Angiogenesis and Atherosclerosis
The Mandate Broadens
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Among the most exciting developments in vascular medicine is the targeting of specific molecular regulators of blood vessel growth and development for therapeutic benefit. Angiogenesis, whether physiological, such as occurs during embryogenesis, or pathological, such as occurs during wound healing and tumor progression, requires a highly coordinated series of events that includes endothelial cell proliferation, migration, tube and lumen formation, and in some cases, recruitment of smooth muscle and other adventitial cells. A large number of studies in cellular systems and animal models have led to identification and characterization of specific factors that can either stimulate or inhibit angiogenesis. These studies were mainly stimulated by Folkman’s hypothesis that tumor growth beyond a few millimeters requires recruitment and formation of a new microcirculation, and thus, that blood vessel-directed therapy might be effective in treating cancer. Translational research has now been initiated by several groups to test the hypothesis that local delivery of angiogenic agents, such as vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF), by various strategies, including viral vectors, naked DNA, or purified recombinant proteins, may improve blood flow to ischemic tissues in patients with advanced atherosclerosis. In some cases, preliminary data have been reported and appear promising.

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Specific antiangiogenic therapies are also now in the clinical-trial stage for a broadening group of common diseases, including cancer, macular degeneration, and rheumatoid arthritis. Recent studies in rodent models suggest that antiangiogenic agents may limit adipose mass in hereditary obesity and may slow progression of atheroma. The latter result, reported in the April 6, 1999, issue of Circulation, showed that atheromatous lesions beyond a certain size, like malignant tumors, contain an increased number of vasa vasorum and that 2 different angiogenesis inhibitors, the tumour necrosis factor (TNF-α) and the VEGF inhibitor endostatin, can reduce lesion progression. The studies substantiate the hypothesis published 15 years ago that neovascularization by vasa vasorum in coronary vessels contributes to atherogenesis.

In the current issue of Circulation, Chen et al have identified a new therapeutic target for vascular disease that may relate to the same biological regulatory mechanisms that control angiogenesis. They show that a specific neutralizing monoclonal antibody directed against thrombospondin-1 (TSP-1) improved “healing” of a balloon-injured rat carotid artery. The antibody was delivered locally into the lumen at the time of the injury and then by constant infusion into the vessel wall adventitia for 2 to 4 weeks. The authors documented successful delivery of the antibody into the vessel wall by immunohistochemical techniques and demonstrated a statistically significant increased rate of reendothelialization in the treated arteries along with a decrease in the ratio of intima-to-media thickness. These effects were associated with an increased number of proliferating cells at the lumen (presumably migrating endothelial cells) and a decreased number of proliferating cells in the media (presumably smooth muscle cells).

So what is TSP-1, and why should targeting this molecule lead to accelerated reendothelialization and reduced neointima formation? TSP-1 is a 450 000-Da matrix glycoprotein of long-standing interest to vascular biologists because of its pattern of expression and its ability to modulate many important vascular cell functions. It is present in large amounts in platelet α-granules, from which it is secreted at sites of platelet activation. It is also made and secreted by endothelial cells and smooth muscle cells in a highly regulated manner. Its expression is rapidly and dramatically upregulated in response to vascular injury or exposure of cells to platelet-derived growth factor (PDGF) or bFGF; thus, it is present in large amounts in atheroma and in the matrix of a balloon-injured vessel. Of most relevance to angiogenesis and repair of arterial balloon injury are the observations that TSP-1 is a highly potent inhibitor of angiogenesis in vivo and is capable of blocking capillary endothelial cell proliferation, migration, and tube formation induced by angiogenic factors. It also enhances smooth muscle proliferation and migration in response to PDGF. Antibodies to TSP-1 thus could potentially augment the endothelial cell migration and proliferation necessary for reendothelialization while blocking the smooth muscle cell migration and proliferation associated with accelerated restenosis.

