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Will the Real Plaque Vasculature Please Stand Up? Why We Need to Distinguish the Vasa Plaquorum From the Vasa Vasorum

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Abstract

Many studies of experimental atherosclerosis and pathologic observations of human specimens have provided evidence supporting a correlation between vascularization of the atherosclerotic plaque and its natural growth and progression toward acute failure, associated with clinical events. The growing interest in the topic is illustrated by several excellent recent reviews discussing the molecular mechanisms that might play a role in the formation of plaque vasculature and that could explain some of the observed associations with pathologic features of experimental and human atherosclerotic lesions. At the same time, these reviews also emphasize that the field is still largely in uncharted territory. Hoping to spark some new investigations, we are taking this opportunity to question some of the common assumptions and to highlight less explored mechanisms. Finally, we are proposing to adopt the term vasa plaquorum to refer to the neovasculature located within the atherosclerotic plaque to distinguish it clearly from vasa vasorum, the native, supporting vasculature of the artery. We suggest that this new nomenclature offers a potential solution to eliminate ambiguity regarding implicit, but frequently neglected, differences between these structures. We think these points are relevant for future efforts to tailor diagnostic tools and therapeutic interventions targeting plaque neovascularization for the clinical management of atherosclerosis.

• Plaque Neovascularization and the Natural History of Atherosclerosis

Numerous pathologic investigations of human atherosclerotic plaques coupled with clinical observations have suggested an important contribution of plaque neovascularization to the natural progression of atherosclerosis (Kolodgie et al.2003). This relation was tested in several experimental atherosclerosis models, resulting in formulation of two major roles. First, development of neovasculature is likely to enhance the growth and maintenance of atherosclerotic plaques. Many pathologic observations support this relation, including the reported association between lesion thickness and extent of vascularization both in human disease (Kumamoto et al. 1995, Zhang et al. 1993) and in experimental atherosclerosis (Moulton et al. 1999). Second, plaque neovascularization is thought to be the major culprit behind intraplaque hemorrhage (Fryer et al. 1987, McCarthy et al. 1999, Virmani et al.

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2005), an important mode of plaque failure associated with symptomatic atherosclerosis (Fleiner et al. 2004). Neovascularization and inflammation were repeatedly associated with clinical events (Tenaglia et al. 1998), with angiogenesis being an early sign of vulnerability, further supporting its role in the progression toward plaque destabilization (Fleiner et al. 2004). The neovessel density was found to be increased in ruptured plaques and to be an independent risk factor for aortic plaque rupture (Moreno et al. 2004).

Vasa Vasorum or Vasa Plaquorum?

In reviewing the existing literature, we noted that the current nomenclature could be a potential source of confusion and thus a probable barrier to understanding. We propose that the common use of *vasa vasorum* (i.e., vascular structures of the vessel) to refer to the neovasculature found in atherosclerotic plaques creates confusion that is likely to affect our major assumptions and subsequent investigational or interventional actions. Vasa vasorum are a naturally occurring vascular structure that runs along the outer surface of a large artery or a vein (Williams and Heistad 1996), essential to the proper functioning of these vessels (Ritman and Lerman 2007). We thus believe it is important to restrict the use of the term *vasa vasorum* to refer to these physiologic vascular structures to differentiate them from the pathologic neovascularization that develops within the diseased arterial wall, specifically within atherosclerotic plaques. We will refer from now on (even when we cite references that used the old nomenclature) to such neovascular structures as *vasa plaquorum*, that is, the vasculature of the plaque, consistent with their most distinctive attribute: localization within the atherosclerotic plaque. Some recent efforts have attempted to distinguish functionally between the vasa vasorum and the neoangiogenic vasculature of the plaque (Goertz et al. 2007), but these implicit differences might be obscured by the broad use of the *vasa vasorum* nomenclature.

We will present logical and practical arguments demonstrating that this distinction is not merely academic, but enables us to avoid implicit associations that might jeopardize our opportunity to better understand the nature and function of vasa plaquorum and the ensuing opportunity to design appropriate clinical management options.

