Angiogenesis in Atherogenesis
Joerg Herrmann, Lilach O. Lerman, Debabrata Mukhopadhyay, Claudio Napoli, Amir Lerman

Abstract—Atherogenesis is the pathobiological process, which underlies atherosclerotic cardiovascular disease and evolves in the 3 stages of initiation, progression, and complication to clinical significance. Of note, this process is associated with neovascularization, and it was not until recently that the implications of angiogenesis in atherogenesis were delineated. This article gives an updated overview on this topic and briefly reflects on the similarities with neovessel formation in carcinogenesis. (Arterioscler Thromb Vasc Biol. 2006;26:000-000.)

Key Words: atherosclerosis ■ cancer ■ neovascularization ■ oxidative stress ■ pathology

Atherosclerosis has been recognized as a degenerative disease of arteries ever since its first recordings. Within the changing era of the 18th century, Boerhaave revolutionized the view on atherosclerosis by suggesting that it was in fact the consequence of constriction and hardening of the small arteries that feed the muscular layer, collectively termed vasa vasorum.1 However, it was not until the end of the 20th century for this view to be substantiated.2,3 Interestingly, as indicated in the current review, neovascularization in atherosclerosis is reminiscent of angiogenesis in carcinogenesis.

Pathoanatomic Sequence
The development of an atherosclerotic and a cancerous lesion involves three distinct pathoanatomic stages (Figure 1). In the initiation stage, “injury” to cell function occurs to such a degree that overall tissue structure sustains alterations. As shown in an animal model of early atherogenesis, this initial stage is already associated with formation of new vasa vasorum in areas of subsequent atherosclerotic plaque development.4,5 The overall appearance of these vasa vasorum neovessels is fairly disorganized and similar to the vascular network surrounding a cancer lesion site (Figure 2). In the progression stage, there is extension of adventitial neovessels to the media and eventually into the enlarging plaque, whereby the composition of the neovessels is reduced from full vessels with a smooth muscle cell layer to a ring of capillary endothelial cells. The main lumen contributes only up to 30% to the neovascularization of the atherosclerotic plaque.6 Similarly, cancer growth is accompanied by neovascularization around and within the cancerous lesion. Eventually, most advanced atherosclerotic lesions are fairly neovascularized, especially the “vulnerable” plaque regions.7,8 Moreover, neovascularization seems to characterize the inflammatory, more “active” plaque rather than the calcified, more “inactive” plaque.9,10 In line with this view, neovessels are encountered in 50% of atherectomy samples from acute coronary syndrome patients, ie, 5 times more frequently than in lesions from stable coronary artery disease patients.6,11 Also, carotid endarterectomy sample-based studies noted a highly positive association between plaque neovessel density and symptomatology, and both these parameters were closely related to intraplaque hemorrhage and rupture.12,13 With similar reference to the complication stage of atherosclerosis, Moreno et al noted that neovessel density was overall highest in ruptured atherosclerotic plaques and microvessel count at the plaque base was independently associated with plaque rupture.10 Likewise, neovessels can be seen at the various stages of a cancer lesion, and importantly, prior to progressive growth and heterogenous spread of cancer cells.14–16

Pathophysiological Significance
Using a rodent balloon injury and a perivascular collar model, Khurana et al suggested an angiogenesis-dependent and an angiogenesis-independent phase in neointima formation after mechanical injury to the intima or adventitia.17 Whether this concept can be extended to non-mechanical arterial injury awaits confirmation. Furthermore, it would be intriguing to answer the question whether simulation of angiogenesis without manipulation of any component of the vascular wall initiates an atherosclerotic lesion. In this context, the susceptibility of arteries to atherosclerosis may be influenced by the degree of primary vascularization of the arterial wall with regional differences.18–20

