Journal of the American College of Cardiology
© 2007 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 49, No. 21, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2006.10.083

Elimination of Neoangiogenesis for Plaque Stabilization

Is There a Role for Local Drug Therapy?

Frank D. Kolodgie, PHD,* Jagat Narula, MD, PHD, FACC,† Chun Yuan, PHD,‡ Allen P. Burke, MD, FACC,* Aloke V. Finn, MD,§ Renu Virmani, MD, FACC*

Gaithersburg, Maryland; Irvine, California; Seattle, Washington; and Boston, Massachusetts

Emerging data suggest that intraplaque hemorrhage is critical in promoting atherosclerotic lesion instability. Because red blood cell membranes are a rich source of free cholesterol and accumulated red blood cells within plaques promote inflammation, intraplaque hemorrhage is associated with expansion of the necrotic core. Plaque hemorrhage results from the development of immature neointimal vasa vasorum. Therefore, it is proposed that molecular therapies designed to eliminate pathologic neovascularization within developing lesions will interrupt the process of hemorrhage and decrease the rate of necrotic core expansion. The elimination of intraplaque neovascularization would involve targeting of pre-existing and new vessel development. The concept of vascular regression has met some success in other neovascular-dependent diseases, including macular degeneration and malignancies. The efficacy of this novel approach is dependent on gaining critical knowledge of the environment required to support development and maturation of the vasa vasorum within varying plaque types. A multitargeted approach involving selective local antiangiogenic agents should contribute to prevention of plaque progression and its clinical consequences. (J Am Coll Cardiol 2007;49:2093–101) © 2007 by the American College of Cardiology Foundation

Approximately 75% of acute coronary events and 60% of recently symptomatic carotid artery disease are caused by disruption of an atheromatous plaque (1–3). Although the underlying mechanism for conversion of an asymptomatic fibroatheroma to a lesion vulnerable to rupture is not established, the significance of intraplaque hemorrhage in lesion stability has recently been proposed as an important contributor (4–6). A series of manuscripts in the current issue of the *Journal* propose the association of hypercholesterolemia with the development of increased vasa vasorum around coronary vessels and intraplaque neovascularization. The newly formed vasculature in the plaque is demonstrated to be incompetent, because red blood cells (RBCs) constantly leak into the microenvironment, exaggerated particularly during intraplaque hemorrhage. The RBC membranes are rich in free cholesterol, and the extent of RBC leakage is suggested to be responsible for the rapid expansion of the necrotic core. It is therefore proposed that elimination of the intraplaque neovascularization should substantially decrease the accumulation of RBC-derived cholesterol in plaques, which may slow the development of the necrotic core and promote plaque stabilization.

From the *CVPath Institute, Gaithersburg, Maryland; †University of California, Irvine, California; ‡University of Washington, Seattle, Washington; and the §Massachusetts General Hospital, Boston, Massachusetts. Supported in part by a research grant from the National Institutes of Health (R01 HL61799-02). Dr. Virmani has also been sponsored by Medtronic AVE, Guidant, Abbott, W. L. Gore, Amersham Health, diaDexus, Takeda, Atrium Medical Corporation, Invatec, ev3, TopSpin Medical (Israel), Boston Scientific, Novartis, Paracor Medical, and Orbus Medical Technologies and has consulted for Medtronic AVE, Guidant, Abbott Laboratories, W. L. Gore, Terumo, TopSpin Medical (Israel), CryoVascular Systems, C. R. Bard, and Volcano Therapeutics. Dr. Narula has been sponsored by Toshiba-America Medical Sytems and Theseus Imaging and has received honoraria and is a member of the speakers bureau for GlaxoSmithKline, Novartis, and Toshiba-America. Amir Lerman, MD, served as Guest Editor of this article.

Manuscript received August 8, 2006; revised manuscript received October 18, 2006, accepted October 30, 2006.

Plaque Volume and Necrotic Core Size Determine the Plaque Vulnerability

Histopathologic features of lesions critical to acute coronary events include plaques with markedly attenuated and inflamed fibrous caps underlined by a relatively large necrotic core. Over 80% of plaques associated with acute rupture demonstrate more than 50% cross-section vascular area narrowing, with greater than 75% narrowing found in at least one-half of lesions (7). Not only are ruptured plaques sizable in circumference, they longitudinally span a median length of 9 mm, resulting in relatively large plaque volumes. Such plaques also contain large necrotic cores, which commonly occupy more than 25% of the plaque area. The necrotic core size and plaque volume interact synergistically, increasing the odds of plaque rupture. This association is so strong that other factors, including inflammation, only marginally contribute to lesion vulnerability. The increased

JACC Vol. 49, No. 21, 2007
May 29, 2007:2093–101

Abbreviations and Acronyms

Ang = angiopoietin

RBC = red blood cell

VEGF = vascular endothelial growth factor

plaque burden may not consistently produce obstructive disease, because more often the outward and expansive vascular remodeling obviates any compromise in luminal integrity. The developing necrotic cores accumulate lipids not only from circulating lipoproteins but also from the cholesterol-enriched RBC membranes deposited upon intraplaque hemorrhage (4,6).

Plaque hemorrhage is a fairly common event in coronary atherosclerosis. In patients dying from plaque rupture, intraplaque hemorrhage can be observed in up to 5 other sites of the coronary tree (6). In patients dying from stable severe coronary atherosclerosis, plaque hemorrhages are less frequent. The least number of sites of hemorrhage are seen in patients dying of noncoronary causes or plaque erosion. This suggests that lesions with hemorrhage may be prone to disruption if not adequately treated. These hemorrhagic sites also show accompanying increase in neointimal vasa vasorum.