• Origin of Vasa Plaquorum—Challenges and Opportunities

The first implication of using the term *vasa vasorum* to describe the plaque neovasculature is the assumption that the two are one and the same. Even if the two are indeed related, as suggested by observations supporting the hypothesis that vasa plaquorum originate from vasa vasorum, numerous studies have indicated that the vasculature supporting an artery and that within its plaque differ both in anatomic structure and in their response to a variety of stimuli (Ritman and Lerman 2007). Clearly establishing a distinction between the two will provide an opportunity to specifically target potential interventions to vasa plaquorum, while sparing vasa vasorum.

The two accepted origins of vasa plaquorum, as a sprouting growth of vasa vasorum or as an ingrowth of the luminal endothelium, were based on earlier interpretations of plaque specimens and casts (Zamir and Silver 1985). These were more recently supported by observations using functional perfusion imaging modalities such as micro-computed

tomography (microCT) (Ritman and Lerman 2007) or fluorescence microangiography (Figure 1), illustrated in an experimental model of lesion neoangiogenesis (Khatri et al. 2004). The most commonly accepted origin of vasa plaquorum is the sprouting of the adventitial vasa vasorum penetrating into the atherosclerotic arterial wall toward the plaque (Figure 2) in response to angiogenic factors upregulated by hypoxia within the lesion. Vasa vasorum were shown to expand significantly during the early phases of experimental atherosclerosis in various animal models (Kwon et al. 1998, Moulton et al. 2003), providing indirect support for a role in the development of plaque neovascularization, as it is conceivable that vasa vasorum growth reflects an increased need for vascularization of the developing atherosclerotic lesion. Extent of vasa vasorum was also recently shown to correlate with the intima-media thickness of human carotid lesions (Magnoni et al. 2009). However, a direct connection between vasa vasorum growth and extent of vasa plaquorum development was not demonstrated in these studies. On the other hand, Langheinrich et al. (2006) found with the use of microCT analysis that both the total vasa vasorum volume and the percentage of vasa vasorum showing connectivity with aortic plaques increased over the time course of lesion development in a mouse model of atherosclerosis.

The second, less frequently observed origin of vasa plaquorum is the luminal endothelium. Of note, Ritman and Lerman (2007) elegantly illustrated with the use of microCT that the vasa vasorum of normal coronary arteries originate from the lumen themselves. In an early study aimed at examining the pathology of intraplaque hemorrhage in human carotid artery endarterectomy specimens, Fryer et al. (1987) observed an apparent continuity between vasa plaquorum and luminal endothelium. In a study directly comparing the connectivity between vasa plaquorum and the lumen or the adventitia of human coronary artery plaques, the former was found to occur several fold less frequently (Kumamoto et al. 1995). Adventitial-derived vasa plaquorum were found to associate with severe arterial stenosis, consistent with vascular growth necessary to maintain the significant thickening of the wall. However, the lumen-derived vasa plaquorum were found most frequently in lesions with 40% and 50% stenosis; more importantly, this was the type associated with intraplaque hemorrhage. Given that moderate stenosis and intraplaque hemorrhage are currently accepted as characteristics of the vulnerable plaque (Virmani et al. 2002), these head-to-head pathologic observations support the idea that vasa plaquorum anatomically connected with the lumen are more likely to give rise to intraplaque hemorrhage, whereas adventitia-derived vasa plaquorum support plaque growth. Such a distinction could be very useful if different processes were to be targeted via vasa plaquorum interventions. However, the situation is far from clear-cut; and different mechanisms may control the susceptibility to rupture at other anatomic sites. In a study of aortic lesions, Moreno et al. (2004) reported that microvessels at the base of the plaque were independently correlated with plaque rupture. In the same study, these authors confirmed the association of neovessel density, cap and shoulder macrophage infiltration, and intraplaque hemorrhage. There are no specific reports of clinical detection of vasa plaquorum originating from the lumen. However, in a study examining the use of angiography to predict plaque progression, Casscells et al. (2003) reported that detection of *plaque blush*, defined as a brief retention of contrast agent after angiography by a fissured plaque or by microvessels accessible from the lumen, was a strong early predictor of future events. In recent studies of mouse experimental atherosclerosis that used fluorescence

microangiography to investigate the spatial organization of vasa plaquorum in a carotid ligation model, we found connectivity with the lumen of the lesion but not with the adventitial capillaries (Lessner and Dawdy 2008).

