Fibroblast growth factors (FGFs) were among the first molecules discovered to stimulate the growth of endothelial cells. Now, more than 15 years after their initial discovery,1 we still do not understand what endogenous role they play in embryonic and pathological angiogenesis. Both acidic FGF (FGF-1) and basic FGF (FGF-2) lack a typical secretion signal, their spatiotemporal expressions do not always correlate with active angiogenesis, and genetic loss of either or both growth factors does not cause major vascular defects.2,3 This is in contrast to their effect in stimulating angiogenesis in ischemic myocardium in experimental animal models.4 However, to make matters even more confusing, thus far initial clinical results with FGF-2 in patients have not yielded the expected success in long-term improvement of myocardial perfusion.5 These conflicting data mandate a better understanding of the role of these growth factors in vivo. In this issue of Circulation Research, Fernandez et al6 provide novel in vivo evidence that FGF-1 stimulates the branching of the myocardial arteries. In addition, FGF-1 stimulates the formation of sustainable, functional vessels by preventing their regression. Both effects are attractive, stated goals of therapeutic angiogenesis in ischemic heart disease.

The three-dimensional organization of the vascular network has fascinated many scientists for a long time. Aristotle, one of the first of these scientists, wrote, “the system of blood vessels in the body may be compared with those of water-courses which are constructed in gardens: they start from one source, or spring, and branch off into numerous channels, and then into still more, and so on progressively, so as to carry a supply to every part of the garden.”7 Now, two millennia later, we have learned a great deal about how individual growth factors and receptors stimulate endothelial growth or migration in vitro, but still we have not determined what molecules control the organization and branching of blood vessels in vivo. Nevertheless, a better understanding of this process is of great clinical interest. Indeed, new vessels would only improve myocardial perfusion if they were correctly branched in an organized pattern and properly connected to the preexisting vasculature.

Branching not only occurs in the vasculature; there are other channels in the body that extensively branch. Genetic studies in the fruit fly Drosophila melanogaster and in the mouse have revealed essential insights regarding how the airways branch in complex tubular networks.8,9 Initially, epithelial buds sprout from the gut into the surrounding mesenchyme to form the trachea and primary bronchi. The latter sprout secondary bronchi, which sprout tertiary bronchi, and so on. Branching continues for a total of 6 to 8 generations in the mouse and for about 20 generations in humans, forming the estimated 17 million branches of the human lung. At least for the early branch generations, the patterns are highly stereotyped, implying fixed developmental control. The pattern of the terminal branches is not rigidly fixed but variable and regulated by tissue oxygen need. In both the fly and the mouse, FGF signaling has been implicated in airway branching. Just before primary branching commences, a cluster of cells arrayed around the tracheal sacs at positions where primary branches will bud expresses FGF-like ligands (branchless in the fly; FGF-10 and possibly FGF-10, KGF in the mouse). These FGF ligands then bind an FGF receptor on nearby tracheal cells, signaling the migration of the tracheal cells as the primary branches bud. As primary branches extend toward the FGF-signaling cells, cells at the growing tip are exposed to high levels of the FGF signal. This induces expression of secondary branch genes (for instance, the transcription factor pointed in the fly), which drives formation of secondary branches. Precisely controlled spatiotemporal production of inhibitors (sprouty in the fly; sprouty homologues, bone morphogenic protein-4, and Sonic hedgehog or Shh in the mouse) blocks FGF signaling in more distant tracheal cells, thereby limiting secondary branching to positions closest to the FGF source.9 In addition, by shutting off FGF-10 expression in mesenchyme near growing tips, Shh splits the initial FGF-10 expression cell clusters into 2 smaller clusters, allowing 2 new buds to sprout. Notch signaling has been implicated in the singling-out process of cells that are allowed to bud.10 Indeed, branchless upregulates the expression of Delta, a ligand for Notch, at the tip of the tracheal branches. Activated Notch then restricts the branchless signal to the tip of the branches by downregulation of branchless in neighboring cells via lateral inhibition. Other growth factors that stimulate branching are platelet-derived growth factor (PDGF) and hepatocyte growth factor (HGF), whereas transforming growth factor-β (TGF-β) inhibits branching.