As for established plaques, Moulton et al were able to demonstrate that treatment of hypercholesterolemic apoE−/− mice with the angiogenesis inhibitors TNP-470 and endostatin reduced plaque neovascularization and plaque size by...
Even though this may indicate a causal contribution of plaque neovascularization to plaque growth, it cannot be fully excluded that these 2 inhibitors exerted angiogenesis-independent effects and that plaque neovascularization followed reduction in plaque size. Of further note, the data by Moulton et al pointed out that along a 60% reduction in CD31 \(^{+}\) capillaries in intima and adventitia, the 31% reduction of plaque area was not related to a reduction of intimal smooth muscle cell content but to a 51% reduction in plaque macrophage content, supporting the notion that plaque neovascularization may contribute to atherogenesis by providing a vascular network for inflammatory cell infiltration.\(^{22,23}\) It has to be mentioned, though, that the fractional contribution of plaque neovessels versus main lumen to the accumulation of macrophages in the plaque is yet to be quantified. Nevertheless, fostering inflammation and metabolism, plaque neovascularization may also contribute to temperature heterogeneity of the vascular wall and the atherosclerotic plaque.

As highlighted by different groups, neovessels within the atherosclerotic plaque are characterized not only by paucity of tight junctions and a discontinuous basement membrane but also by a relative lack of smooth muscle cells.\(^{24,25}\) Thereby these neovessels are not only leaky but also unable to control intraluminal pressure and therefore prone to rupture.\(^{26}\)

Leakage of plasma lipoproteins from vasa vasorum and plaque neovessels may contribute to plaque formation in an extension of the intima-filtration or response-to-retention theory of atherosclerosis.\(^{19}\) Furthermore, sequestration of red blood cells may contribute to the cholesterol load of the plaque.\(^{27}\) Of note, intraplaque hemorrhage has been related to both plaque neovessels originating from the adventitia and plaque neovessels originating from the main lumen.\(^{9}\) Considering the fragility of these neovessels, theory is that intramural pressure changes can greatly affect neovascular blood flow to and from the vascular wall and atherosclerotic plaque, leading to inner layer ischemia with infarction or congestion with plaque hemorrhage, and ultimately even to plaque rupture.\(^{28–32}\) As angiogenesis involves extracellular matrix proteolysis, neovascularization may already reflect a weakening of the mechanical properties of the atherosclerotic plaque.\(^{33–35}\)

It is of notice that contrary to the neovessels within the intimal plaque area, neovessels originating from the adventitial vasa vasa and entering the media are characterized by a smooth muscle cell layer, as outlined by Heisted et al.\(^{24}\) It relates to this anatomic characteristic that these neovessels are not merely passive and fragile tubes but rather vasoactive, functional vessels, as underscored by Scotland et al.\(^{36}\) Hence, these vessels may sustain luminal capacity against intramural pressures but may also be influenced by vasoactive factors within the vascular wall such as endothelin-1 (ET-1), leading
to vasoconstriction and intramural hypoxia, which in turn stimulates oxidative stress and inflammation. Plaque neovascularization, therefore, contributes to the progression as well as to the vulnerability of atherosclerotic lesions.37

As pioneered by Folkman et al, tumor volume remains limited to 1 to 2 mm³ unless a switch to angiogenesis allows further growth.14,15 The main role of cancer angiogenesis is thought to reside in the supply of oxygen and nutrients even though its effectiveness can be limited.38 Ultimately, the most important implication of tumor neovascularization is the generation of metastatic potential.15 This view is supported by the reduction not only of tumor growth but also of metastases in studies with the aforementioned angiogenesis inhibitors.39

Considering these findings, it is not surprising that a number but not all studies indicated a correlation of the extent of microvascular density with prognosis in solid and hematologic malignancies.40,41

