This Review is part of a thematic series on Cardiovascular Aging, which includes the following articles:

- The Intersection Between Aging and Cardiovascular Disease [Circ Res. 2012;110:1097–1108]
- Mitochondria and Cardiovascular Aging [Circ Res. 2012;110:1109–1124]

Effects of Aging on Angiogenesis

Abstract: Aging is a dominant risk factor for most forms of cardiovascular disease. Impaired angiogenesis and endothelial dysfunction likely contribute to the increased prevalence of both cardiovascular diseases and their adverse sequelae in the elderly. Angiogenesis is both an essential adaptive response to physiological stress and an endogenous repair mechanism after ischemic injury. In addition, induction of angiogenesis is a promising therapeutic approach for ischemic diseases. For these reasons, understanding the basis of age-related impairment of angiogenesis and endothelial function has important implications for understanding and managing cardiovascular disease. In this review, we discuss the molecular mechanisms that contribute to impaired angiogenesis in the elderly and potential therapeutic approaches to improving vascular function and angiogenesis in aging patients. (Circ Res. 2012;110:1252-1263.)

Key Words: aging ■ angiogenesis

Perhaps because of its inevitability, aging is the risk factor most likely taken for granted despite its potent impact on cardiovascular disease, which generally outweighs all the other risk factors combined. Yet the obviousness of this association masks a more subtle question, why is aging associated with cardiovascular disease? Clearly, one contributor is that aging provides greater cumulative exposure to harmful stimuli such as oxidized lipids or hemodynamic stress. Even so, the exact mechanisms linking such exposures to cardiovascular disease often are incompletely understood. However, recent work suggests pathways and processes intrinsic to the aging process also play important roles in cardiovascular diseases associated with aging. For example, pathways central to vessel formation, such as hypoxia-inducible factor-1α (HIF-1α), PGC-1α, and endothelial nitric oxide synthase (eNOS), interact with multiple aging-related pathways (Figure 1). These include telomerase, sirtuins, and the p16/p19 regulators of cell senescence. The current review focuses on how aging impairs angiogenesis. Because the elderly are at higher risk for ischemic injury and other diseases in which angiogenesis is crucial to the healing response, this impairment increases end-organ...
Overview of Angiogenesis

Blood vessel growth in the adult is categorized into three related processes. Angiogenesis is the growth of small capillary size vessels, whereas arteriogenesis is the growth of larger supplying vessels. Vasculogenesis refers to the process whereby progenitor cells form vascular structures de novo. Blood vessel growth occurs in response to both physiological and pathological stimuli. Physiological angiogenesis occurs in response to exercise to accommodate the increased oxygen and metabolic demands of the active muscle tissue and to supply the hypertrophied muscle.

Angiogenesis accompanies both physiological and pathological cardiac hypertrophy, and an imbalance between blood vessel and cardiac muscle growth has been suggested to be a key event leading to cardiac failure.

Initiation of the angiogenesis and formation of early vascular structures appears dependent on endothelial cells. The number and functional capacity of circulating endothelial or vascular progenitor cells also appear to influence angiogenesis and correlate with vascular outcomes more generally. The nature and exact roles of circulating progenitors are still being evaluated, and it remains unclear whether they are essential for the angiogenesis or are simply a marker of vascular health or regenerative capacity.

Angiogenesis also is essential for recovery after tissue damage. It occurs in healing wounds and, in combination with arteriogenesis, is important in recovery after cardiac and skeletal muscle ischemia. However, angiogenesis also can contribute to pathological events such as tumor growth and neovessel growth in atherosclerotic plaques. As discussed, aged individuals appear to have simultaneously impaired physiological angiogenesis and to be at higher risk for processes associated with pathological vessel formation. Identification of distinct regulatory mechanisms controlling beneficial or physiological angiogenesis and pathological angiogenesis would be of great interest.

