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to the sixth, we have also entered the third era of treatment of ischemic disease. Until the latter part of the 20th century, pharmacological therapy was the sole means that physicians had to alleviate ischemic symptoms in patients with coronary and peripheral ischemia. The advent of mechanical revascularization revolutionized the care of these patients during the past 25 years, prolonging life and improving function beyond what had been possible with optimal medical management. Recently, the concept that new blood vessels could be grown to enhance tissue perfusion, barely a footnote in the cardiovascular literature 10 years ago, is now achieving widespread acceptance. “How,” not “whether,” is now the prevailing question. Biological revascularization, the third era in the treatment of ischemic disease, is now within our reach.1

The notion that stem cells, present in adults, were participants in physiological and pathological neovascularization has had at least two profound effects on cardiovascular investigators: (1) An entire field of research dedicated to harnessing the potential of stem cells for therapeutic purposes was born, and (2) the mechanisms governing embryonic blood vessel formation, previously the exclusive domain of developmental biologists, suddenly appeared as potential therapeutic targets.

The possibility that embryonic signaling pathways might participate in adult neovascularization was recently highlighted by Pola et al,3 demonstrating the role of the hedgehog family of proteins in the response to ischemia and the potential therapeutic effect of local sonic hedgehog administration. In the present issue of Circulation, Dufourcq et al4 illuminate another novel signaling pathway influencing blood vessel formation. Like many developmental signaling pathways, this one derives its nomenclature from initial observations made in Drosophila, resulting in designations that seem diabolically contrived to dispel the interest of clinicians. We will attempt to reverse this natural reaction by breaking the code and, most importantly, by highlighting the fascinating results of this study.

Wnt is a family of secreted proteins that, acting via surface receptors such as “Frizzled,” activate the cytoplasmic protein “Disheveled” to modulate the ability of β-catenin to activate the transcription of target genes (Figure).5 The name Wnt derives from the fusion of wingless and Int-1, genes first discovered in the fruit fly Drosophila that were noted to exhibit significant sequence homology. Wingless referred to the phenotype of flies in which the gene was missing, whereas Int was so named because it became activated when the mouse mammary tumor virus inserted—or integrated—next to it in the genome. Similarly, Frizzled and Disheveled refer to the appearance of fruit flies with null mutations of these genes, hence the nomenclature. Wnt family members are secreted glycoproteins that have been shown to be multipotent factors, modulating proliferation, differentiation, and migration in various contexts, similar to better known secreted proteins such as fibroblast growth factors (FGF) and transforming growth factor-β.6,7

Recently, soluble proteins were discovered with sequence homology to the Wnt binding site of the Frizzled receptor and were thus named soluble Frizzled-related proteins (sFRP).

In an elegant series of experiments, Dufourcq et al demonstrate physiological and pathological roles of one of the sFRP family members, FrzA, and the mouse orthologue, sFRP-1, in postnatal neovascularization. The authors have
previously shown that sFRP-1/FrzA is strongly expressed during the angiogenic phase in a murine model of hindlimb ischemia. However, little is known about the functional role of FrzA and its mechanism for angiogenesis.

With the use of a murine model of ovarian hyperstimulation, the authors now demonstrate a role of sFRP-1/FrzA in physiological angiogenesis. They document expression of sFRP-1/FrzA in endothelial cells, lutein cells, and pericytes during cyclic ovarian neovascularization and go on to show that with further corpus luteum maturation, sFRP-1 expression remained strong in lutein cells and pericytes, whereas sFRP-1 expression decreased in endothelial cells. The spatiotemporal expression of sFRP-1 during cyclic ovarian blood vessel maturation therefore suggests a relationship between sFRP-1 expression and vessel formation—in other words, FrzA-mediated vessel maturation. They also performed recombinant AdFrzA gene transfer in a chick chorioallantoic membrane (CAM) model, in a mesenchymal cell graft model, and in a tumor cell engraftment model to demonstrate FrzA-induced neovascularization. Gene transfer of AdFrzA significantly induced neovascularization compared with Adβgal transfection in a CAM model. AdFrzA gene transfer also significantly augmented new vessel growth in mesenchymal and glioma cell xenografts. Finally, and perhaps most significantly, FrzA induced a more robust angiogenic response compared with treatment with vascular endothelial growth factor (VEGF) in the tumor model. FrzA once again appeared to enhance maturation of neovessels.