We suggest the plaque itself as a third potential origin of vasaplaquorum. In this scenario, recruitment of vascular progenitor cells and/or in situ differentiation of plaque cells could initiate de novo formation of a vascular plexus through a process that recapitulates embryonic vasculogenesis. We emphasize that there are few facts related to the atherosclerotic plaque that support such a possibility at this time; however, many useful clues regarding major potential pathways and master switches are suggested by the cancer and stem cell literature (Li et al. 2006, Schmidt et al. 2007). Thus, we believe research testing this hypothesis in relation to atherosclerosis not only might reveal interesting new information but could create new opportunities for specific therapeutic targeting of vasa plaquorum.

Suggestions of association between blood-derived CD34⁺ cells, which might possess endothelial and hematopoietic progenitor potential (Barber and Iruela-Arispe 2006), inflammatory infiltrates, and vasa plaquorum came from an earlier study of human endarterectomy specimens. Milei et al. (1998) reported detection of CD31⁺ and CD34⁺ channels in highly vascularized lipid cores. Interestingly, the two marker patterns seemed to segregate, with the CD34⁺ neovessels found in more than half the cases at the plaque's base and at its shoulder, in the company of macrophages. However, in this early study, no additional surface markers were investigated to confirm that these were true progenitor cells. Whereas fibrous cap ruptures were characterized by mononuclear infiltrates, intraplaque hemorrhage without frank cap rupture was always associated with highly vascularized lipid cores. Mobilization of blood-derived progenitor cells is known to increase in response to hypoxia and angiogenic factors and to contribute in pathologic situations to the formation of new vascular structures in addition to classic angiogenesis (Li et al. 2006). Both the microenvironment and epigenetic events can control endothelial (Aird 2006) and hematopoietic transformation (Eilken et al. 2009). Atherosclerosis offers a rich repertoire of potential stimuli that could influence differentiation and behavior of endothelial and other plaque cells, including local factors, which constitute the biochemical and mechanical environment of the plaque, and systemic factors, such as metabolic status, aging, and genetics.

Thus, the vasa plaquorum neovessels could arise *within* the rich infiltrates of blood-derived cells that characterize the highly vascularized atherosclerotic lesion, expanding to connect either with the lumen of the artery or with the vasa vasorum, or developing independently of both. Initiation could occur through in situ differentiation of blood-derived endothelial, hematopoietic, and/or myeloid precursors. Another possibility that has not been rigorously investigated in this context is transdifferentiation of more mature cells of the hematopoietic or myeloid lineages. Circulating monocytes, macrophages, and dendritic cells were all shown to be capable of assuming an endothelial phenotype under a variety of conditions likely to be found in the atherosclerotic plaque (Rehman et al. 2003, Zhao et al. 2003). Of note, a recently published report demonstrated the existence of hemogenic endothelial cells by showing cells already expressing endothelial markers giving rise to red blood cells (Eilken et al. 2009). Interestingly, the tumor literature indicates that the endothelial marker

CD31 is frequently expressed by tumor macrophages and leukocytes involved in extravasation (McKenney et al. 2001); and both CD31 and CD34 expression were used as immunohistochemical markers of endothelial transdifferentiation in human cutaneous melanoma (Folberg et al. 2000). Vascular mimicry, a phenomenon first described in tumors (Maniotis et al. 1999), involving de novo generation of vessel-like structures without participation by endothelial cells, could also occur in atherosclerotic plaques. Such pseudovascular structures have a different response to various stimuli compared with angiogenic structures, possibly explaining their resistance to typical angiogenic inhibitors such as endostatin and TNP-470 in vitro (van der Schaft et al. 2004). These specific inhibitors were shown in a mouse model of atherosclerosis (Moulton et al. 1999) to reduce plaque growth and intraplaque neovascularization. However, the authors reported only little effect when the treatments were performed during the early stages of atherosclerosis. Such observations are consistent with the hypothesis that mechanisms apart from classic angiogenesis are at play in the early stages of vasa plaquorum formation. Thus, elucidating the various potential cellular contributors (angiogenic vs pseudovasculature) to vasa plaquorum structure, as well as the mechanisms governing their function, could provide great opportunities for the development of new therapeutic targets that would spare normal endothelium. Neovascular structures in the plaque might present challenges because of nonhomogeneous perfusion and variations in capillary permeability. In addition, their functional connectivity with the rest of the vasculature may be impaired. These abnormalities likely would decrease their susceptibility to therapeutic interventions, as reported in relation to drug delivery and impaired therapeutic effect in some tumors (Jayson et al. 2002).

