Organized blood vessel formation is essential for development and physiological function of organs. Blood vessel formation is composed of complicated and sequential processes initiated by vasculogenesis in which endothelial progenitor cells differentiate, proliferate, and subsequently assemble into primitive tubular networks. Vasculogenesis is followed by angiogenesis, wherein vascular networks remodel into more complex networks through dilatation, sprouting, and bridging. Another form of blood vessel growth after birth is arteriogenesis. Arteriogenesis is defined as structural enlargement and remodeling by growth of preexisting arteriolar connections. During arteriogenesis, smooth muscle cells migrate and assemble along the preexisting tubes to form mature, stabilized vessels with vasomotor functions. Although vasculogenesis has been observed solely in the embryonic stage, the identification of circulating endothelial precursor cells indicates the contribution of vasculogenesis in blood vessel formation in adult tissue.

Coronary blood vessels originate from endothelial precursor cells migrating to the epicardium in the embryonic stage, during which coronary vasculogenesis takes place. The vascular tubes formed by vasculogenesis grow by angiogenic sprouting and mature in the interaction with pericytes. The result is coronary capillary formation. After capillary formation, vascular plexuses, which appear in the outflow tract region, generate continuous tubes and penetrate into the aorta. Along these preexisting vessels, recruited smooth muscle cells migrate and cover the tubes from epicardial to endocardial direction. Then vessels form coronary veins and arteries. During the early postnatal period, marked capillary growth proceeds in myocardium, and maturation of arteries occurs mostly after birth.

Because most of the works in this field have focused on polypeptide angiogenic factors until recently, angiogenic growth factors, such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), have been highlighted as key regulators of blood vessel formation and maintenance in development and physiology. Novel genetic approaches using gene ablation and transgene expression have made enormous contributions in understanding that VEGF is an indispensable requirement for both vasculogenesis and angiogenesis. FGF also promotes angiogenesis, as revealed in various types of angiogenesis assays both in vitro and in vivo, whereas the ablation of FGF did not cause severe vascular defects. Angiopoietins also work as an angiogenic factor in maturing and stabilizing vessels at later stages of vascular development. On the other hand, recent advances have identified angiostatin, an internal fragment of plasminogen, and endostatin, a proteolytic fragment of collagen XVIII, as angiogenesis inhibitors. Both angiostatin and endostatin have been shown to possess multiple antiangiogenic effects in vitro and in vivo, including an induction of tumor regression in vivo. Additionally, multiple factors, such as extracellular matrix and mechanical factors like shear stress, are considered to modulate vessel formation. Hypoxia is also a strong inducer of angiogenesis, mostly through hypoxia-inducible factor-dependent expression of VEGF, its receptors, and other angiogenic factors.

In this issue of Circulation Research, Tomanek et al demonstrate in vivo interplay of VEGF and basic FGF (bFGF) in coronary vessel development through a unique approach. Tomanek et al tried to partially block the actions of VEGF and bFGF in rat myocardium by neutralizing antibodies against VEGF and bFGF during the early stage of the postnatal period, when angiogenic sprouting leading to capillary growth actively progresses. Although the antibodies were administered through intraperitoneal injection, the delivery of the antibodies to the myocardium was verified. The ventricular weights and histology of the hearts, including cardiomyocytes, vascular cells, and extracellular matrix, in the treated rats were unaffected by the neutralizing antibodies against VEGF and bFGF. The complete ablation and suppression of VEGF signaling by the treatment with anti-VEGF, anti-FGF, or a combination of both antibodies. Tomanek et al could then observe a physiological remodeling of coronary vessels attributable to modified actions of the growth factors. However, a pathological remodeling secondary to changed myocardial oxygen demand could not be observed. In this model, it was shown that both VEGF and bFGF are essential for capillary growth. The careful analysis on the morphometry of coronary vessels revealed the decrease of capillary growth to approximately 80% of control by the treatment with anti-VEGF, anti-FGF, or a combination of both. The complete loss of VEGF signaling by the ablation of VEGF or its functional receptor VEGFR-2 failed to form a vasculature and had fewer endothelial cells. Inducible Cre-loxP-mediated VEGF gene targeting in newborn mice resulted in impaired organ development. Therefore, the inhibition of VEGF and bFGF by the neutralizing antibodies must be partial, and the partial reduction of VEGF during the

Harmonic Interplay of Angiogenic Growth Factors in the Development of Coronary Blood Vessels

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early postnatal stage, when angiogenesis is active, can be expected to form decreased capillaries in which endothelial cells are a major component. Accumulating evidence of the angiogenic property of bFGF supports this observation.15–19 The interplay of VEGF and bFGF in the promotion of angiogenesis has been characterized, and angiogenic actions of bFGF are shown to depend partially on VEGF.23 In the study by Tomanek et al.,22 the effects of anti-VEGF and anti-bFGF antibodies are not additive on the reduced capillary growth. This may be because the actual neutralization in vivo of each growth factor is not equivalent.