Although the authors did not examine the effect of FrzA gene transfer in tissue ischemia, these in vivo data suggest the potential of FrzA for therapeutic angiogenesis in ischemic diseases. In vitro, FrzA protein augmented chemotaxis of endothelial cells as well as VEGF protein. FrzA also stimulated tube formation of endothelial cells on Matrigel. These in vitro findings support the angiogenic potential of FrzA. One of the most intriguing results of the study is that addition of VEGF did not induce FrzA-mRNA expression in cultured endothelial cells or smooth muscle cells nor did transfection of AdFrzA induce VEGF-mRNA expression in endothelial cells. Hypoxic conditions also did not affect the FrzA expression. Finally, the authors show that FrzA protein does not upregulate phosphorylation of Akt in endothelial cells. These findings suggest that the angiogenic potential of FrzA is independent of VEGF and Akt activation. These observations thus indicate that FrzA may augment postnatal neovascularization through a novel and potentially complementary signaling pathway.

The concept of therapeutic angiogenesis by administration of angiogenic genes or proteins has been established in numerous preclinical models. The initial report that intracoronary injection of basic FGF could improve cardiac function and reduce infarct size in a canine model of myocardial infarction launched a new field of cardiovascular investigation. Subsequently, intraarterial administration of recombinant VEGF-1, as well as intramuscular injection of naked DNA encoding VEGF-1 and VEGF-2, were shown to
enhance perfusion in the rabbit hind limb ischemia. Numerous reports have documented the potential for a variety of cytokines to induce neovascularization and enhance perfusion in ischemic tissues.\textsuperscript{13–17}

Recently, pilot clinical trials of therapeutic angiogenesis with some of these growth factors have been reported. Rajagopalan et al.\textsuperscript{18} demonstrated improvement of lower extremity blood flow reserve after adenovirus-mediated gene transfer of VEGF-121 in patients with critical limb ischemia. Simons et al.\textsuperscript{19} showed trends toward symptomatic improvement after single intracoronary administration of recombinant FGF-2 in patients with coronary artery disease. Grines et al.\textsuperscript{20} documented improvement of exercise tolerance after single intracoronary injection of Ad5-FGF4 in patients with stable angina pectoris. We have performed clinical trials of VEGF-1 and VEGF-2 naked DNA transfer in patients with critical limb and myocardial ischemia. VEGF-1 gene transfer was associated with reduced limb necrosis/enhanced ulcer healing and augmented angiographic collateral circulation.\textsuperscript{21} We have also performed operative and catheter-based intramyocardial gene transfer of VEGF-1 or VEGF-2 plasmid in patients with coronary artery disease.\textsuperscript{23,24} A phase I study of VEGF-1 gene therapy showed reduction of ischemia by SPECT imaging as well as improved symptoms but was limited by the absence of a control group, necessitated by the operative approach for gene delivery. A subsequent double-blind, placebo-controlled, phase II study of VEGF-2 gene transfer also documented significant improvement of subjective symptoms evaluated by the Canada Cardiovascular Society Classes and strong trends favoring efficacy of VEGF2 versus placebo treatment on exercise duration. Although subjective symptoms have been significantly improved in these phase I/II trials of therapeutic angiogenesis, other studies have failed to demonstrate improvement of objective findings such as myocardial perfusion and exercise tolerance. Larger phase III studies will be necessary to fully evaluate safety and therapeutic efficacy of these angiogenic factors.

Analysis of the data generated in all of these pilot studies reveals two common features: (1) In each study the effect of a single agent was evaluated, and (2) certain patients are nonresponders. The absence of a response in certain individuals is a consistent feature of all therapies and is the basis for the concept of pharmacogenomics, the science of designing drugs on the basis of genetic features of individual patients. Lacking, as yet, this tailored approach to drug development, physicians have traditionally tried combining drugs to achieve therapeutic effects in patients with conditions refractory to single agents.

A combination of ≥2 growth factors that act on endothelial cells through different pathways may be one the solutions to failures of single agents noted in early trials. Dufourcq et al report that the angiogenic potential of FrzA is independent of VEGF and Akt activation. This finding may suggest the usefulness of this novel factor in combination therapy with VEGF. Of course, the precise mechanism of FrzA-induced angiogenesis should be clarified and the therapeutic potential of sole therapy of FrzA should be evaluated first. Does the angiogenic activity of FrzA differ from other factors? Does FrzA induce vasculogenesis by inducing stem cell recruitment and incorporation at sites of neovascularization? We have previously reported the therapeutic potential of endothelial progenitor cells in animal models of hindlimb\textsuperscript{25} and myocardial ischemia\textsuperscript{26} in dogs. Circulation. 1994;89:2183–2189.

The participation of the Wnt/\beta-catenin pathway in postnatal angiogenesis, documented by Dufourcq et al in this issue, provides another candidate to augment perfusion in ischemic tissue by capitalizing on what was previously thought to be a developmental pathway for cell signaling. In the era of biological revascularization, we expect that therapy will increasingly attempt to recapitulate ontogeny by molecular interventions, taking full advantage of embryonic plasticity while mooting the debate regarding the use of embryonic tissue.

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