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How can we relate these insights in airway branching to the branching of vessels, in particular in the heart? The myocardium in the early embryo is initially avascular as it receives oxygen via diffusion from the ventricular cavity. Subsequently, when the myocardial wall becomes thicker, a vascular network in the myocardial wall develops to supply oxygen to the cardiomyocytes. The vascular precursor cells that establish this network are derived from the outside heart. Indeed, mesothelial cells located at the right side of the sinus venosus (proepicardial organ) invade the embryonic epicardium and differentiate in situ into endothelial precursor cells (angioblasts). These endothelial cells subsequently assemble into a primitive labyrinth of capillaries. Coronary vascular smooth muscle cells are also recruited from the epicardial layer and migrate alongside the preexisting endothelial channels. Both endothelial and smooth muscle cells migrate in a stereotypic epicardial-to-endocardial direction. The number and location of the epicardial coronary arteries are usually fixed within one species, although interindividual variability may occur. Like airways, myocardial vessels branch into smaller terminal vessels.

Several mechanisms have been identified that could result in the branching of vessels. First, new vessel branches can sprout toward a cluster of cells in the surrounding mesenchyme that produce the angiogenic stimulus. Second, vessels can split into individual daughter vessels by the formation of transendothelial cell bridges. Third, vessels can branch via intussusception on the basis of insertion of interstitial tissue columns into the lumen of preexisting vessels. The subsequent growth of these columns and their stabilization, in part via ingrowth of periendothelial cells, results in partitioning of the vessel lumen and remodeling of the vascular network.

Several angiogenic signals have been identified to affect one or more of these branching processes, although the distinction among these processes is often blurred. For instance, gene-targeting factors, such as VEGF and Ang1, have survival properties for endothelial or smooth muscle cells. For the sake of clarity and brevity, some typical examples are illustrated. Vascular endothelial growth factor (VEGF) contributes to the complexity of the vascular network by stimulating vascular splitting and sprouting. Embryos lacking a single VEGF allele have fewer vascular sprouts. In addition, mice that only express the short VEGF120 isoform die of ischemic heart disease and suffer a markedly reduced coronary perfusion reserve. This results from a significantly impaired branching of myocardial vessels. Expression of PDGF-BB, which is known to affect airway branching, was reduced in these hearts, but it is possible that VEGF also acts as a branching factor by directly affecting endothelial or smooth muscle cells. Angiopoietin-1 (Ang1) and its receptor Tie2 induce angiogenic sprouts but may also affect microvascular intussusceptive growth. Indeed, gene inactivation of Ang1 and Tie2 resulted in dilated capillary-like vessels of uniform size that failed to remodel in a mature, complex network of large vessels ramifying into smaller branches. Renin is a branching factor for renal arteries, but its cellular mechanism remains unknown. In their transgenic study, Fernandez et al observed that FGF-1 is a branching factor for myocardial arteries. Such a role of FGF-1 in the vasculature is consistent with the role of the FGF-related signals in airway branching. A challenge for the future will be to unravel whether FGF-1 also induces a similar finely tuned cascade of cellular and molecular events in the heart as branchless and FGF-10 accomplish in the lung. For instance, are Notch, Delta, and Jagged molecules whose role in angiogenesis is only now starting to be revealed, also involved in singling out sprouting endothelial cells via lateral inhibition in response to FGF-1? The transgenic FGF-1 mice should provide useful models to address these questions.

Another remarkable finding of the study by Fernandez et al is that the myocardial arterial density was normal in transgenic neonates but higher in adult mice overexpressing FGF-1. The myocardial arterial network expands almost 10-fold during the first postnatal weeks in the mouse, when expression of VEGF and FGFs is upregulated. Genetic studies have shown that changes in the level or isoform of VEGF can have drastic effects on myocardial angiogenesis and arteriogenesis. Thus, it is somewhat surprising that overexpression of FGF-1 did not cause an additional increase in myocardial vessels during this neonatal period. Perhaps this relates to the low level of transgene expression (from 1.3- to 1.9-fold more than the levels of endogenous FGF-1) or the lack of a secretion signal in front of the transgene. Another reason may be that the myocardial vasculature becomes more sensitive to small changes in FGF-1 once the endogenous levels of FGF-1 have become minimal in the adult stage.