Pathways to Angiogenesis

Stimuli such as hypoxia or the increased metabolic demands associated with exercise work through multiple pathways to regulate angiogenesis, in many instances through modulation of secreted angiogenic peptides, such as vascular endothelial growth factor (VEGF; Figure 1). Hypoxia increases expression of transcription factors or coactivators such as HIF1α and PCG-1α that, in turn, induce production of angiogenic

Non-standard Abbreviations and Acronyms

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>ACTH</td>
<td>adrenocorticotropic hormone</td>
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<tr>
<td>Akt</td>
<td>serine-threonine kinase, also known as protein kinase B</td>
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<tr>
<td>Asc2P</td>
<td>ascorbic acid 2-phosphate</td>
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<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
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<td>eNOS</td>
<td>endothelial nitric oxide synthase</td>
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<tr>
<td>Flik-1</td>
<td>fetal liver kinase 1, also known as vascular endothelial growth factor receptor-2 (VEGFR-2)</td>
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<td>Fit-1</td>
<td>fms-related tyrosine kinase 1, also known as vascular endothelial growth factor receptor-1 (VEGFR-1)</td>
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<tr>
<td>HIF1α</td>
<td>hypoxia-inducible factor-1 alpha</td>
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<td>IGF</td>
<td>insulin-like growth factor</td>
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<td>mRNA</td>
<td>messenger ribonucleic acid</td>
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<tr>
<td>NADP(H)</td>
<td>nicotinamide adenine dinucleotide phosphate</td>
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<tr>
<td>NO</td>
<td>nitric oxide</td>
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<tr>
<td>p16(ink4a)</td>
<td>cyclin-dependent kinase inhibitor 2A</td>
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<tr>
<td>p19(Arf)</td>
<td>cyclin-dependent kinase inhibitor 2A</td>
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<td>p21(Cdc42/Rac)-activated kinase 3</td>
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<tr>
<td>p53</td>
<td>tumor protein 53</td>
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<tr>
<td>p66shc</td>
<td>Src homology 2 domain-containing-transforming protein C1A</td>
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<tr>
<td>PGC-1α</td>
<td>peroxisome proliferator-activated receptor gamma coactivator 1-alpha</td>
</tr>
<tr>
<td>Rac1</td>
<td>Ras-related C3 botulinum toxin substrate 1</td>
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<tr>
<td>Ras</td>
<td>rat sarcoma, a family of small GTPase proteins</td>
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<td>ROS</td>
<td>reactive oxygen species</td>
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<tr>
<td>SIRT1–7</td>
<td>family of sirtuin homologs for the yeast Sir2 (silent mating type information regulation 2) gene</td>
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<td>TERC</td>
<td>telomerase RNA component</td>
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<tr>
<td>TERT</td>
<td>telomerase reverse transcriptase</td>
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<tr>
<td>Tie-2</td>
<td>TEK tyrosine kinase endothelial-2</td>
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<td>VEGF-A</td>
<td>vascular endothelial factor-A</td>
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<td>VEGF-D</td>
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growth factors. The responsible mechanisms perhaps have been best-elucidated for HIF1α. Hypoxia induces prolyl hydroxylation of HIF1α, which stabilizes the protein, which translocates to the nucleus, inducing transcription of angiogenic growth factors such as VEGF-A. The transcriptional coactivator, PGC-1α, has been shown recently to mediate a HIF1-independent pathway of angiogenesis that also occurs in response to hypoxia but is regulated at the level of PGC-1α transcription. Interestingly, PGC-1–driven angiogenesis appears to be particularly important in exercise-induced angiogenesis. Elucidating exactly how the HIF1-regulated and PGC-1–regulated angiogenic pathways are integrated in different pathophysiological contexts will be of great interest. As noted, impairment of both these pathways may contribute to reduced angiogenesis in the elderly.