- **The Chicken and the Egg**

The neovasculature of the atherosclerotic plaque has been widely reported to occur in association with certain pathologic features and processes. Although pathology excels at providing essential clues, it is not the easiest tool to use for determining temporal and causal relationships, which are important for diagnostic purposes and therapeutic interventions. The use of experimental models has added significantly to our knowledge, although by its nature this approach limits the context of the observations.

- **The Macrophages—Again**

The frequently noted close spatial relation between vasa plaquorum and inflammatory infiltrates, specifically macrophages (Fleiner et al. 2004, Milei et al. 1998), also provides an interesting challenge in assessing a “chicken and egg”-type relationship. One common interpretation is that the adjacent inflammatory infiltrate results from development of leaky neovasculature, enhancing transmigration of inflammatory cells (de Boer et al. 1999). There is indirect evidence of increased permeability in vasa plaquorum, reminiscent of tumor neovasculature, which is known to have many gaps with endothelial cell and basement membrane fenestrations (Dvorak 2002). A leaky endothelium with an increased permeability for blood-derived material (Zhang et al. 1993), including circulating lipoproteins, inflammatory cell infiltrates, and extravasated red blood cells, is generally thought to contribute to the growth of plaques through formation of complex acellular and cellular

deposits (Virmani et al. 2005). Macrophage-derived foam cells also contribute with a variety of secreted factors, including growth and angiogenic factors, proteases, and reactive oxygen species. These cells are a major driver of atherosclerotic lesion growth (Lessner et al. 2002) and progression toward instability (Fleiner et al. 2004, Galis et al. 1994, Virmani et al. 2002).

Whereas the relationship between macrophages and angiogenesis is likely a symbiotic one, in which macrophages enhance angiogenesis and angiogenesis brings more macrophages (Moulton et al. 2003), the order of arrival could point to an important causal relation. First, by examining specimens of experimental and human atherosclerotic lesions, one can see rich inflammatory infiltrates before and in the absence of any evidence of neovascularization (Moulton et al. 1999). This is not surprising, as macrophages routinely penetrate deep into tissues, based on their ability to produce matrix-drilling proteases (Moldovan 2002), a capacity that is enhanced within atherosclerotic lesions (Galis et al. 1995). In fact, it is more likely to see many macrophages without any neighboring neovessels than to find neovessels without any adjacent macrophages. Thus, it appears that macrophages arrive first. If this is true, the next step will be to consider the main mechanisms by which macrophages may contribute to the development of vasa plaquorum. First, macrophage-derived soluble growth factors are known to be essential for angiogenesis. Furthermore, we have demonstrated that the macrophage-derived matrix metalloproteinase (MMP)-9 is essential for angiogenic branching (Johnson et al. 2004). More recently, it was shown that MMPs released by macrophages have the capacity to directly orchestrate vascular patterning via proteolytically processing vascular endothelial growth factor (VEGF) and the extracellular matrix (Lee et al. 2005). Gerhardt et al. (2003) and Lundkvist et al. (2007) have demonstrated a role for soluble and matrix-bound VEGF isoforms in controlling directed migration of endothelial sprouts during angiogenesis, in combination with Notch pathway signaling, which determines the tip vs stalk cell identity. Macrophages produce reactive oxygen species and occur in areas of hypoxia (Sluimer et al. 2008), a combination recently shown to have the ability to induce in vitro differentiation of endothelial precursors (Milovanova et al. 2009). In relation to arterial lesions, we tested the hypothesis that increased oxidative stress, such as that expected in a macrophage-rich environment, can trigger lesion angiogenesis. We artificially created this type of environment in the carotid artery neointimal lesions of mice overexpressing the p22phox subunit of nicotinamide adenine dinucleotide phosphate oxidase (Khatri et al. 2004). These lesions did not contain any inflammatory cells; but they were characterized by high levels of hydrogen peroxide, increased expression of VEGF and MMPs, and development of a florid neovasculature (Figure 1). Of note, no angiogenesis occurred in neointimal lesions of similar thickness in wild-type mice. Besides providing paracrine influences on angiogenesis, we might discover that monocyte-macrophages can also become direct builders and participants in the formation of neovascular channels. Our studies in a mouse model of atherosclerosis indicated that a significant number of infiltrated monocyte-macrophages, commonly considered terminally differentiated, were capable of proliferating inside the lesion (Lessner et al. 2002). Clearly, there is a lot to be gained from elucidating the role of precursors and mature monocyte-macrophages in the development of vasa plaquorum.