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The biology of TSP-1, however, is complex, and the effects seen in response to delivery of anti–TSP-1 into an injured vessel are likely to be much more complicated than those described above. TSP-1 interacts with many matrix constituents, including heparan sulfate proteoglycans, fibronectin, and collagen, and probably plays a role in matrix stability and remodeling. Evidence from TSP-1 null mice suggests that this is particularly important in bone matrix, but the role of TSP-1 in vascular wound matrix remodeling and stability has not yet been well characterized. TSP-1 can also interact with several specific cellular adhesion receptors, including CD36, αv integrins, heparan sulfate proteoglycans, and integrin-associated protein (IAP; CD47). It can function as an adhesion molecule, promoting tumor cell–matrix interactions and platelet aggregation, but it also has antiadhesive properties for some cells, leading, for example, to disruption of endothelial cell focal adhesion plaques. On macrophages, TSP-1 mediates recognition and phagocytosis of apoptotic leukocytes, thus participating in the later stages of the inflammatory response and limiting proinflammatory influences. TSP-1 interacts with several proteases, including plasminogen, urokinase, thrombin, cathepsin, and elastase, promoting plasmin generation but inhibiting cathepsin. It also binds transforming growth factor–β (TGF–β) with high affinity and can efficiently convert the latent form into the active form. This latter effect is probably very important in vascular injury responses. TGF–β is a highly active cytokine and, like TSP-1, is also found in large amounts in injured vessels. It promotes collagen production and matrix deposition by vascular cells, regulates plasmin generation, and has many important anti-inflammatory properties. Studies of the TGF–β null mouse and the TSP-1 null mouse have shown, in fact, that the 2 phenotypes are similar and that TSP-1 is the critical in vivo activator of TGF–β.

Two critical questions arise from these observations. How can a single molecule effect so many disparate functions, and to which of the many functions attributable to TSP-1 can the “healing” effects of anti–TSP-1 treatment be ascribed? The answer to the first question is the most straightforward and relates directly to the structural organization of the protein. TSP-1 is a homotrimeric protein structurally organized as a series of discrete modules in linear array (Figure). The most N-terminal of these modules is a domain responsible for heparin binding and for disulfide bond–dependent trimerization of the TSP monomers. Immediately adjacent to the heparin binding domain is a cysteine-rich region with homology to procollagen. After that are 3 copies of a domain called the type I repeat, sequences of 50 to 54 amino acids that are homologous to malaria proteins and properdin. Adjacent to this region are 3 copies of type II repeats bearing epidermal growth factor–like homology, and after that are 7 type III repeats bearing homology to calcium binding sites in many other proteins. These are followed by a unique globular carboxy-terminal domain. Studies using protease digestion, monoclonal antibodies, synthetic peptides, and recombinant TSP-1 fragments have shown that each of these domains is responsible for specific functions. Thus, the apparently contradictory studies showing that TSP-1 has both proadhesive and antiadhesive properties are explained by the existence of multiple TSP-1 cellular adhesion receptors, each with specificity for a different TSP domain (Figure) and each with its own unique pattern of cellular expression. The TSP type I repeats contain the amino acid sequence CSVTCG, which has been shown to mediate binding to CD36. The last type III repeat contains the integrin binding domain, RGDA, and the carboxy-terminal domain contains the binding site for IAP. The TGF–β binding and activation domain also appears to be in the type I repeats, but it is not known whether it is identical to the CSVTCG sequence required for CD36 binding.