Intraplaque Hemorrhages Contribute to Enlargement of Necrotic Core

A strong association of the extent of intraplaque hemorrhage (identified by glycophorin A staining, a protein exclusive to the erythrocyte membrane) with increasing necrotic core size has been reported in coronary lesions prone to rupture (4). Whether the relationship of intraplaque hemorrhage and lesion instability offered any clinical significance was further explored in case-control magnetic resonance imaging (MRI) study of 29 patients followed over 18 months. Of these 29 patients, 15 demonstrated MRI evidence of intraplaque hemorrhage in carotid plaques at baseline; the remaining 15 patients with comparably sized plaques did not reveal intraplaque hemorrhage (5). The percent change in plaque volume (6.8% vs. −0.15%; $p = 0.009$) and lipid-rich necrotic core volume (28.4% vs. −5.2%; $p = 0.001$) was significantly higher in the hemorrhage group than in the nonhemorrhage plaques. Further, the patients with intraplaque hemorrhage at baseline were more likely to have new plaque hemorrhage (43% vs. 0%; $p = 0.006$). A follow-up study tested whether plaque characteristics studied by MRI were possible predictors of future ipsilateral cerebrovascular events. In that prospective study design, serial carotid MRI scans were performed every 18 months in 154 asymptomatic patients who had ultrasonically verified 50% to 79% carotid stenosis (8). During a mean follow-up of 3 years, 12 carotid cerebrovascular events occurred, which correlated with MRI characteristics of thin or ruptured fibrous caps (hazard ratio [HR] 17.0; $p \leq 0.001$), intraplaque hemorrhage (HR 5.2; $p = 0.005$), large hemorrhagic areas (HR for every 10 mm^2 increase 2.6; $p = 0.006$), large necrotic cores (HR for every 10% increase 1.6; $p = 0.004$), and large plaque dimensions (HR for every 1-mm increase 1.6; $p = 0.008$). Although not confirmatory, these clinical MRI studies suggested that hemorrhage into the carotid atherosclerotic lesion increases plaque volume and necrotic core size, important hallmarks of plaque vulnerability to rupture.

Evidence of RBC-Derived Cholesterol Accumulation

Experimental studies of simulated hemorrhage in at least the skin (9) and brain (10) suggest complete resolution of the lesion by 7 days to 14 days, depending on the extent of hemorrhage. The increase in erythrophagocytosis by macrophages leads to the eventual development of foam cells (11), which in most tissues outside the coronary vasculature, eventually disappear with no pathologic consequence. One exception, however, involves the maxillary sinus, where increased intrasinus pressure due to drainage obstruction may affect venous and lymphatic drainage outflow and lead to venular microhemorrhages; continued arterial flow into the sinus mucosa further contributes to relatively large localized hemorrhages (12). In that circumstance, the lymphatic drainage may be insufficient to completely remove the lipid components of the RBC, and cholesterol crystals precipitate. Hemosiderin-laden macrophages and occasional multinucleated foreign-body giant cells surround the cholesterol clefts in maxillary sinus.

In contrast to organs, the removal of the lipid components of RBC membranes in atherosclerotic plaques may parallel that of the maxillary sinus, because phagocytic clearance of dead macrophages in advanced lesions is inherently defective (13–15). It is presumed that the rapid influx of macrophages in response to the hemorrhage itself would lead to postapoptotic macrophage necrosis and enhanced inflammation in atherosclerotic lesions (16). The cumulative effect of a late lesional hemorrhagic event leads to the generous expansion of the necrotic core, which together with the proinflammatory response of surviving macrophages promotes further inflammation, plaque instability, and thrombosis.

As proof of principle, we developed an animal model simulating intraplaque hemorrhage to assess the role of RBC-derived cholesterol in lesion progression (4). The direct injection of 25 μl to 50 μl packed RBCs into quiescent aortic atherosclerotic plaques produced excessive macrophage infiltration along with free cholesterol crystals and hemosiderin-laden macrophages. In contrast, control (noninjected) lesions showed the characteristics of a regressed lesion with far fewer lesional macrophages and no free cholesterol. Neutral lipids identified by oil red O were also significantly greater in injected plaques.

The pathologic response to the extravasation of blood involves a cellular reaction in tissues adjacent to where monocyte/macrophages are strongly drawn to the lesion. The signals for this perifocal migration of inflammatory cells are not fully understood, but proteins in the coagulated blood likely contribute to cellular activation (17,18). Alter-

natively, the migration of macrophages may be promoted by multispecific receptors on erythrocyte membranes, which can bind a wide array of chemokines in the blood, including monocyte chemotactic peptide 1 (19). Further, lipid oxidation products from senescent RBCs or iron-catalyzed reactions may liberate potent chemoattractants (20). Nonetheless, the influx of macrophages is the main cellular component in response to hemorrhage in tissues, highlighting the importance of inflammation in the eventual resorption of the lesion.

Plaque Hemorrhage Is Associated With Neointimal Neovascularization and Vasa Vasorum Proliferation

A major source of micro- and macrohemorrhages within atherosclerotic lesions is a network of immature blood vessels that develop within the intima of a plaque. Most of neointimal vessels are endothelialized but rarely possess mural pericytes or smooth muscle cells. The lack of mural cells and poorly formed endothelial cell junctions likely contribute to the leakiness of neovasculature (21,22). Postmortem studies confirm that intraplaque hemorrhage and plaque rupture are proportional to an increase in neovascular density (4,23). Vessel density is increased twofold in vulnerable plaques and fourfold in disrupted plaques compared with severely obstructive stable lesions (6). The newly formed vessels closer to the media are relatively more mature compared with the vessels closer to the lumen (Fig. 1) (22). In contrast, vasa vasorum close to lumen structurally only contain a single endothelial lining and demonstrate diffuse perivascular expression of von Willebrand factor. These vessels are associated with remnants of RBC (identified by

