in Vascular Calcification

Vascular calcification is a complication of vasculopathies that develop as a result of diabetes, hypercholesterolemia, or hypertension and interact with fluctuations in phosphate levels as a result of dialysis to treat chronic kidney disease (CKD) [88]. This fluctuating hyper- and hypophosphatemia promotes chronic vascular inflammation and VSMC apoptosis [89•]. Vascular calcification has emerged as a powerful prognostic indicator of cardiovascular morbidity and mortality [89•, 90]. Inflammatory cytokines and oxidative stress signaling have been shown to contribute to activation of osteogenic differentiation programs in VSMC and ECs [88, 91]. Several pathways have been shown to contribute to vascular calcification including TGF β pathway components [92, 93], Dkk1 [94], and FGFs [89•, 95]. FGF2 was shown to play a role in inducing osteogenic differentiation of VSMC in vitro by activating the osteogenic transcription factor Runx2 in an ERK- and ROS-dependent manner [95]. Recently, the role for the endocrine FGF, FGF23, in vascular calcification has emerged. FGF23 is released by osteocytes in response to serum phosphate levels and circulating 1,25(OH) $_2$ D $_3$, the active form of vitamin D [89•, 96]. FGF23 binds to FGFR/Klotho heterodimer complexes in the kidney to facilitate excretion of phosphate by down-regulating phosphate transporters in the proximal tubule. FGF23 also lowers 1,25(OH) $_2$ D $_3$ levels by inhibiting the expression of *CYP27B1* and increasing the expression of *CYP24A1*, two enzymes involved in vitamin D metabolism. By regulating phosphate excretion in the kidney and phosphate absorption in the intestine through regulating vitamin D, increased levels of FGF23 maintains phosphate in the normal range during excess dietary phosphate and lower FGF23 levels maintain normal serum phosphate during conditions of low dietary phosphate levels. High FGF23 levels have also been associated with increased cardiovascular disease independent of CKD, including atherosclerosis, myocardial infarction, and heart failure [89•].

Mechanistically, incubation of VSMC with medium containing high phosphate concentrations results in their osteogenic differentiation [97]. Vascular calcification can be induced in mouse models with high phosphate diets [98, 99]. Perhaps the most compelling experimental evidence for a role of FGF23 in regulating vascular calcification is that gene targeting of either *Fgf23* [96] or *Klotho* [54], or administration of neutralizing antibodies to FGF23 [100] in mice results in vascular calcification. Despite these findings, some studies suggest that FGF23 may have a protective effect on the vasculature in CKD. Recent studies show that Klotho is expressed in human arteries and that in CKD patients, Klotho is downregulated [53••]. In vitro studies on human VSMC showed that Klotho is downregulated by inflammatory cytokines, uremia, and dysregulated metabolic conditions. Knockdown of Klotho also accelerated osteogenic differentiation of VSMC through upregulation of Runx2 and downregulation of Myocardin-SRF transcriptional complexes [53••]. Deficiency of Klotho induced by procalcific conditions could be restored by vitamin

D receptor activation and restored FGF23 responsiveness. Thus, additional studies are needed to explore the complex interplay between serum phosphate and FGF23 to determine the possible therapeutic benefits of FGF23 in treating cardiovascular disease.

Summary and Conclusions

The study of the FGF family and their receptors has revealed multiple effects in multiple cell types in development and disease, including the vasculature (Fig. 1). While the early promise of using FGF1, FGF2, and other paracrine FGFs therapeutically to treat vascular diseases have been mostly disappointing, these early disappointments reveal that much remains to be learned about the roles of FGFs in the cardiovascular system. New drugs that inhibit FGFR signaling have served as useful tools in elucidating the *in vivo* functions of FGFs and their receptors and will guide future development of new approaches to manipulate FGF signaling to treat cardiovascular diseases and promote wound healing. The recent identification of the FGF23 signaling pathway as a major regulator of vascular calcification and perhaps other cardiovascular diseases such as atherosclerosis warrants intense investigation. While many challenges remain to be explored, new discoveries in FGF biology will lead to a better understanding of their roles in cardiovascular disease and the development of novel approaches to treat these diseases.

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Fig. 1.