As shown by Tomanek et al.,22 bFGF inhibition, but not VEGF inhibition, significantly decreased arteriolar growth. This observation is not surprising, because bFGF plays important roles to promote the differentiation and recruitment of smooth muscle cells,2,15 which are essential for arteriolar growth. Again, because it is impossible for us to evaluate to what extent and how long VEGF and bFGF actions were neutralized in vivo by the antibodies, there is a possibility that we underestimate the involvement of VEGF in arteriolar formation. The dual inhibition of both VEGF and bFGF caused a shift in dimension of arterioles from smaller to larger but not a decrease in arteriolar growth. Therefore, controlled actions of both VEGF and bFGF seem to regulate arteriolar diameter. According to the dual growth factor inhibition, both arteriolar growth and the vascular morphology—including wall thickness, which indicates relevance to the recruitment of smooth muscle cells—were unaffected. The shift in diameter of arterioles might be attributable to an adaptation of vessels to maintain coronary circulation, whereas additional studies are required to clarify the determinants of vascular dimension and the involvement of growth factors.

In the study by Tomanek et al.,22 several questions remain to be answered. Is harmonic interplay of VEGF and bFGF necessary and sufficient for the maturation and maintenance of coronary vessels in adult rats after the early postnatal stage? Once coronary vessels undergo physiological remodeling in the postnatal stage, are the maturation and the maintenance of coronary vessels perturbed in the later adult stage? To address these issues, longer observations of coronary vascular trees in treated rats are necessary. The VEGF or bFGF inhibition for a longer duration or during the later postnatal stage is required. In addition, the involvement of other angiogenic factors as well as angiogenesis inhibitors should be investigated. The answers to the aforementioned questions are crucial elements in the ability to predict the outcome of long-term growth factor inhibition in blood vessel development.

Recently, the results of early clinical trials in therapeutic angiogenesis for peripheral vascular disease and coronary artery disease have been reported,24 and limited but promising results have had a tremendous impact on additional investigations. On the other hand, the results deserve more intensive research on potential risks involved. There is the possibility for improvement by modifying doses, routes, and duration of administration. The choice of angiogenic agents, genes, or proteins, whether single or multiple, is also essential. We should note that therapeutic angiogenesis has been focused mainly on the induction of capillary formation. To construct and maintain physiological blood circulation, capillaries as well as arterioles with vasomotor functions must be properly formed.4 However, newly formed capillaries are prone to be destroyed because of the lack of surrounding smooth muscle cells. Therefore, arteriogenesis should coincide with angiogenesis. Strategies to trigger not only angiogenesis but also arteriogenesis could be more beneficial to therapeutic angiogenesis. Combined and sequential administration of multiple factors would be ideal. Along this line, the study by Asahara et al.25 provides evidence that angiopoietins enhance the angiogenic actions of VEGF in vivo. Multiple angiogenic/antiangiogenic factors, including unknown factors, are biologically potent and constitute a complex orchestration for physiology of the vascular network.8 Timing, duration, and controlled targeting to the organs of angiogenic actions are key factors for optimal outcomes and prevention of deleterious effects. One such example reported that unregulated, continuous delivery of VEGF to the myocardium caused the formation of hemangioma and death in mice.26 Growth factor antagonism is a novel therapeutic strategy for cardiovascular diseases, such as atherogenesis and restenosis.27–30 Certainly profound insights are required into therapeutic angiogenesis/antiangiogenesis by modulating actions of angiogenic/antiangiogenic factors to obtain favorable and more physiological results. The present study by Tomanek et al.22 leads us to appreciate the conclusion that actions of angiogenic growth factors must be strictly regulated and harmonized for development during the early postnatal period of functional coronary vessels possessing normal structures.

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