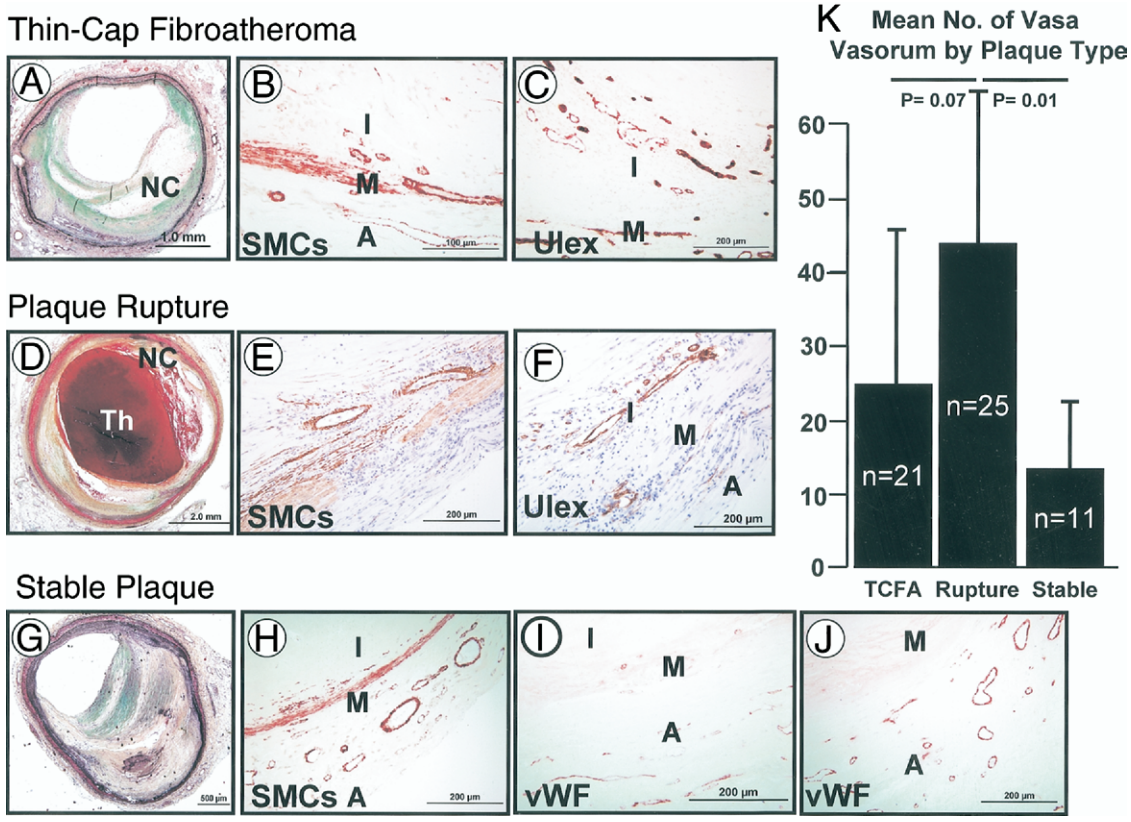

Figure 1 **Neovascularization in Stable and Unstable Coronary Plaques**

Unstable atherosclerotic plaques thin-cap fibroatheroma **(A to C)** and rupture **(D to F)** are associated with marked neoangiogenesis. The microvessels close to the adventitial and medial layers **(B and E)** tend to be in contact with surrounding smooth muscle cells compared with intimal vessels closer to the lumen, which are characterized by a single lining of luminal endothelium **(C and F)**. The main pathologic feature of the vulnerable plaque is an intact thin fibrous cap heavily infiltrated by macrophages **(A)**. In plaque rupture **(D)**, the fibrous cap is disrupted with a superimposed luminal thrombus. The adventitial vessels in unstable plaques often show perivascular smooth muscle cells **(B and E)**. In contrast, the vasa vasorum close to the necrotic core are abnormal, consisting mostly of endothelial cells overlying a disrupted "leaky" basement membrane. **(G to J)** Stable plaques, on the other hand, although severely narrowed, contain mostly collagen, proteoglycans, and calcium, and show fewer vasa vasorum in the intima, media, and adventitia. **(K)** Bar graph showing the mean number of vasa vasorum for stable and unstable plaques. The values within the bars represent the number of lesions examined. Note that unstable thin-cap fibroatheromas (TCFA) and ruptures show significantly greater densities of microvessels compared with stable plaques. Endothelial markers: *Ulex europeaus* (Ulex) and anti-von Willebrand factor (vWF) antibody immunohistochemical staining; smooth muscle cell (SMC) marker: α-actin. **(A, D, and G)** Movat pentachrome staining. Modified from Virmani et al. (6). A = adventitia; I = intima; M = media; NC = necrotic core; Th = thrombus.

glycophorin A and hemosiderin) and are typically seen in close vicinity to the necrotic core. The micro- and macrohemorrhages deposit variable amounts of RBC-derived free cholesterol (4). In the stable plaques, there is lower density of leaky microvessels, absence of intraplaque hemorrhage, smaller necrotic cores, and accordingly smaller amount of free crystalizable cholesterol (Fig. 1).

Plaque Neovascularization and Vasa Vasorum Proliferation Are Associated With Hypercholesterolemia and Inflammation

The vasa vasorum normally penetrate into the vessel wall at regular intervals and then bifurcate circumferentially around the vessel. In contrast, in hypercholesterolemia a dense network of vasa vasorum forms within the adventitia (24), which is also associated with an extensive growth of neointimal microvessels. Neovascularization correlates with the extent of atherosclerosis and is driven, at least partly, by intimal hypoxia. On the other hand, the extent of vasa vasorum decreases significantly with lesion regression and upon antiangiogenic intervention.

Hypercholesterolemia may trigger the up-regulation of growth factor receptors on endothelial and smooth muscle cells, facilitating the development of neointimal angiogenesis. Hypercholesterolemia is associated with inflammation, which is also an important stimulus for angiogenesis (25); interventions for containment of inflammation attenuate microvessel formation (26). The reduction in hypercholesterolemia and inflammatory state are equally important in clinical studies evaluating the regression of atherosclerosis (27), and reduction in both factors was found to be most effective.

Neointimal Neovascularization, Immature Vessel Leakiness, and Intraplaque Hemorrhage

Immature microvessels in developing atheroma are poorly supported by pericytes and comprise a potential source of large collections of extravasated RBC, contributing to intraplaque hemorrhage. The adventitial vasa vasorum demonstrate more complete maturation, and their newer branches traverse the medial layer into the deep intima, where they become more fragile and leaky (28). The neovascular leakiness in plaques is most probably due to inherent structural defects within vessels, proteolytic damage from ongoing inflammation, the release of selective signaling molecules capable of disrupting endothelial cell-to-cell contact, and/or mechanical forces. The inherent structural defect is exemplified in experimental murine tumors where defective cellular linings composed of disorganized, loosely connected, branched, overlapping, or sprouting endothelial cells have been identified in areas of hemorrhage (29). In atherosclerosis, leaky intraplaque vasa vasorum have been characterized by ultrastructural visualization of defects between endothelial junctions (22) or the identification of perivascular von Willebrand factor (vWf) immunoreactivity around vessels (4,21,30) as a sign of a less intact or leaky endothelium. Factors that induce vessel growth also influence leakiness, and the principal regulator of vascular permeability in pathologic angiogenesis is VEGF and its related receptors and cofactors. The VEGF signaling pathway involves an interaction between growth factor receptors and certin integrins (31). The VEGF receptors (VEGFR) may play a critical role in cell-to-cell adhesion and communication through their direct interaction with cell-to-cell adhesion molecules. In endothelial cells, VEGFR2 associates with vascular endothelial (VE) cadherin and provides regulation of junctional integrity and represents one of the potential targets for local therapy.