Cellular interactions in normal and diseased vasculature. In the normal homeostatic vessel (*left*), basal signaling of FGFs via FGFR1 and FGFR3 promote junctional integrity in endothelial cells (*blue*) via stabilization of VE-cadherin and catenins, which mediate stability of adherens junctions. These signals promote vascular integrity and homeostasis. Several layers of quiescent, contractile vascular smooth muscle cells (*green*) surround the endothelium. During vascular pathology (*right*), disruption of the FGF pathway by either increased or decreased signaling results in dysfunction of vascular cells. *Top* Endothelial cells with overactive FGF signaling have altered cell surface receptors and cytokine production. As a result, these paracrine signals recruit inflammatory cells stimulate and underlying smooth muscle cells. *Bottom* Vascular smooth muscle cells respond to FGF by proliferation and migration, potentially contributing to neointimal lesion formation. In addition, under conditions such as high phosphate, FGFs contribute to osteogenic differentiation of vascular cells. These cellular and molecular changes promote a vascular environment leading to atherosclerosis, vascular stenosis, or vascular calcification

Table 1

FGF signaling pathway components active in vascular cells

Gene	Vascular expression	Vascular activity/functions	Mutant mouse phenotypes
FGF1	EC, VSMC [2]	Mitogen, chemotactic for EC, VSMC [3, 34]	<i>Fgf1</i> ^{-/-} mice show normal development and viability [36]
FGF2	EC, VSMC [2]	Mitogen, chemotactic for EC, VSMC [3, 34]	<i>Fgf2</i> ^{-/-} mice have mild phenotype in vascular tone [37], delayed wound healing [38]
FGF5	EC, VSMC [2]	Arteriogenic [39]	Abnormally long hair in <i>Fgf5</i> ^{-/-} mice [40]
FGF7	EC, VSMC [2]	EC migration [41]	Defects in hair development in <i>Fgf7</i> ^{-/-} mice [42]
FGF8	EC, SMC [2]	Cardiovascular morphogenesis [43]	<i>Fgf8</i> ^{-/-} mice have outflow tract defects, defects in VSMC of large vessels [43]
FGF16	EC, VSMC [2]	Promotes EC migration [44]	<i>Fgf16</i> ^{-/-} mice have defects in cardiac morphogenesis [45]
FGF18	EC, VSMC [2]	Promotes EC migration [44]	<i>Fgf18</i> ^{-/-} mice show postnatal lethal, defects in osteogenesis and chondrogenesis [46], and the lung [47]
FGFR1 (FGFR1IIIc)	EC, VSMC [2]	Activates MAPK and Akt pathways in EC and VSMC [3, 14]	<i>Fgfr1</i> ^{-/-} mice are embryonic lethal [48], compound deletion of <i>Fgfr1/Fgf2</i> in ECs results in defects in wound healing [49••]
FGFR2 (FGFR2IIIc)	EC, VSMC [2]	Activates MAPK and Akt pathways in EC and VSMC [3, 14]	<i>Fgfr2</i> ^{-/-} mice are embryonic lethal [50], compound deletion of <i>Fgfr1/Fgf2</i> in ECs results in defects in wound healing [49••]
FGFR3 (FGFR3IIIc)	EC, VSMC [2]	Activates MAPK and Akt pathways in EC and VSMC [3, 14]	Defects in skeleton and hearing in <i>Fgfr3</i> ^{-/-} mice [51]
FGFR5 (<i>FGFR1L</i>)	EC [2]	Unknown	Defects in kidney development of <i>Fgfr1L</i> ^{-/-} mice [52]
Klotho	EC, VSMC [53••]	Prevents vascular calcification [53••]	Premature aging, vascular calcification in <i>Kl</i> ^{-/-} mice [54]
Syndecan-4	EC, VSMC [7]	Transmembrane protein that binds FGFs to propagate signals via FGFR1, involved in FGFR endocytosis [7]	<i>Sdc4</i> ^{-/-} mice have decreased neointimal lesion formation in injured vasculature [55], increased mortality after myocardial infarction [56]
Spry1	EC, VSMC [2]	Inhibits EC proliferation and tubulogenesis [57, 58], promotes VSMC contractile phenotype [59]	Defects in kidney development [60]; gain of function mutants embryonic lethal with hematopoietic and vascular defects [61]
Spry2	EC, VSMC [2]	Inhibits EC and VSMC proliferation [57]	Spry2/Spry4 double mutants lethal at E12.5 with vascular defects [62]
Spry3	EC, VSMC [2]	Unknown	Unknown
Spry4	EC, VSMC [2]	Inhibits migration and adhesion of EC [63]	Viable with increased vascular density, resistant to hindlimb ischemia due to accelerated neovascularization [64]
Sef (IL17RD)	EC, VSMC [2]	Binds and inhibits FGFR1 kinase activity [65]	Postnatal cortical bone thickening [66], not yet tested in adult pathology

Listed are components of the FGF signaling pathway that are found in endothelial cells or smooth muscle cells, and their vascular-related functions and mouse model phenotypes