Angiogenesis in the Elderly

Recovery after ischemia or infarction in any organ requires blood vessel growth. The incidence of stroke, claudication, and myocardial ischemia or infarction all increase in elderly patients, and they have worse outcomes when ischemia or infarction does occur. For example, after acute limb ischemia, the elderly have both higher mortality and an increased rate of limb amputation. Elderly patients have reduced capillary density and reduced angiogenesis in response to ischemia (Figure 2). In addition to reducing the endogenous angiogenic response, aging may also limit the response to exogenous interventions such as the angiogenic growth factors delivered in clinical trials. Although preclinical trials are generally conducted in young and otherwise healthy animals, the clinical targets for such interventions are often elderly patients with multiple comorbid diseases, many of which further negatively impact vessel formation. This may help explain why promising preclinical results often fail to translate into clinical benefits.

Recent evidence suggests multiple pathways can drive blood vessel growth, and there may be important differences between angiogenesis that occurs in different contexts. This could help explain apparent discrepancies between the degrees of angiogenesis impairment in distinct settings. For example, angiogenesis is impaired in the elderly in response to ischemia or infarction, but may be intact in the response to exercise. Conversely, the elderly are at increased risk for most forms of neoplasia, which often depends on angiogenesis. However, such clinical observations obviously reflect multiple complex processes, including those driving tumor formation itself, and some have suggested that reduced angiogenic capacity actually may limit growth of some tumors in the elderly. Clarifying these issues ultimately will require a deeper understanding of the role of specific angiogenic pathways in each of these settings. Currently, however, it is clear that aging impacts virtually all the angiogenic pathways identified to date.

Intersections Between Mechanisms of Aging and Angiogenesis

To a remarkable degree, the pathways integral to aging intersect with and modulate those regulating angiogenesis. One might ask why this should be the case and, of course, it is impossible to answer such questions definitively. However, from a teleological perspective, it seems likely that angiogenic pathways evolved to support tissues during periods of growth and/or high metabolic demand. Downregulating the growth of blood vessels in less active tissues that are not growing could help match blood supply with metabolic needs. The maladaptive consequences of this overall for an aged individual, particularly in the context of chronic diseases such as atherosclerosis or ischemic injury, would have little influence on reproductive success and therefore would not be subject to negative evolutionary selection. Thus, it is perhaps not surprising that aging-related pathways have an important impact on angiogenesis.

Cellular Senescence

Senescent cells cease to proliferate and undergo other functional changes in association with aging. In senescent endo-
Theleral cells, reduced proliferation may limit the capacity to form new vascular structures and decreases in endothelial function may contribute to atherosclerotic plaque formation. Interestingly, initial signs of endothelial senescence can even be found in the young.27 The life span and proliferative capacity of endothelial cells can be modified by external stimuli.28 Enhancing endothelial proliferation and angiogenesis may provide a therapeutic strategy for ischemic complications of atherosclerosis, but could increase the susceptibility to malignancies.

Specific pathways linked to regulation of cellular senescence include cyclin-dependent kinase inhibitors and regulation of telomere length. These pathways likely interact on multiple levels, and both appear to regulate angiogenesis.

**Cyclin-Dependent Kinase Inhibitors**

The cyclin-dependent kinase inhibitors, p16(Ink4a), p19(Arf), and p21, function upstream of the tumor suppressor gene, p53, among other pathways, and have been implicated in senescence of endothelial cells and endothelial precursor cells.29,30 These molecules arrest the cell cycle in G1, preventing replication. Mutations in these genes have been observed in malignancies.31 Aging and many known inducers of cellular senescence such as reactive oxygen species (ROS) increase p16 activity.32 inhibiting p16 and related proteins has been proposed as a strategy to escape cellular senescence and loss of proliferative capacity.33 In aging, increased p19(Arf) expression suppresses VEGF-A production,34 whereas exogenous VEGF-A downregulates p16 and p21, restoring the proliferative capacity of senescent endothelial cells.35 In population genetic studies, one of the loci most robustly linked to the phenotype observed in the fourth-generation TERT/mER mice was intercrossed, producing generations of telomerase-deficient mice with progressive reductions in telomere length. Although the first intercrossed generation had largely intact telomeres, the fourth generation of intercrossed TERT−/− mice had severe telomere dysfunction.54 Interestingly, these mice had development of multi-system organ dysfunction, including spontaneous cardiomyopathy.54 Network analyses revealed profound repression of PGC-1α-dependent and β-dependent gene expression. As noted elsewhere, PGCs play an important role in angiogenesis, and it is tempting to speculate that this may contribute to the phenotypes observed. However, PGC-1α is also crucial to cardiac mitochondrial biogenesis and maintenance of cardiac function under stressed conditions.55,56 The more severe phenotype observed in the fourth-generation TERT−/− than PGC-1α−/− mice may reflect the additional p53-dependent pathways being activated in this setting.54