The integrity of endothelial cell-to-cell junctions and vascular barrier functions is regulated by a series of adhesion molecules that make up tight, gap, and adherens junctions (32). These gaps are large enough for the extravasation of RBC and probably result from disruption of some or all of the junctional proteins. The VEGF blocks gap junctional communication between adjacent endothelial cells by altering connexin-43 phosphorylation via VEGFR2 and Src kinase activation. The VEGF also disrupts tight junctional communication by altering phosphorylation of zonula occludens 1 (ZO-1) and occludens (33) through an Src-dependent pathway (34). Several adherens junction proteins (VE-cadherin, β-catenin, γ-catenin/plakoglobin, and p120-catenin) become tyrosine phosphorylated downstream of VEGFR2 after VEGF stimulation, leading to the loosening of endothelial cell-to-cell contact in vitro. Other signaling pathways initiated by VEGF/VEGFR2, such as eNOS, PLC, or protein kinase C, may also allow vascular leakage, and their inhibitors block VEGF-induced permeability (34). In addition, a sustained VEGF-induced permeability response is mediated by activation of the urokinase plasminogen activator and receptor system (34). Together with VEGF, the angiopoietins (Ang) and their receptors tyrosine kinase 1 and 2 (Tie-1 and -2) also play a key role in the maintenance and control of vascular leakage (35). Whereas Ang-1 via Tie-2 is involved in maintaining the integrity of the endothelium, Ang-2 suppresses the effects of angiopoietin-1, resulting in vessel destabilization.

Eliminating Angiogenesis for Plaque Stabilization

Based on the principle that RBC leak is the major determinant of plaque vulnerability, the targeted inhibition of plaque angiogenesis may constitute a valuable therapeutic approach toward plaque stabilization (36). Although the greater part of angiogenesis research is historically linked to cancer biology, emerging evidence suggests that similar molecules are involved in nonneoplastic disorders, including chronic inflammatory and ophthalmic diseases (37). Ocular diseases such as neovascular age-related macular degeneration (choroidal bleeds) are characterized by aberrant angiogenesis accompanied by catastrophic effects caused by hem-

orrhage and increased vascular permeability. Vitreous levels of VEGF have been shown in humans to rise simultaneously with growth and leakage of new vessels (38,39). Intimal vasa vasorum within the coronary artery may share a common pathogenesis with diseases such as macular degeneration, because there is abnormal and haphazard growth of microvessels which are leaky and hemorrhagic. Conceptually, the selective targeting of neoangiogenesis poses a viable approach for the elimination of pre-existing and new growth of microvessels, because the selective removal of angiogenic stimuli has been shown to cause vessels to regress, as demonstrated in tumors (40) and heart (41), in particular when vessels have only recently been assembled and are still immature.

The vast array of angiogenic inducers, including growth factors, chemokines, angiogenic enzymes, endothelial specific receptors, and adhesion molecules, presents a wide range of potential therapeutic targets for the elimination of pathologic neovascularization. The expanding list of candidate drugs contains agents primarily targeted against endothelial growth factors (VEGF and downstream mediators) and its receptors (Fig. 2). Endogenous inhibitors of angiogenesis include cleavage products of matrix components such as arresten, canstatin, and tumstatin from collagen IV, vatstatin from collagen VIII, restin from collagen XV, and endostatin from collagen XVIII (25). Other compounds include synthetic inhibitors of cell invasion (marimastat, neovastat, AG-3340), adhesion (Vitaxin), and proliferation (TNP-470, thalidomide, combretastatin A-4) and compounds that interfere with angiogenic growth factors (interferon-alpha, suramin, and analogues) or their receptors (SU6668, SU5416) (42). For a review of potential inhibitors of angiogenesis tested in experimental plaques please see Moulton (43).

Besides lipid lowering, statins possess reported pleotropic effects in humans either promoting or inhibiting angiogenesis in some chronic diseases. This dual role is complex and related to the organ site, cell type, disease process, and possibly statin concentration. Randomized control trials for cardiovascular disease show that statins produce an unex-