The CD36–TSP-1 interaction is particularly relevant to angiogenesis and vascular biology. CD36 is an 88 000-Da glycoprotein expressed on platelets, macrophages, adipocytes, and certain specialized epithelia (retinal pigment and breast). It too is a complex multifunctional protein, serving as a scavenger receptor on macrophages for oxidized LDL and apoptotic cells and as a fatty acid transporter on adipocytes and muscle. On endothelium, its expression is limited to microvessels, the vessels from which new vessels arise during an angiogenic response. CD36 is the receptor that mediates some of the antiangiogenic properties of TSP-1. Studies by Bouck and colleagues, collaborating with our group, have shown that blockade of the TSP-1–CD36 interaction with recombinant CD36 peptides or inhibitory antibodies blocked the ability of TSP-1 to inhibit endothelial cell proliferation, migration, and tube formation. Synthetic peptides from the type I TSP repeat or from related sequences in other molecules have been shown to bind CD36 and to inhibit angiogenesis. Furthermore, transfection of CD36 cDNA into TSP-1–unresponsive macrovascular endothelial cells conferred responsiveness to TSP-1 in in vitro angiogenesis assays. Studies on a CD36 null mouse generated in our laboratory have shown that angiogenesis is not inhibited by TSP-1 in an in vivo corneal angiogenesis assay. A second region of the TSP-1 molecule from the procollagen homology domain also has antiangiogenic activity, implicating TSP-1–matrix interactions in angiogenesis as well. Although αv integrins have been implicated in endothelial cell survival and apoptosis, the integrin binding region of TSP-1 has not been shown to have antiangiogenic activity.

The question of which of the many functions attributable to TSP-1 is responsible for the healing effects of anti–TSP-1 treatment is a more difficult one. There are 2 pieces of evidence suggesting that CD36 might not be involved. If, in fact, the cells responsible for reendothelialization are migrating in from the periphery of the injury, they are not likely to express CD36. Macrovascular endothelial cells, such as in the
carotid artery, are CD36 negative. Second, the monoclonal antibody used by Chen et al., C6.7, recognizes the carboxyterminal IAP binding domain on TSP-1, not the CD36 binding region in the type I repeats. IAP is a membrane-spanning protein shown to modulate integrin function and signaling on vascular cells. C6.7 can block chemotaxis and spreading of endothelial cells on RGD-containing substrates, presumably by blocking the ability of TSP-1 to mediate IAP-dependent integrin activation. On the other hand, recent studies suggest the existence of a circulating pool of endothelial cell progenitors with high angiogenic potential. If reendothelialization is mediated in part by homing of these cells to the lesion, with subsequent proliferation and differentiation, it is possible that TSP could function by blocking these phenomena in a CD36-dependent manner. Also, although C6.7 does not recognize the type I repeat, it might interfere with CD36 binding and function by steric effects. Thus, although the studies by Chen et al. are intriguing, significantly more characterization at the molecular level will be required to determine whether the observed effects are due to antiendothelial proliferation/migration activities, pro–smooth muscle cell proliferation activities, TGF-β activation, integrin interactions, IAP interactions, matrix stability, protease activity, or even effects on macrophage activation.

The provocative preliminary studies published in Circulation this year by Chen et al. and Moulton et al. may help in defining the precise clinical scenarios suitable for proangiogenesis- or antiangiogenesis-based therapies for patients with atherosclerosis. The use of antiangiogenic therapies to halt progression of lesions is exciting and logical but may be limited by a restricted temporal window of opportunity and, in certain circumstances such as angioplasty, may be associated with exacerbating restenosis after balloon angioplasty. The use of proangiogenic factors as an in vivo bypass strategy is equally logical and is supported by intriguing preliminary data. On the basis of studies similar to the one reported by Chen et al., this strategy might also be of benefit for the angiogenesis-related process of reendothelialization of a balloon-injured artery. In this regard, TSP-1 might be an attractive therapeutic target because of its additional effects on smooth muscle cells, TGF-β, and the inflammatory response, which could limit the exaggerated proliferative responses in the vessel wall that lead to accelerated restenosis. The caveats of these studies include the potential risk of promoting plaque instability and lesion progression if, as suggested by Barger et al. and others and as supported by the recent studies of Moulton et al., neovascularization in the lesion is proatherosclerotic. It is possible that as we learn more about the mechanans of the arterial healing effects of TSP-1, “designer” molecules based on structure/function relationships could be developed to maximize the therapeutic effects of TSP-1 while minimizing potential negative effects.

**References**


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