In separate experiments, DePinho et al engineered mice with tamoxifen-inducible TERT fused to the mutant estrogen receptor (mER) under the transcriptional control of the endogenous TERT promoter. In the absence of tamoxifen, TERT-mER is inactive and homozygous mice have short dysfunctional telomeres and progressive end-organ degeneration,53 similar to the TERT−/− mice.54 Tamoxifen treatment of TERT-mER mice induced TERT reactivation, which reduced DNA damage and eliminated degenerative phenotypes in many organs.53 Cardiovascular phenotypes at baseline or in response to TERT activation have not yet been reported in this model but will be of great interest.

In addition to the cell autonomous effects of senescence, the presence of senescent cells appears to have broader negative effects contributing to organ dysfunction in aging. Van Deursen et al57 engineered a genetic mouse model called INK-ATTAC that enabled them to remove senescent p16(Ink4a)-expressing cells in response to treatment with a specific drug. They used this model in combination with mice hypomorphic BubR1, a component of the mitotic checkpoint, which has a premature aging phenotype and shortened life spans.58 Of note, the BubR1 hypomorphs have vascular phenotypes similar to those associated with aging in humans, including arterial thinning with fibrosis as well as impaired vasodilation and NO synthase activity consistent with endothelial dysfunction.59 Removal of senescent cells from the many cell types, including endothelial and vascular smooth muscle cells. Ectopic overexpression of TERT was shown to protect human endothelial cells from senescence and to maintain their angiogenic phenotype.48 TERT expression also improves eNOS function in senescent endothelial cells.49 TERT gene transfer has been used to improve the proliferative and migratory capacity of endothelial precursor cells50 and leads to formation of more durable vascular structures in vivo.51 Thus, impaired telomerase activity and reduced telomere length appear to be important contributors to endothelial senescence and likely impaired angiogenesis in the elderly. Interestingly, telomerase overexpression has been shown to suppress p16 and p21 activity.52

In a fascinating series of experiments, DePinho et al have explored the role of TERT in a variety of aging phenotypes and the capacity for TERT expression to rescue these phenotypes.53,54 TERT−/− mice were intercrossed, producing generations of telomerase-deficient mice with progressive reductions in telomere length. Although the first intercrossed generation had largely intact telomeres, the fourth generation of intercrossed TERT−/− mice had severe telomere dysfunction.54 Interestingly, these mice had development of multi-system organ dysfunction, including spontaneous cardiomyopathy.54 Network analyses revealed profound repression of PGC-1α-dependent and β-dependent gene expression. As noted elsewhere, PGCs play an important role in angiogenesis, and it is tempting to speculate that this may contribute to the phenotypes observed. However, PGC-1α is also crucial to cardiac mitochondrial biogenesis and maintenance of cardiac function under stressed conditions.55,56 The more severe phenotype observed in the fourth-generation TERT−/− than PGC-1α−/− mice may reflect the additional p53-dependent pathways being activated in this setting.54
BubR1 hypomorphs delayed onset and progression of age-related phenotypes in multiple tissues, including adipose, skeletal muscle, and eye. This suggests senescent cells are causally involved in the development of aging-associated tissue dysfunction, and their adverse effects extend beyond dysfunction of the senescent cells themselves.