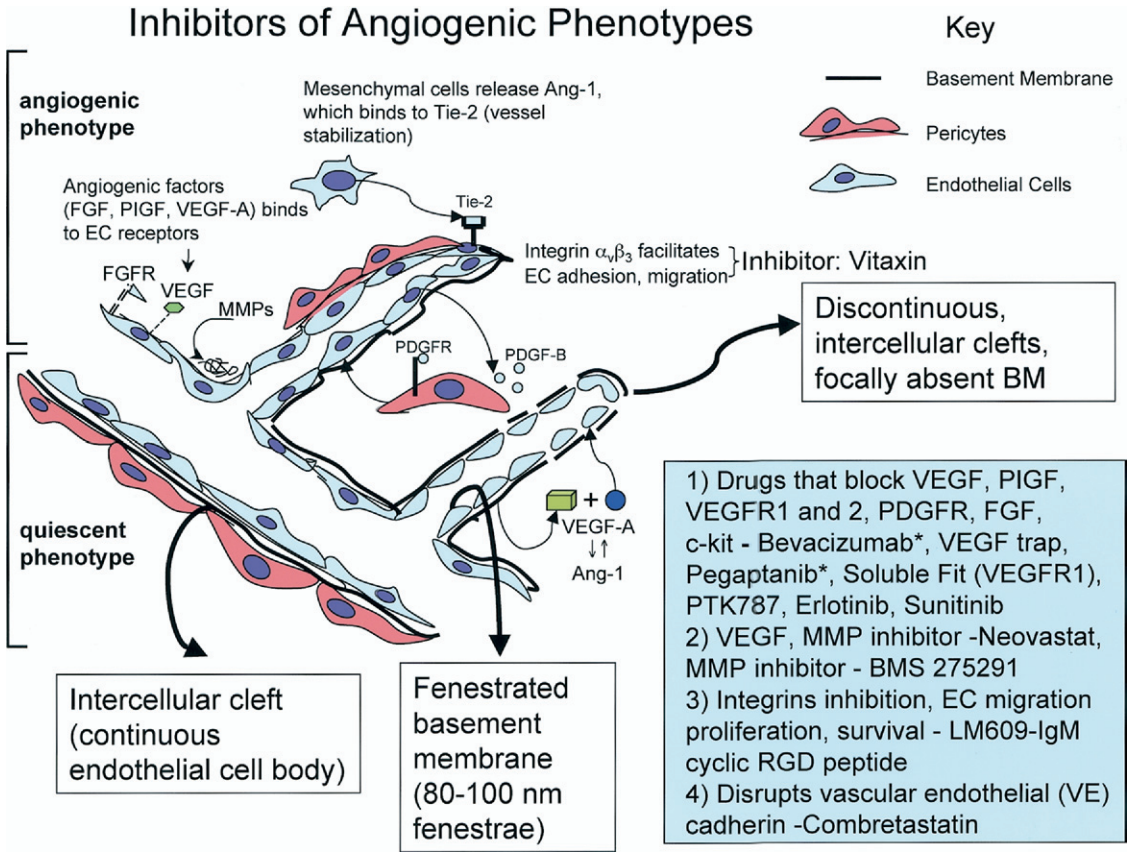

Figure 2 **Diagram Illustrating Molecules Critical to Angiogenesis and Potential Therapeutic Targets**

The scheme highlights the ability of growth factors to positively regulate the "angiogenic switch" to stimulate new vessel formation. Vessel stabilization is mediated by a coordinated response involving platelet-derived growth factor and receptor (PDGR/PDGFR) and angiopoietin (Ang) 1. Modified from Moulton et al. (43). BM = basement membrane; EC = endothelial cell; FGF = fibroblast growth factor; FGFR = fibroblast growth factor receptor; Ig = immunoglobulin; MMP = matrix metalloproteinase; PIGF = placenta growth factor; RGD = arginine-glycine-aspartic acid; Tie-2 = tunica internal endothelial cell kinase; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor.

pected benefit for reducing colorectal carcinoma and melanoma (44). It has been suggested that statins exert a biphasic dose-dependent effect on angiogenesis, at low doses inducing angiogenesis and at high doses inhibiting angiogenesis (45). Animal models with combined ischemia and tumors indicate that the dose may not be central to the antiangiogenic effect, because there was augmented blood flow to hind-limb ischemic tissue with a marked reduction in tumor growth (46).

Because neovascularization is the major response to hypoxia, the mere existence of intraplaque vessels strongly supports the notion of hypoxic conditions in human atherosclerosis. The reaction to the stress of hypoxia is mediated in large part by the hypoxia-inducible factor 1 (HIF-1), a transcription factor that drives the expression of genes controlling cell survival and governs the formation of new blood vessels. Hypoxia-inducible factor 1 consists of a constitutively expressed subunit HIF-1β and an oxygen-related subunit HIF-1α (or its paralogs HIF-2α and -3α). The existence of HIF-1α in human coronary plaques has been described (47). The regulation of HIF-1α is complex, with multiple pathways involving transcription, translation, post-translational modification, and interaction with other transcription factors involved. Although selective molecules critical to HIF-1 signaling have been identified, only a few examples of HIF inhibitors that potentially target selective pathways associated with HIF activation have been described, among which include echinomycin and synthetic polyamides, which inhibit HIF-1 DNA binding, and chetomin, which blocks recruitment of coactivator p300/CBP (48). The potential targeting of HIF-1 for eliminating plaque angiogenesis remains to be explored.

Whether compounds known to be effective in tumors would be beneficial for eliminating plaque angiogenesis depends on shared coordinated pathways in vessel growth and maturation in different vascular beds. This is not always the case: One example highlighting fundamental differences in vascular growth responses in different tissues involves the effect of Ang-1, which stimulates angiogenesis in the skin but suppresses vascular growth in the heart (25). On the other hand, the identification of organ-specific targets would potentially improve the safety and perhaps efficacy of angiogenic therapies, because the drugs could be delivered with a degree of specificity to target sites based on the expression of selective molecules unique to different vascular beds. In this respect, patients with malignancies currently treated with antiangiogenic drugs may provide an opportunity to assess whether those agents impact cardiovascular disease processes, thus expanding the current knowledge base of how different organ systems respond to these drugs.

The molecular and cellular elements of the plaque microenvironment that dictate the growth and development of new vessels are not well characterized. It is becoming apparent, however, that intraplaque vasa vasorum exists in various stages of vessel maturation, likely driven by extracellular matrix molecules and surrounding cells by the release of various cytokines and growth factors. The degree of vessel maturation may impact the resistance or responsiveness to antiangiogenic therapy. For example, inhibitors of angiogenesis directed against cell proliferation will likely be ineffective in quiescent endothelial cells. The concept of selective molecular targeting for the removal of vessels, as proposed for other organ systems (49), may circumvent injury to luminal endothelium of large vessels, minimizing the risk of thrombosis. Therefore, optimized therapy for eliminating plaque angiogenesis will produce desirable effects on pathologic neovascularization without influencing normal quiescent vessels. Molecular mapping studies, such as that provided by Hiltunen et al. (50), would further identify candidate genes/proteins for selective antiangiogenic therapy targeted against the atherosclerotic plaque. As basic knowledge increases about the control of angiogenesis and its role in plaque development and instability, it may be possible in the future to develop specific antiangiogenic agents that offer a potential therapy for cancer and angiogenic diseases.

Potential Imaging of Neovascularization for Localized Therapy

The development of antiangiogenic drugs for treating noncancerous diseases such as atherosclerosis will require improved monitoring of lesion vascularity to adequately assess treatment benefits. Although contrast-based intravascular ultrasound technique is being perfected for the assessment of vasa vasorum in the plaques (51,52), molecular imaging strategies such as $\alpha_v\beta_3$ molecular targeting agents (radiolabeled arginine-glycine-aspartic acid peptides) (53) or direct imaging of intraplaque hemorrhage using MRI (5,8) or annexin-A5 (54) may become useful tools for the identification of plaque vascularity. A recent study demonstrated the ability of $\alpha_v\beta_3$ integrin-targeted paramagnetic nanoparticles to deliver the potent antiangiogenic drug fumagillin to established plaques, thus achieving site-specific delivery of the drug to plaque-associated neovascularization at a fraction of levels used for systemic treatments (55). Further, the same strategy using paramagnetic nanoparticles without drug provides an integrated noninvasive monitoring of plaque microvascular density as a surrogate marker of atherosclerosis. That study highlights the potential of a combined method of drug delivery and imaging, which could serve as a unique tool for noninvasive characterization, drug delivery, and monitoring in atherosclerosis.