Instead of causing an additional increase in myocardial vessels, FGF-1 prevented the age-related regression in myocardial arterial density beyond 6 weeks of age. Because no differences in vascular cell proliferation were detected, these findings raise an outstanding question of whether continuous low levels of FGF-1 are essential for survival of endothelial or smooth muscle cells in the coronary vasculature. FGF-1 could affect survival of vascular cells directly or indirectly via stimulating the production of survival factors by cardiomyocytes or other cardiac cells. Many vascular growth factors, such as VEGF and Ang1, have survival properties for endothelial or smooth muscle cells. For instance, gene-targeting studies of the endothelial junctional molecule VE-cadherin revealed that embryonic vessels fail to expand, remodel, and branch because VE-cadherin critically regulates the survival function of VEGF. Furthermore, impaired survival, not proliferation, of vascular cells prevented branching of the coronary vasculature in mice that only expressed the short VEGF120 isoform. Survival of endothelial and smooth muscle cells is also important once the vasculature has been established in the adult. Quiescent endothelial cells have prolonged lifetimes (several years) and may require the continuous presence of minimal amounts of survival factors. An imbalance between survival and apoptotic factors could contribute to the regression of terminal myocardial vessels (rarefication) in pathological conditions of hypertension and diabetes.

Several questions remain unanswered. For instance, why did only the arteries and not the capillaries regress in the wild-type mice, and why was there no effect of FGF-1 on capillaries? Vessels without a muscular coat have been proposed to be more susceptible to regression. What is the role of endogenous FGF-1? The observed effect after overexpression of an FGF-1 transgene does not necessarily implicate a relevant role for endogenous FGF-1 in vascular development. What will happen if FGF-1 transgene expression drops? Will the vessels then regress, or will the established flow in these vessels induce sufficient release of vascular survival factors? Will FGF-1 have similar effects in the ischemic myocardium, which expresses more and distinct angiogenic factors and receptors than normal myocardia? What are the functional consequences of the more
extensively branched myocardial vascular network on cardiac function during hemodynamic stress or ischemia? Would FGF-1 have affected collaterals between epicardial coronary arteries if such collaterals existed in the mouse? Will continued expression of FGF-1 result in intimal thickening of the coronary arteries? What about atherosclerosis? Will the FGF-1 mice be more prone to plaque neovascularization?

The findings by Fernandez et al. may have implications for therapeutic angiogenesis. First, this study demonstrates that FGF-1 improved myocardial perfusion by stimulating arteriogenesis and not angiogenesis (i.e., the number of arteries was increased). Although capillaries directly deliver oxygen to the cells, arteries are essential conduit vessels, providing and regulating the bulk blood flow to the tissue. Therapeutic angiogenesis may remain insufficient if it is not associated with arteriogenesis. Second, FGF-1 prevented regression of established vessels. Most studies have studied the short-term effects of therapeutic angiogenesis, but it remains outstanding whether therapeutic angiogenesis will lead to a sustainable new vascular network. Although short-term angiogenesis is already a formidable challenge, we should be aware that stimulating growth of new vessels is not an endstage and could even be harmful if these vessels regress after ending the treatment. Perhaps therapeutic angiogenesis should involve a sequential treatment scheme: initially, short-term administration of a higher amount of one or several angiogenic factors to stimulate the growth of additional vessels and, subsequently, long-term administration of low amounts of a survival factor to prevent regression of the new vessels. At the very least, this study enlightens expectations that FGF-1 has some attractive properties for therapeutic stimulation of new blood vessels in the heart.

References


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Fibroblast Growth Factor-1 Stimulates Branching and Survival of Myocardial Arteries: A Goal for Therapeutic Angiogenesis?

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