Early investigations into the realm of therapeutic angiogenesis focused on the ability to augment perfusion of ischemic tissue by the administration of single angiogenic cytokines, either as fully formed proteins or as gene therapy. The use of single agents, although teleologically unlikely to result in an optimal effect, reflected both a practical reality and the need to discern the mechanisms resulting from these early attempts to modify a complex process. Much was learned from these pioneering laboratory and clinical studies. Of note, the very term angiogenesis, classically used to describe the expansion of existing vasculature by sprouting and migration in various contexts, similar to better known transforming growth factor-β (TGF-β) receptors such as fibroblast growth factors (FGF) and vascular endothelial growth factor (VEGF). These receptors are secreted glycoproteins that have been shown to be members of the TGF-β superfamily, which are secreted by a variety of cell types, including endothelial cells, smooth muscle cells, and immune cells. The TGF-β superfamily includes several members, such as activin, bone morphogenetic proteins (BMPs), and connective tissue growth factor (CTGF). These proteins are involved in a variety of processes, including cell proliferation, differentiation, migration, and angiogenesis.

In an elegant series of experiments, Dufourcq et al demonstrated physiological and pathological roles of one of the sFRP family members, FrzA, and the mouse orthologue, sFRP-1, in postnatal neovascularization. The authors have shown that sFRP-1 is expressed in the developing vasculature and is required for proper angiogenesis. The loss of sFRP-1 results in angiogenic defects, including the absence of blood vessels and the formation of abnormal vessels. These findings suggest that sFRP-1 plays a critical role in the regulation of angiogenesis and that it may be a potential therapeutic target for the treatment of angiogenic diseases.
previously shown that sFRP-1/FrzA is strongly expressed during the angiogenic phase in a murine model of hindlimb ischemia.\textsuperscript{8} 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 spatio-temporal 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\textsuperscript{9} launched a new field of cardiovascular investigation. Subsequently, intraarterial administration of recombinant VEGF-1,\textsuperscript{10} as well as intramuscular injection of naked DNA encoding VEGF-1\textsuperscript{11} and VEGF-2,\textsuperscript{12} 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.13–17

Recently, pilot clinical trials of therapeutic angiogenesis with some of these growth factors have been reported. Rajagopalan et al18 demonstrated improvement of lower extremity blood flow reserve after adenovirus-mediated gene transfer of VEGF-121 in patients with critical limb ischemia. Simons et al19 showed trends toward symptomatic improvement after single intracoronary administration of recombinant FGF-2 in patients with coronary artery disease. Grines et al20 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.21,22 We have also performed operative and catheter-based intramyocardial gene transfer of VEGF-1 or VEGF-2 plasmid in patients with coronary artery disease.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 ischemia25 and myocardial ischemia. An attractive feature of employing a progenitor cell approach is the innate ability of these cells to orchestrate a variety of signaling pathways involved in vessel formation that in some measure resembles embryonic vascular development.

The participation of the Wnt/β-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 muting the debate regarding the use of embryonic tissue.

References


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