Is There a Role for Local Antiangiogenic Therapy?

Whether targeted therapy for vascular regression in other organ systems can be directly applied to atherosclerosis is debatable, because several lines of evidence suggest that neovascularization differs depending on its location within the body and the underlying disease process. Moreover, important criteria regarding the target lesion, drug delivery

platform, and timing of therapy must be established before a strategy to treat angiogenesis in plaques can go forward. The identification of target lesions is a critical issue, because current technologies have yet to achieve the goal of characterizing plaque morphology to the degree necessary to correctly identify rupture-prone lesions according to pathologic criteria. Given these limitations, patients who may benefit from antiangiogenic agents are those who are at high risk for recurrent cardiac events, because these individuals possess plaques whose near-term risk of causing acute coronary syndromes is high. Further, the majority of thin-cap fibroatheromas exist in the proximal and middle left anterior descending artery, particularly at branch points (56), and the somewhat restricted existence of these lesions is no coincidence, because flow-related shear stress likely plays a critical role in lesion burden and vulnerability (57). Finally, as shown in earlier experimental studies, angiogenic inhibitors will likely be more effective in advanced plaques, where plaque neovascularization is more prevalent (36).

The focal and rather predictable nature of vulnerable plaques raises the issue of whether appropriate measures of plaque stabilization could involve localized therapy. Local delivery of antiangiogenic agents has met with recent clinical success in the treatment of age-related macular degeneration (58). The localized delivery of antiangiogenic agents may limit potential adverse effects of the drug and expand the selection of drugs that given systemically would be toxic, let alone reduce the amount and cost of the drug. One could envision that either stent- or catheter-based therapy may be an effective means of delivering drugs to eliminate neovascularization in the clinical setting. Indeed, as shown in a recent feasibility study, stent-based delivery of antiangiogenic agents, such as with the VEGF-specific antibody (bevacizumab), may be a viable treatment option (59).

The efficacy of the drug will inferentially dictate whether it needs to be given as a single dose or repeatedly. Conventional wisdom favors a repeated dosing, because VEGF antagonists are typically given systemically for long periods to inhibit tumor angiogenesis (60,61). In the case of plaque angiogenesis it may be potentially convenient to obtain controlled-release delivery locally through stents. Moreover,

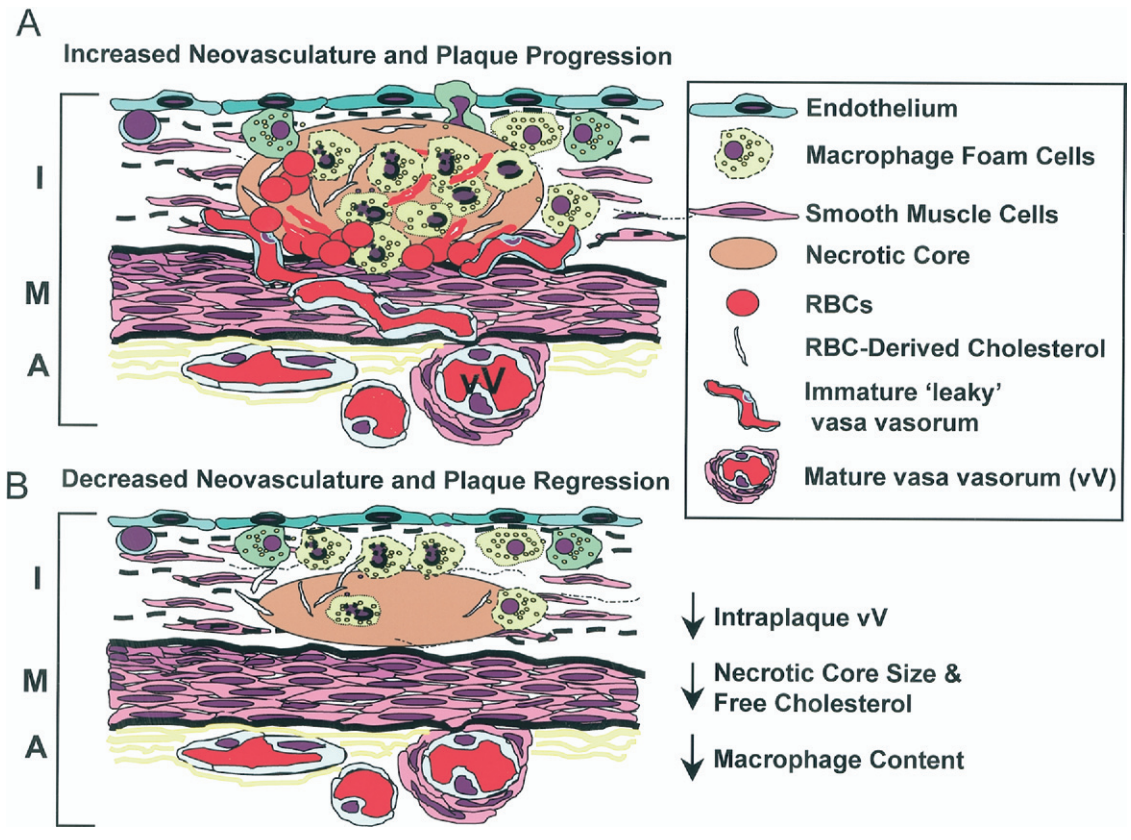

Figure 3 **Diagram Illustrating the Potential Pathologic Outcome After Neovascular Regression of Developing Human Coronary Atheroma**

(A) In advanced unstable atheroma, intraplaque vasa vasorum leak, allowing red blood cells (RBCs) to spill into the surrounding microenvironment. Red blood cell membrane-derived cholesterol and associated macrophage infiltration in response to hemorrhage contribute to necrotic expansion. **(B)** Targeted therapy to eliminate intraplaque microvasculature should reduce plaque burden and necrotic core size by eliminating RBC membrane-derived cholesterol and secondary macrophage response. The alterations in lesion substrate should favor plaque stabilization with a reduction of plaque size. A= adventitia; I= intima; M= media.

similarly to cancer therapy, a combined therapeutic approach in plaques inhibiting multiple pathways of angiogenesis will more likely be effective.