**Angiogenic Growth Factors**

Angiogenesis describes the formation of new capillaries from post-capillary venules, typically stimulated by tissue ischemia/hypoxia via action of hypoxia-inducible factor 1 (HIF-1)42–44 (Figure 3). Promoter regions for the stabilized HIF-1 dimer belong to gene sequences encoding, for instance, for vascular endothelial growth factor (VEGF), which stimulates endothelial cell proliferation, migration, and survival, and...
Figure 3. Illustration of the hypoxia-inducible factor (HIF) activation pathway, highlighting hydroxylation and acetylation of HIF-1α depending on cellular oxygen tension, i.e., normoxia and hypoxia. Hydroxylation of the proline residues 402 and 564 of HIF-1α allows recognition by the ubiquitin system, and thereby labeling for degradation by the proteasome complex. Growth factors and cytokines stimulate HIF-1α transcription. Modified according to references 44 and 102.
mediates vascular hyperpermeability. By this latter action, VEGF facilitates a fibrin network for the proliferating endothelial cells once proteolytic degradation of the basement membrane via action of matrix metalloproteinase (MMP)-2 and MMP-9 allows their sprouting into the extracellular matrix.\(^{45}\) The endothelial cells continue to divide proximal to the tip of the capillary sprout, which migrates toward the angiogenic stimulus.\(^{1}\)

Increased expression of HIF-1\(\alpha\), VEGF, and MMP-2 and MMP-9 in the coronary artery wall was shown in the aforementioned model of experimental hypercholesterolemia, coinciding with vasa vasorum neovascularization.\(^{46}\) Moreover, the systemic administration of recombinant VEGF increases plaque area and endothelial cell content in other models of experimental atherogenesis while other reports questioned the overall significance of VEGF for neointima formation.\(^{47,48}\) On a mechanistic note, the effect of VEGF may be enhanced by fibroblast growth factor (FGF)-1, especially as the expression of both these growth factors appears to be stimulated by oxidized low-density lipoprotein.\(^{49}\) Importantly, overexpression of FGF-1 or FGF-2 induces neovascularization along with intimal thickening.\(^{49,50}\)

Another important pro-angiogenic factor is ET-1, which can induce VEGF expression in vascular smooth muscle cells via the ET-B receptor and directly stimulate endothelial cell proliferation via the ET-A receptor.\(^{51,52}\) Activation of nuclear factor kappa B (NF\(\kappa\)B) may stimulate the expression of ET-1, MMP-2, and MMP-9, and thus contribute to vasa vasorum and plaque neovascularization.\(^{47,48}\) The notion that NF\(\kappa\)B expression is primed along the vascular tree may provide further explanation for the focality of vasa vasorum neovascularization.\(^{56}\)

As for atherosclerotic lesions in humans, Penn et al and Park et al showed that DNA extracts from human coronary artery plaques transformed NIH 3T3 fibroblasts.\(^{57,58}\) In addition, injection of these transformed fibroblasts into athymic nude mice lead to the formation of tumors, similar to fibrosarcoma found in nude mice after injection of ras-transformed cells. Moreover, Alpern-Elran et al were able to highlight the angiogenic potential of atherosclerotic plaques.\(^{59}\) They noted induction of sustained ingrowth of new vessels in the rabbit cornea in 45% of all plaque fragments taken from carotid endarterectomy samples compared with 2.4% of control tissues. Of interest, histological differentiation highlighted that the cellular zones were 4.5 times more angiogenic than the acellular plaque zones. In addition, boiling of atherosclerotic plaques obliterated the angiogenic effect, suggesting angiogenic growth factors as the ultimate mediators. Indeed, a number of studies highlighted the expression of VEGF, especially in plaques and plaque regions, which are rich in neovessels. Importantly, increase in VEGF expression seemingly precedes angiogenesis in atherogenesis.\(^{60,65}\) Of biological significance, atherosclerotic plaques also express VEGF receptors.\(^{64,65}\) VEGF-R\(\text{2}\) is considered to be the central mediator of endothelial cell proliferation in angiogenesis and mediates VEGF-induced adhesion molecule expression and vascular permeability.\(^{66,67}\) VEGF-R1 is of significance for macrophage infiltration and activation, which may ultimately influence angiogenesis.\(^{68}\)

### Pro- and Antiangiogenic Factors

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inhibit VEGF-induced angiogenesis in the chorioallantoic membrane assay.80 On the contrary, recent data suggest that ADAMTS-1 may promote atherogenesis by cleaving extracellular matrix proteins such as versican and promoting vascular smooth muscle cell migration.81