Oxidative Stress
Oxidative stress is increased in aging, which is associated with increases in oxidatively damaged proteins, lipids, and DNA. Multiple mechanisms contribute to the increase in oxidative stress seen in aging, including increased production and imbalances in endogenous antioxidant/oxidant pathways. Mitochondrial production of ROS is increased in aging animals, which may reflect mitochondrial dysfunction from multiple causes, including age-related accumulation of mitochondrial mutations.

Oxidative stress affects blood vessel growth on multiple levels. Redox imbalance can lead to both telomere-dependent and telomere-independent cellular senescence, reducing proliferative capacity and altering endothelial cell function. Superoxide, the initial oxygen free radical generated by mitochondria, inhibits angiogenesis through multiple mechanisms, including acting as a nitric oxide (NO) scavenger and inhibiting eNOS activity. Thus, increased superoxide in aged endothelium impairs both endothelium-dependent vasodilation and collateral vessel formation. As noted at multiple other points in this review, eNOS acts as a central integrator of signaling pathways related to aging and angiogenesis, as well as atherosclerosis (Figure 3).

The ROS generated by the NADPH family of oxidases also modulate cell signaling and appear necessary for VEGF-induced angiogenesis. However, excessive production of ROS inhibits endothelial cell proliferation. In some settings, ROS can induce hyperstimulation of endothelial cells by inducing VEGF production and amplifying its intracellular effects by increasing expression of its downstream signaling targets, increasing phosphorylation of Akt and eNOS. For example, in a model of prohibitin-1 deficiency, ROS stress was shown to lead to hyperactivation of Akt and Rac1, leading to cytoskeletal rearrangements and decreased endothelial cell migration and endothelial tube formation and in vivo angiogenesis. Oxidative stress decreased telomerase activity and increased telomere erosion and the premature onset of replicative senescence in human endothelial cells.

Antioxidants have demonstrated beneficial effects in a variety of experimental models, although clinical trials exploiting this approach in cardiovascular disease have been universally negative. Thioredoxin overexpression in the mitochondria of endothelial cells has been shown to improve endothelial cell function and reduce atherosclerosis. ROS scavenging also has been used as a strategy to prevent telomere-dependent cellular senescence. Asc2P, an oxidation-resistant form of vitamin C, was shown to slow telomere shortening and extend the life span of endothelial cells in vitro. Treatment of endothelial cells with the antioxidant N-acetylcysteine delays replicative senescence by inhibiting TERT nuclear export and by preserving TERT activity. Aspirin also may have antioxidant effects that mitigate telomerase downregulation, in addition to its well-recognized effects on prostaglandin synthesis and platelets. In humans, vitamin C has been shown to reverse the attenuation of vascular reactivity induced by angiotensin II, although it has not been demonstrated to improve cardiovascular outcomes. Genetic deletion of p66shc, a cytoplasmic signal transducer involved in the transmission of mitogenic signals from activated receptors to Ras, protects cells from oxidative stress and extends life span in mice. Interestingly, SIRT1 represses p66shc expression. Deletion of p66shc also protects against aging-related vascular dysfunction and atherosclerosis. Once again, regulation of eNOS activation appears to play an important role in these effects. Together, these data support a role for aging-related oxidative stress in impaired vascular function and angiogenesis in the elderly. However, it should be noted that these data come from experimental models and thus far have failed to translated into clinically effective therapeutic strategies.

Nitric Oxide
NO is an essential mediator of blood vessel reactivity and angiogenesis (Figure 3). It regulates both the endogenous angiogenic response to ischemia and angiogenesis induced by exogenously administered VEGF-A, and it is involved in endothelial progenitor cell mobilization and function. In normal blood vessels, shear stress is an important stimulus for NO production by eNOS in endothelial cells. Although initially discovered because of its endothelial-dependent induction of vasodilation through its effects the underlying...
smooth muscle cell layer. NO has many important functions in the vasculature. It affects telomerase activity, inhibits platelet aggregation, and suppresses smooth muscle cell proliferation. Reduced NO also has been suggested to enhance sensitivity to apoptotic stimuli in endothelial cells. Exogenous NO can form peroxynitrite from its reaction with superoxide anion and further increase oxidative stress. The NO protein expression is increased and activation by cellular localization, binding to intracellular activating proteins, and dimerization are all decreased in aging cells, leading to reduced production of NO.