Conclusions

It is proposed that molecular therapies designed to eliminate pathologic neovascularization within atherosclerotic lesions will interrupt the process of intraplaque hemorrhage, a critical event leading to plaque instability (Fig. 3). The removal of intraplaque vessels should alleviate the risk of rupture by slowing the rate of necrotic core expansion through eliminating the accumulation of RBC-derived cholesterol and associated macrophage response. The selective elimination of intraplaque neovascularization will likely involve the targeting of pre-existing and new vessel development and expansion. Although this treatment strategy is in its infancy, the concept of vascular regression has met some success in other neovascular-dependent diseases, such as cancer and age-related macular degeneration. The success of this novel approach is dependent on gaining critical knowledge of the environment required for supported development and maturation of the vasa vasorum within plaques. A multitargeted approach involving selective antiangiogenic agents, along with improved imaging methods for visualizing plaque neovascularization to monitor treatment effects, will likely be required to prevent plaque progression and its clinical consequences.

Atherosclerosis is a systemic disease with multifocal discrete vascular lesions; diffuse vascular involvement, however, is relatively rare. It is therefore obvious that local therapy would play only a supplemental role to systemic intervention. For such a strategy to become successful it would be necessary that noninvasive and invasive imaging strategies are perfected to precisely identify imminently unstable plaques. This approach will be mandatory, because de novo institution of statin therapy has a significant lag time to become efficacious at the vessel wall level.

Reprint requests and correspondence: Dr. Renu Virmani, Director, CVPath Institute, 19 Firstfield Road, Gaithersburg, Maryland 20878. E-mail: rvirmani@cvpath.org.

REFERENCES

1. Burke AP, Farb A, Malcom GT, Liang YH, Smialek J, Virmani R. Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 1997;336:1276–82.
2. Milei J, Parodi JC, Alonso GF, Barone A, Grana D, Matturri L. Carotid rupture and intraplaque hemorrhage: immunophenotype and role of cells involved. Am Heart J 1998;136:1096–105.
3. Redgrave JN, Lovett JK, Gallagher PJ, Rothwell PM. Histological assessment of 526 symptomatic carotid plaques in relation to the nature and timing of ischemic symptoms: the Oxford Plaque Study. Circulation 2006;113:2320–8.
4. Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349:2316–25.
5. Takaya N, Yuan C, Chu B, et al. Presence of intraplaque hemorrhage stimulates progression of carotid atherosclerotic plaques: a high-resolution magnetic resonance imaging study. Circulation 2005;111: 2768–75.
6. Virmani R, Kolodgie FD, Burke AP, et al. Atherosclerotic plaque progression and vulnerability to rupture: angiogenesis as a source of intraplaque hemorrhage. Arterioscler Thromb Vasc Biol 2005;25: 2054–61.
7. Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2000;20:1262–75.
8. Takaya N, Yuan C, Chu B, et al. Association between carotid plaque characteristics and subsequent ischemic cerebrovascular events: a prospective assessment with MRI—initial results. Stroke 2006;37: 818–23.
9. Lalonde JM, Ghadially FN. Ultrastructure of experimentally produced subcutaneous haematomas in the rabbit. Virchows Arch B Cell Pathol 1977;25:221–32.
10. Koeppen AH, Dickson AC, McEvoy JA. The cellular reactions to experimental intracerebral hemorrhage. J Neurol Sci 1995;134 Suppl: 102–12.
11. Ishihara T, Sano J, Yamanami S, et al. Foamy cells associated with phagocytosis of glutaraldehyde-treated red blood cells and red cell membranes. Acta Pathol Jpn 1987;37:627–37.
12. Leon ME, Chavez C, Fyfe B, Nagorsky MJ, Garcia FU. Cholesterol granuloma of the maxillary sinus. Arch Pathol Lab Med 2002;126: 217–9.
13. Hegyi L, Skepper JN, Cary NR, Mitchinson MJ. Foam cell apoptosis and the development of the lipid core of human atherosclerosis. J Pathol 1996;180:423–9.
14. Kockx MM, Herman AG. Apoptosis in atherogenesis: implications for plaque destabilization. Eur Heart J 1998;19 Suppl G:G23–8.
15. Steinberg D, Lewis A. Conner Memorial Lecture. Oxidative modification of LDL and atherogenesis. Circulation 1997;95:1062–71.
16. Tabas I. Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency. Arterioscler Thromb Vasc Biol 2005;25: 2255–64.
17. Davis GE. The Mac-1 and p150,95 beta 2 integrins bind denatured proteins to mediate leukocyte cell-substrate adhesion. Exp Cell Res 1992;200:242–52.
18. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. Cell 1992;69:11–25.
19. Darbonne WC, Rice GC, Mohler MA, et al. Red blood cells are a sink for interleukin 8, a leukocyte chemotaxin. J Clin Invest 1991;88: 1362–9.
20. Neote K, Darbonne W, Ogez J, Horuk R, Schall TJ. Identification of a promiscuous inflammatory peptide receptor on the surface of red blood cells. J Biol Chem 1993;268:12247–9.
21. Jeziorska M, Woolley DE. Local neovascularization and cellular composition within vulnerable regions of atherosclerotic plaques of human carotid arteries. J Pathol 1999;188:189–96.
22. Virmani R, Narula J, Farb A. When neoangiogenesis ricochets. Am Heart J 1998;136:937–9.
23. Burke AP, Farb A, Malcom GT, Liang Y, Smialek JE, Virmani R. Plaque rupture and sudden death related to exertion in men with coronary artery disease. JAMA 1999;281:921–6.
24. Kwon HM, Sangiorgi G, Ritman EL, et al. Enhanced coronary vasa vasorum neovascularization in experimental hypercholesterolemia. J Clin Invest 1998;101:1551–6.
25. Carmeliet P. Angiogenesis in health and disease. Nat Med 2003;9: 653–60.
26. Wilson SH, Herrmann J, Lerman LO, et al. Simvastatin preserves the structure of coronary adventitial vasa vasorum in experimental hypercholesterolemia independent of lipid lowering. Circulation 2002;105: 415–8.
27. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 2005;352:29–38.
28. Takaba M, Endo S, Kurimoto M, Kuwayama N, Nishijima M, Takaku A. Vasa vasorum of the intracranial arteries. Acta Neurochir (Wien) 1998;140:411–6.
29. Hashizume H, Baluk P, Morikawa S, et al. Openings between defective endothelial cells explain tumor vessel leakiness. Am J Pathol 2000;156:1363–80.