The molecular basis of angiogenesis in carcinogenesis has been reviewed extensively with similarities to the above.82 For instance, overexpression of VEGF has been confirmed for common solid and hematologic malignancies with different isoform patterns that may relate to overall prognosis.83,84 Stimulation of the FGF/FGF-receptor system has been reported in a number of solid and hematologic cancer tissues as well, and interference with this system has been shown to reduce tumor progression in experimental models.85–87 Likewise, there is evidence for stimulation of the endogenous endothelin system and NFκB in cancer with pathophysiological significance.88–90 Finally, increased expression of MMPs has been shown to enhance metastatic potential.91

Angiogenic Pathomechanisms

As for the mechanisms underlying plaque neovascularization, one of the most obvious is tissue hypoxia (Figure 4). Of interest, arteries whose thickness is beyond the 150 to 200 μm diffusion capacity of oxygen are characterized by adventitial vasa vasorum, which can even extend into the outer media in arteries media if the number of lamellar units exceeds 29 or the “critical depth” of 500 μm.92 Plaque neovascularization may, therefore, be the logical extension of the “anatomic” link between vasa vasorum, artery size, and tissue partial oxygen tension. Bjoernhelden et al supported this theory by demonstrating areas of hypoxia in the atherosclerotic plaque.93 Additional studies indicated that microvessels were seen in the atherosclerotic lesions if the intima exceeded more than half of the entire wall thickness or 300 μm, resembling Geiringer’s “critical depth of coronary arteries” for a surrounding vascular network.92

Increase in HIF-1α expression in experimental models of atherogenesis may point in the direction of intramural hypoxia.94 However, Santilli et al were unable to prove reduction of oxygen supply to the arterial wall of hypercholesterolemic rabbits, pointing to the possibility that HIF-1α stabilization may not necessarily be the consequence of ischemia.95 In an extension of this thought, in a carotid ligation model, Khatri et al were able to demonstrate increased HIF-1α and VEGF expression, neointima formation, and neovascularization by overexpression of the p22phox NAD(P)H oxidase subunit in vascular smooth muscle cells, an effect that was reversed by H2O2 scavenging.96 Hence, increased tissue oxidative stress may be sufficient to cause angiogenesis in addition to initiating atherogenesis. By inducing endothelial dysfunction of the vasoactive vasa vasorum, oxidative stress may also contribute to intermittent intramural hypoxia.36

Another potential mechanism for the creation of a “proangiogenic milieu” in atherogenesis is inflammatory cell infiltration.97 In hypercholesterolemic apoE−/− mice, plaque neovascularization correlated highly with the extent of inflammatory cells but not with lesion size.21,22 Furthermore, a number of studies on human atherosclerotic plaque speci-
mens outlined a close correlation of inflammatory cell and neovessel density. Of note, in the primary atherosclerosis model of hypercholesterolemic apoE−/− mice, antibodies directed against VEGF-R1 but not against VEGF-R2 reduced plaque size by up to 50% and macrophage infiltration by 40%. Also, transfection with the murine soluble VEGF-R1 inhibited early inflammation and late neointimal formation in hypercholesterolemic mice after cuff-induced periarterial injury, and a similar effect on neointima formation was observed in mice with additional VEGF-R1 kinase deficiency. Based on these findings, one may conclude that the inflammatory aspects of VEGF-R1 signaling are of predominant significance for atherogenesis. Another link between inflammation and atherosclerosis is provided by toll-like receptor signaling. In particular, deficiency in the common adaptor molecule myeloid differentiation factor 88 (MyD88) and its upstream receptor toll-like receptor 4 (TLR-4) has been shown to lead to a reduction in atherosclerosis through a decrease in macrophage recruitment to the artery wall in association with reduced cytokine and chemokine levels. Currently, no data are available on the selective effect of anti-inflammatory agents on plaque neovascularization to dissect the causal interaction between plaque inflammation and neovascularization. Hence, for now, plaque neovascularization may be cause and consequence of plaque inflammation.