- **Hypoxia and Vasa Vasorum**

The correlation between lesion volume in human and animal model arteries with extent of neovascularization (Kumamoto et al. 1995, Langheinrich et al. 2006, Moulton et al. 1999, Zhang et al. 1993) supports a role for hypoxia in the development of vasa plaquorum. The most commonly proposed hypothesis is that pathologic thickening of the intimal layer leads to tissue hypoxia, which encourages vasa vasorum sprouting across the arterial wall toward the inner layer. Evidence of hypoxia was demonstrated in experimental atherosclerotic lesions in the rabbit model (Bjornheden et al. 1999) and in human endarterectomy specimens (Sluimer et al. 2008), with the most strongly affected areas occurring at the base of the lesions, closest to the adventitia. All the major players that mediate hypoxia-induced angiogenesis in tumors (Pugh and Ratcliffe 2003) were also detected in human (Sluimer et al. 2008) and experimental (Moulton et al. 2003) atherosclerotic plaques. The hypoxia-driven expansion of atherosclerotic plaques has been likened to that of growing tumors (Herrmann et al. 2006).

All these observations are consistent with hypoxia-induced recruitment of vasa vasorum into the thickening arterial wall, with its offspring vasa plaquorum further enhancing lesion growth by reducing hypoxia and facilitating infiltration of blood-derived matter into the lesion. Thus, blocking vasa vasorum access into the arterial wall would appear to be a sensible way to intervene. Because most seem to agree on this point, we will only present some facts that do not fit this widely held presumption. Paradoxically, blocking or removing vasa vasorum of healthy vessels leads to intimal thickening (Martin et al. 1991).

Furthermore, the lower density of vasa vasorum on the myocardial side of the coronary arteries has been suggested as a possible factor influencing formation of atherosclerotic plaques predominantly in this anatomic location (Gossel et al. 2003). In addition, despite the widely held view that vasa vasorum deliver factors that contribute to the growth of the lesion, hemodynamic considerations eloquently reviewed by Ritman and Lerman (2007) indicate that substances diffuse *into* the vasa vasorum from within the vessel wall and not the other way around. Coronary vasa vasorum are functional end arteries not connected via a plexus. This characteristic may significantly impact the spatial distribution of perfusion and drainage of the coronary vessel wall. Thus, the proliferation of vasa vasorum seen in early atherogenesis could be a repair response reflecting the need for increased blood flow to maintain adequate oxygenation throughout the vessel wall. Based on this perspective, therapies that facilitate rather than block flow through the vasa vasorum might limit lesion thickening and growth.

- **Of Mice and Men**

As in the case of plaque rupture (Schwartz et al. 2007), finding a reliable experimental model of plaque neovascularization has been a somewhat elusive goal. Recent reviews have eloquently discussed this specific challenge (Rader and Daugherty 2008, Zadelaar et al. 2007). Most of the animal models studied to date show evidence of intraplaque vessel growth late in the time course of lesion development, requiring long-term studies. Furthermore, neovascularization may not occur consistently in all lesions, even at the same anatomic location (Moulton et al. 1999). In comparing results across species, it is important

to keep in mind that both the driving forces and the mechanisms of plaque vascularization may vary in different animal models and at different anatomic locations.