Redox imbalance in aging endothelial cells can lead to uncoupling of eNOS function. In an oxidative environment, NO can form peroxynitrite from its reaction with superoxide anion and further increase oxidative stress. Tetrahydrobiopterin, a cofactor for eNOS, is reduced in aged endothelium, which may further reduce NO relative to superoxide anion, contributing to endothelial dysfunction and reduced vascular reactivity. In addition, compensatory upregulation of inducible NO synthase in senescent cells may further increase ROS production. The central role of eNOS and NO in regulation of endothelial function and angiogenesis underscores the importance of all these adverse effects of aging (Figure 3).

**HIF1α**

As noted, ischemia-induced angiogenesis is impaired in aging. It seems likely that impaired HIF1α activity is an important contributor to this, although the precise mechanisms are complex and incompletely understood. Exogenous HIF1α delivery is being explored as a therapeutic strategy for ischemia, underscoring the importance of understanding aging-associated changes in this pathway. In mice, adenoviral expression of constitutively active HIF1α improved perfusion after ischemia in old animals to levels similar to those in young mice, suggesting the downstream mechanisms are intact in aging animals. Interestingly, exercise appears to restore HIF1α activity and ischemia-induced neovascularization in aged animals through a PI3 kinase–dependent mechanism. In sedentary animals, multiple mechanisms likely contribute to reduced HIF1α activity in aging tissues. Increased endogenous glucocorticoid levels have been shown to decrease the transcription of HIF1α. Importin-1α levels are also decreased, leading to decreased nuclear transport of HIF1α. The role of SIRT1 in this context is complex. HIF1α is deacetylated and inactivated by SIRT1. Hypoxia leads to downregulation of SIRT1, providing another mechanism for enhancing HIF1 activity in this setting. Based on this alone, one might expect that aging-associated decreases in SIRT1 would paradoxically enhance angiogenesis. As discussed, we do not have a direct experimental answer to this question. However, it is important to keep in mind that the final role of SIRT1 reflects the potentially distinct effects of multiple SIRT1 targets (including eNOS) in different cell lineages. Thus, although reduced HIF1α activity is likely a contributor to impaired angiogenesis in aging, an integrated model of the roles of HIF-1α–dependent and HIF-1α–independent pathways in distinct contexts (ischemia, exercise, neoplasia) ultimately will be required to resolve some of these apparent discrepancies.

**Reduced Production or Response to Vascular Growth Factors**

VEGF-A is required for embryonic blood vessel growth and is a major regulator of both physiological and pathological angiogenesis in adult tissues. VEGF-A also has been shown to be essential for exercise-induced angiogenesis in skeletal muscle. VEGF production is reduced in aged animals and expression levels of VEGF receptors are lower. In humans, VEGF mRNA and protein levels are lower in aged individuals at baseline, after exercise, and after ischemia in comparison to younger control subjects. Several other growth factor pathways also have been shown to be impaired in senescent endothelial cells, including expression of platelet-derived growth factor and the response to basic fibroblast growth factor. Although the levels of angiopeptin-1 and angiopeptin-2 are unchanged, Tie-2 receptor expression appears be decreased in aging skeletal muscles. Impaired HIF1α activation is likely the main reason for reduced VEGF expression, whereas mechanisms underlying altered expression of other growth factors are largely unknown.

Although the growth factor response to ischemia seems to be impaired, exercise induces apparently normal or even enhanced growth factor factor production in aging tissues. Swimming increases VEGF, Flt-1, and Flk-1 levels, and increases capillary density in aged rat myocardium similar to young controls. Previous exercise improves recovery after hind limb ischemia in old rats by increasing HIF1α and VEGF-A expression, leading to increase in capillary density in the ischemic limb.