As for angiogenesis in carcinogenesis, the paradigm of a merely hypoxia-mediated process has been challenged over time as well. Increase in reactive oxygen species as well as inflammatory mediators and cells have been considered to contribute to tumor neovascularization. Also, HIF-1 transcriptional activity may be stimulated not only by hypoxia in tumors but also by growth factor (oncogene)-mediated stimulation of the PI3K/Akt/mTOR pathway or loss of tumor suppressor gene signaling, including p53, VHL, or PTEN, and potentially modulated by inflammatory mediators such as IL-1 and tumor necrosis factor-α. Furthermore, tumor-associated macrophages have been shown to express HIF-1, in particular the HIF-2α subunit isoform, which activates a different set of genes. Recently, it has been indicated that the tumor vasculature may be part of the cancer phenotype, as tumor DNA was confirmed in endothelial cells, lining tumor neovessel. This process overlaps with so-called mosaic vessels, where both endothelial and tumor cells contribute to vessel formation. In addition, some tumors may be vascularized without significant angiogenesis, using existing vessels, so-called vascular co-option, or even by forming vessels on their own without any endothelial cells involvement, so-called vascular mimicry. Bone marrow-derived stem and endothelial progenitor cells, potentially stimulated by cytokine release from the tumor, may, furthermore, contribute to tumor angiogenesis; its relative extent, however, remains to be defined. The contribution of progenitor cells to hypoxia-driven vasa vasorum neovascularization has been indicated for pulmonary arteries but is yet to be confirmed for angiogenesis in atherogenesis. So far, there are no reports on non-angiogenesis pathways contributing to the increase vessel density of atherosclerotic plaques.

**Antiangiogenic Approaches**

Until now, we have seen many clinical trials to enhance arteriogenesis but no clinical study to assess the impact of antiangiogenic therapy on the outcome of patients with ASCVD. As outlined, vasa vasorum and plaque neovascularization relate to different processes, and targeting these processes will eventually address both, angiogenesis and atherogenesis. Indeed, 3-hydroxy-3-methylglutaryl (HMG)-coenzyme A (CoA) reductase inhibitors (statins), one of the cardinal classes of drugs used for patients with atherosclerosis, have been shown to reduce vascular wall oxidative stress, inflammation, angiogenesis, and progression of atherosclerosis. It is of interest that, contrary to the effects of low-dose statin therapy, angiostatic effects of atorvastatin were observed at ≥0.05 μmol/L in in vitro studies, corresponding to the 80 mg per day, high-dose statin therapy approach in humans, which has recently been shown to yield better long-term outcome. The nonlipid-lowering effects of high-dose statins seem to account for the antiangiogenic action of high-dose statin therapy, including modulation of VEGF and MMP production, as well as a direct effect on endothelial cells. Of note, antiangiogenic and anti-tumor effects of statins have been increasingly recognized in oncology and hematology.

Chemotherapeutic approaches, which were recognized to inhibit neointima formation, include rapamycin and paclitaxel. As neovascularization is a characteristic feature of the vascular wall to mechanical injury and even more pronounced after stenting than after balloon angioplasty, one may argue that the suppressive effect on neointima formation may be at least partly caused by the anti-angiogenic effects of these substances. Indeed, in a peripheral balloon injury model, Celetti et al were able to demonstrate that paclitaxel reduced VEGF-enhanced neointima formation along with a reduction of endothelial cell and macrophage accumulation. It has to be mentioned, though, that the effect of these substances was not tested in a model of primary atherosclerosis. Low-dose conventional chemotherapy or "metronomic therapy" by itself may be an effective way to reduce any tumor-related vessel formation and by bone marrow suppression the extent of progenitor cell contribution. The impact of these therapies with lower cardiovascular toxicity potential on atherosclerosis and related angiogenesis remains to be assessed.

Overall, one may constitute that there is an overlap of drug therapies for atherosclerosis with cancer, which includes anti-angiogenic properties. Finally, modulation of common risk factors may be the most effective way to reduce the incidence and the prevalence of both diseases with nicotine as the best example.

**Conclusions**

Neovascularization is a key characteristic of tissue pathology in all stages of atherosclerosis and cancer. Whether the "angiogenic switch" is a central component of the growth of an atherosclerotic lesion similar to carcinogenesis awaits further confirmation. If so, atherosclerosis may be regarded as just another cancer.
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Disclosures
None.

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