Although proliferation of vasa vasorum has been reported in the hypercholesterolemic pig, the extent to which these vessels contribute to the vasa plaquorum is not well established (Kwon et al. 1998). Mouse models of atherosclerosis demonstrate varying degrees of plaque neovascularization (Figure 3), depending both on the transgenic strain used and on the type of diet. Moulton et al. (1999) reported neovascularization in only 13% of advanced aortic lesions in apolipoprotein (apo) E knockout mice maintained on a Western diet (42% fat, 0.15% cholesterol) for 28 to 30 weeks. In contrast, Langheinrich et al. (2006) found a high frequency of neovascularization in aortic lesions in apo E low-density lipoprotein receptor double knockout mice on an atherogenic diet (10% fat, 5% cholesterol) for 16 to 80 weeks. The reason for the apparent discrepancy between strains is unclear, although the double knockout mice develop atherosclerosis somewhat more rapidly than the single apo E knockouts (Bonthu et al. 1997). The rabbit model has received increasing attention recently, particularly for imaging studies with the use of targeted contrast agents (Cornily et al. 2008, Winter et al. 2003). A combination of vascular injury (balloon denudation) and hypercholesterolemia promotes complex lesion development, including intraplaque vascularization, more rapidly than diet alone (Cornily et al. 2008). Intimal capillary density was much higher after 4 months on high-cholesterol diet in animals that underwent double balloon denudation of the aorta than in spontaneously atherosclerotic Watanabe heritable hyperlipidemic rabbits after 6 months on a chow diet (Cornily et al. 2008, Roy et al. 2006). Interestingly, the pattern of neovascularization differed in these two models, with most capillary profiles appearing near the luminal surface of the plaques in the Watanabe heritable hyperlipidemic model (Roy et al. 2006). In contrast, capillaries in the combined diet/balloon denudation model were concentrated at the base of the plaques (Cornily et al. 2008), as typically seen in human specimens, suggesting that different mechanisms of neovascularization may be operative in each case. Much remains to be learned regarding the stimuli driving plaque vascularization in specific arteries as well as the factors that control the pattern of neovessel growth. Thus, there is still considerable work to be done in establishing and characterizing good animal models of plaque vascularization. Although small animal models are convenient experimentally, we should be careful not to assume that plaque neovascularization in these species replicates processes occurring in human pathology. In particular, small animals such as mice have relatively thin-walled elastic arteries that lack vasa vasorum under normal physiologic conditions (Wolinsky and Glagov 1967).

• Implications for Diagnosis and Therapy

Recent developments in clinical imaging of atherosclerosis have been guided by the recognition of plaque destabilization as the main culprit for acute clinical events. Identifying specific pathologic features associated with plaque vulnerability has become the goal of several new or modified imaging modalities (Burke et al. 2003) aimed at early identification of patients at risk. Among these, specific modalities focus on the detection of vasa vasorum. Ultrasound-based techniques under development for the detection of adventitial vasa vasorum in the carotid and coronary arteries were recently reviewed (Granada and Feinstein

2008). At the current clinical stage, these can reveal the extent of adventitial vasa vasorum, which is likely a good surrogate measure for lesion volume. This relationship was recently confirmed by the observed correlation between adventitial signal and intima-media thickness of human carotid lesions (Magnoni et al. 2009). It is not clear whether this type of clinical measurement offers significant advantages compared with direct measurement of plaque volume. However, the development of new contrast agents targeted to signatures of pathologic angiogenesis could provide diagnostic information about vasa plaquorum (Fleiner et al. 2004), which have been suggested as an earlier indicator of plaque vulnerability, together with opportunities for delivering therapeutic agents. In a rabbit model of atherosclerosis, Goertz et al. (2007) used contrast intravascular ultrasound detection techniques based on bubble-specific, nonlinear acoustic signatures to show that the signal in adventitia and not the plaque itself was consistent with the microvascular distribution revealed by histologic sections. Kerwin et al. (2008) reported that the use of dynamic contrast-enhanced magnetic resonance imaging produced adventitial vasa vasorum signals in carotid arteries that correlated with plaque neoangiogenesis detected histologically in the corresponding endarterectomy specimens obtained after imaging.