30. Kockx MM, Cromheeke KM, Knaapen MW, et al. Phagocytosis and macrophage activation associated with hemorrhagic microvessels in human atherosclerosis. Arterioscler Thromb Vasc Biol 2003;23: 440–6.
31. Weis SM, Cheresh DA. Pathophysiological consequences of VEGF-induced vascular permeability. Nature 2005;437:497–504.
32. Suarez S, Ballmer-Hofer K. VEGF transiently disrupts gap junctional communication in endothelial cells. J Cell Sci 2001;114:1229–35.
33. Antonetti DA, Barber AJ, Hollinger LA, Wolpert EB, Gardner TW. Vascular endothelial growth factor induces rapid phosphorylation of tight junction proteins occludin and zonula occluden 1. A potential mechanism for vascular permeability in diabetic retinopathy and tumors. J Biol Chem 1999;274:23463–7.
34. Pedram A, Razandi M, Aitkenhead M, Hughes CC, Levin ER. Integration of the nongenomic and genomic actions of estrogen. Membrane-initiated signaling by steroid to transcription and cell biology. J Biol Chem 2002;277:50768–75.
35. Brindle NP, Saharinen P, Alitalo K. Signaling and functions of angiopoietin-1 in vascular protection. Circ Res 2006;98:1014–23.
36. Moulton KS, Heller E, Konerding MA, Flynn E, Palinski W, Folkman J. Angiogenesis inhibitors endostatin or TNP-470 reduce intimal neovascularization and plaque growth in apolipoprotein E-deficient mice. Circulation 1999;99:1726–32.
37. Folkman J. Angiogenesis. Annu Rev Med 2006;57:1–18.
38. Duh EJ, Yang HS, Haller JA, et al. Vitreous levels of pigment epithelium-derived factor and vascular endothelial growth factor: implications for ocular angiogenesis. Am J Ophthalmol 2004;137: 668–74.
39. Gariano RF, Gardner TW. Retinal angiogenesis in development and disease. Nature 2005;438:960–6.
40. Benjamin LE, Golijanin D, Itin A, Pode D, Keshet E. Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest 1999;103:159–65.
41. Dor Y, Djonov V, Abramovitch R, et al. Conditional switching of VEGF provides new insights into adult neovascularization and pro-angiogenic therapy. EMBO J 2002;21:1939–47.
42. Liekens S, De Clercq E, Neyts J. Angiogenesis: regulators and clinical applications. Biochem Pharmacol 2001;61:253–70.
43. Moulton KS. Angiogenesis in atherosclerosis: gathering evidence beyond speculation. Curr Opin Lipidol 2006;17:548–55.
44. Demierre MF, Higgins PD, Gruber SB, Hawk E, Lippman SM. Statins and cancer prevention. Nat Rev Cancer 2005;5:930–42.
45. Ii M, Losordo DW. Statins and the endothelium. Vascul Pharmacol 2007;46:1–9.
46. Sata M, Nishimatsu H, Osuga J, et al. Statins augment collateral growth in response to ischemia but they do not promote cancer and atherosclerosis. Hypertension 2004;43:1214–20.
47. Chen F, Eriksson P, Kimura T, Herzfeld I, Valen G. Apoptosis and angiogenesis are induced in the unstable coronary atherosclerotic plaque. Coron Artery Dis 2005;16:191–7.
48. Melillo G. Inhibiting hypoxia-inducible factor 1 for cancer therapy. Mol Cancer Res 2006;4:601–5.
49. Campochiaro PA. Ocular versus extraocular neovascularization: mirror images or vague resemblances. Invest Ophthalmol Vis Sci 2006;47: 462–74.
50. Hiltunen MO, Tuomisto TT, Niemi M, et al. Changes in gene expression in atherosclerotic plaques analyzed using DNA array. Atherosclerosis 2002;165:23–32.
51. Carlier S, Kakadiaris IA, Dib N, et al. Vasa vasorum imaging: a new window to the clinical detection of vulnerable atherosclerotic plaques. Curr Atheroscler Rep 2005;7:164–9.
52. Goertz DE, Frijlink ME, de Jong N, van der Steen AF. Nonlinear intravascular ultrasound contrast imaging. Ultrasound Med Biol 2006; 32:491–502.
53. Haubner R. Alpha(v)beta(3)-integrin imaging: a new approach to characterise angiogenesis? Eur J Nucl Med Mol Imaging 2006;33 Suppl 13:54–63.
54. Kietselaer BL, Reutelingsperger CP, Heidendal GA, et al. Noninvasive detection of plaque instability with use of radiolabeled annexin A5 in patients with carotid-artery atherosclerosis. N Engl J Med 2004; 350:1472–3.
55. Winter PM, Neubauer AM, Caruthers SD, et al. Endothelial alpha(v) beta3 integrin-targeted fumagillin nanoparticles inhibit angiogenesis in atherosclerosis. Arterioscler Thromb Vasc Biol 2006;26:2103–9.
56. Kolodgie FD, Burke AP, Farb A, et al. The thin-cap fibroatheroma: a type of vulnerable plaque: the major precursor lesion to acute coronary syndromes. Curr Opin Cardiol 2001;16:285–92.
57. Cheng C, Tempel D, van Haperen R, et al. Atherosclerotic lesion size and vulnerability are determined by patterns of fluid shear stress. Circulation 2006;113:2744–53.
58. Steinbrook R. The price of sight—ranibizumab, bevacizumab, and the treatment of macular degeneration. N Engl J Med 2006;355:1409–12.
59. Stefanadis C, Toutouzas K, Stefanadi E, Kolodgie F, Virmani R, Kipshidze N. First experimental application of bevacizumab-eluting PC coated stent for inhibition of vasa vasorum of atherosclerotic plaque: angiographic results in a rabbit atheromatic model. Hellenic J Cardiol 2006;47:7–10.
60. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.
61. Yang JC, Haworth L, Sherry RM, et al. A randomized trial of bevacizumab, an antivascular endothelial growth factor antibody, for metastatic renal cancer. N Engl J Med 2003;349:427–34.