**Sirtuins**

Sirtuins (SIRT1-7) are nicotinamide adenine dinucleotide–dependent histone deacetylases originally identified as important contributors to the extended life span seen with caloric restriction. There are seven mammalian homologues of the yeast gene originally identified, and sirtuins have been implicated in a wide variety of important biological processes from cellular stress resistance and genomic stability to energy metabolism. Sirtuin overexpression or small molecule activators prolong the life span of several species. Although some of the earliest results recently have been questioned, a robust literature points to important effects of sirtuins in mammalian biology and pathophysiology, as well as their beneficial effects in cardiovascular disease and inflammation. Sirtuins function as cellular sensors of redox imbalance by sensing nicotinamide adenine dinucleotide/NADH balance. Caloric restriction and exercise increase this ratio and activate SIRT1, whereas hypoxia downregulates its activity and, inversely, in oxidative stress SIRT1 inactivates HIF1α, which may be beneficial in cancer but could be maladaptive in ischemic injury.

SIRT1 has been shown to have direct effects on endothelial cells and blood vessel growth. Loss of SIRT1 leads to
premature endothelial cell senescence in vitro, whereas its overexpression protected cells from senescence-associated morphological and molecular changes. Caloric restriction induces eNOS expression, which appears required for induction of mitochondrial biogenesis and SIRT1 expression. SIRT1, in turn, deacetylates and activates eNOS, providing a positive feedback loop linking SIRT1 and eNOS, a central regulator of both angiogenesis and atherosclerosis. Consistent with this, endothelial-specific overexpression of SIRT1 mitigates atherogenesis in apolipoprotein E−/− mice. In contrast, more general SIRT1 overexpression was reported to worsen atherosclerosis in low-density lipoprotein receptor−/− mice through effects on hepatic lipid metabolism, suggesting the atherogenic lipid profile overcomes the protective effects of endothelial SIRT1 expression. Although angiogenesis was not examined in these studies, it might well parallel the observed changes in atherosclerosis. Taken together, these studies suggest sirtuins provide an important link between intrinsic mechanisms of aging and impaired angiogenesis.

**Systemic Factors**

Although this review focuses on the processes and pathways intrinsic to vascular cells that impair angiogenesis in the elderly, systemic factors also play an important role in this context. Although beyond the scope of this review, established risk factors for vascular disease including hypercholesterolemia, diabetes, smoking, and hypertension all have adverse effects on endothelial or progenitor cell function and angiogenesis, independent of aging. It seems likely that in the context of aging negative synergy between these factors and the considerations discussed further compromise angiogenesis. Hemodynamic influences also are important in angiogenesis, particularly after the initial formation of neovessels. Blood flow is required for vessel lumen formation and recruitment of perivascular cells and subsequent secretion of basement membrane and vessel stabilization. The expanding microvascular bed decreases blood pressure downstream, leading to increased blood flow. The diminished vascular reactivity seen commonly in the elderly and in disease states in larger upstream arteries may further compromise angiogenesis by reducing flow-mediated stabilization and result in decreased microvessel density.

Endocrinologic changes seen in aging are also important modulators of angiogenesis. Important changes include reductions in growth hormone, IGF-1 and estrogens, which may further impair the regenerative capacity of the cardiovascular system. Endogenous production of glucocorticoids is increased 20\% to 50\% in aging individuals because of blunting of the glucocorticoid feedback inhibition of ACTH, and it has been shown to inhibit angiogenesis. Menopause results in a dramatic increase in cardiovascular disease risk in women. Estrogen has been shown to be involved in virtually every aspect of the angiogenesis process. Estrogen maintains and stimulates NO production, mitigating endothelial dysfunction. Estrogen increases telomerase activity, protecting both endothelial and smooth muscle cells as well as endothelial progenitor cells from senescence, promoting endothelial progenitor cell migration and proliferative capacity and protecting endothelial progenitor cells from angiotensin II-induced oxidative damage. Estrogen induces telomerase activity by activating the phosphatidylinositol 3-kinase/Akt pathway and stimulating NO production in endothelial cells. Androgens also have been shown to stimulate VEGF production and to induce angiogenesis by stimulating erythropoietin production. However, it is worth noting that randomized controlled trials of hormone replacement therapy in postmenopausal women have not demonstrated the expected reduction in cardiovascular events and in some cases appear to increase adverse outcomes. Interestingly subgroup analyses suggest the effects may vary with age of the recipient.