Therapeutic intent could be directed either at preventing growth of the anatomic structures or at limiting the pathologic functionality of vasa plaquorum. The use of angiogenesis inhibitors, first investigated in a murine model of atherosclerosis (Moulton et al. 1999), has since been frequently suggested. Doyle and Caplice (2007) have recently reviewed the potential benefits and pitfalls of such therapies. A potential alternative is local antiangiogenic therapy (Kolodgie et al. 2007). Interesting insights regarding antiangiogenic therapeutic options and a model of collaboration in assessing their risk-benefit profile are once again offered by the cancer literature (Duda 2006). Unlike the situation in cancer treatment, however, applying broad antiangiogenic therapies in cardiovascular disease presents the challenge that endothelium, the intended victim of such interventions, is essential to the health of the artery that we are trying to preserve.

New options could emerge from scientific knowledge regarding expression of specific molecules by angiogenic endothelium that are absent from normal endothelium. These findings had their origin in the cancer field (Ruoslahti 2004), but have since been confirmed in remodeling of nonmalignant tissues (Jarvinen and Ruoslahti 2007). A similar investigation applied to the atherosclerotic lesion would create the opportunity to selectively target specific molecules that differentiate vasa plaquorum endothelial cells vs normal endothelial cells for diagnostic and therapeutic purposes. It is plausible that molecular markers and functionality of a given vessel network in one atherosclerotic plaque could vary temporally or differ from plaque capillaries in another artery. More recently, the possibility of manipulating angiogenesis therapeutically via microRNA was suggested by several reports identifying microRNAs controlling major players in this process. Kuehbach et al. (2008) reported that miR-221 and miR222 block endothelial cell migration, proliferation, and angiogenesis in vitro by targeting the stem cell factor receptor c-Kit and indirectly regulating expression of endothelial nitric oxide synthase. Wang et al. (2008) found that miR-126 enhances the proangiogenic actions of VEGF and fibroblast growth factor and promotes blood vessel formation by repressing the expression of Spred-1, an intracellular inhibitor of angiogenic signaling. Furthermore, Suarez et al. (2008) demonstrated the role of

the cMyc oncogenic cluster miR-17–92 in the control of postnatal angiogenesis. A broader intervention, which we believe would be upstream of vasa plaquorum formation, would target macrophages and the redox environment in the atherosclerotic plaque.

Another potential therapeutic option would be to mitigate the contribution of vasa plaquorum to plaque destabilization. Although the exact mechanisms by which neovascularization destabilizes the plaque have not yet been elucidated, two features of plaque vulnerability associated with vasa plaquorum are lipid core size and intraplaque hemorrhage, which in fact are thought to be interrelated (Virmani et al. 2002, 2005). As discussed above, the leakiness of the neovasculature is thought to be responsible in part for rapid addition of lipids to the arterial wall. The functionality of the vasa plaquorum may also contribute to plaque vulnerability in ways that cannot be assessed by morphologic observations. In this regard, the vasoactivity of vasa plaquorum may be important in promoting intraplaque hemorrhage. Neovessels invested with smooth muscle cells can actively participate in vasoconstriction (Scotland et al. 2000). We hypothesize that vasospasm could result in intraplaque hemorrhage, which in theory could destabilize a highly vascularized plaque. The task at hand is to define the tone regulator profile for neovessels, which appears to be different from that of similarly sized resistance arteries. Scotland et al. (2000) have suggested that endothelin receptor antagonists may be useful in preserving or restoring nutrient blood flow to vasa vasorum.

• Conclusion

Our brief conjecture is that, in relation to the natural history of an artery, vasa vasorum are beneficial, whereas vasa plaquorum are likely to be detrimental. The ability to specifically identify and target the latter is essential, but will require future research and willingness to challenge some of the current assumptions in the field.

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Figure 1.
Three-dimensional confocal reconstruction of the neovasculature of an experimental lesion in the carotid artery of the p22phox transgenic mouse (fluorescence microangiography).

Figure 2. Human atherosclerotic plaque with evidence of vasa vasorum sprouting (*inset*) into the plaque (Movat pentachrome staining).

Figure 3. Neovessels in atherosclerotic plaque in the mouse carotid artery ligation model demonstrated by CD31 staining for endothelial cells. Red = CD31, green = elastin (autofluorescence), blue = nuclei. Scale bar = 100 micrometers.