**Therapeutic Implications**

As many have noted, aging is generally considered preferable to the most obvious alternative. However, new insights into general mechanisms of aging or specific links between aging and vascular health could provide opportunities for therapeutic intervention. Clinically, this possibility will only become more important as populations age.

In the context of angiogenesis, some of the potential therapeutic targets are not specific to aging. For example, as alluded to throughout the text, NO synthesis lies at the intersection of many important endothelial functions that are impaired both with aging and disease (Figure 3), and thus presents an attractive target for intervention. Exogenous eNOS overexpression restores vascular reactivity and enhances angiogenesis. However, a variety of technical challenges currently limit the clinical applicability of vascular gene therapy approaches. Several molecules related to eNOS activation and NO production have been shown to increase NO production and can be administered systemically. Statins increase NO, both through increasing tetrahydrobiopterin, a cofactor for production of NO by NO synthases, and through activation of the serine-threonine kinase, Akt, which phosphorylates and activation eNOS. Dietary supplementation with l-arginine, the substrate for NO synthesis, rescues the angiogenic response in hypercholesterolemic animals, and nitrite supplementation in drinking water was shown to prevent endothelial dysfunction in aged mice. Scavenging peroxynitrite by antioxidant ebselen was able to both prevent and reverse endothelial cell senescence in vitro. Physical exercise has been shown to improve vascular reactivity and to prevent eNOS uncoupling by inducing endogenous tetrahydrobiopterin, increasing activating eNOS phosphorylation (Ser1177) and decreasing inactivating phosphorylation (Thr495).

Exogenous delivery of angiogenic agents could circumvent the diminished molecular response to ischemia in aged individuals. Administration of exogenous VEGF-A protein or transcriptional activation of VEGF-A restored angiogenesis in old animals. In addition to enhancing sprouting angiogenesis, VEGF treatment has other beneficial effects in aging tissues. Pretreatment with a combination of growth factors, including platelet-derived growth factor-AB, angiopoietin-1, and VEGF, improved the endothelial progenitor cell-mediated vasculogenic responses in aged mice. VEGF delivery also increases expression of TERT in an
Table. Beneficial Effects of Exercise-Related to Angiogenesis

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<td>Oxidative stress</td>
<td>Reduced</td>
<td>Pierce (2011)141</td>
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eNOS indicates endothelial nitric oxide synthase; HIF, hypoxia-inducible factor; NO, nitric oxide; PGC, peroxisome proliferator-activated receptor gamma coactivator; VEGF, vascular endothelial growth factor.

Exercise mitigates many of the aging-related molecular defects seen in endothelial cells and angiogenesis.

NO-dependent mechanism, possibly enhancing the proliferative capacity of endothelial cells.158 High doses of exogenously delivered growth factors also may overcome other aging-related pathological conditions limiting endogenous blood vessel growth such as diabetes and hypercholesterolemia.159 Unfortunately, clinical results based on growth factor delivery have been disappointing.160–162 In part, these results may reflect the challenges of extrapolating from experiments generally performed in young healthy animals to clinical trials in elderly patients with multiple other risk factors and comorbid diseases that impair angiogenic capacity.163 Other considerations including choice of growth factors, therapeutically agent (protein versus viral vector), and delivery methods (intravenous versus direct intramuscular injection) are likely also important.164

As noted, sirtuin activators are being pursued in a variety of systemic diseases and there is reason to believe that they may enhance function of endothelial cells and might prove to be a useful adjunct to enhance angiogenesis in the elderly.

In the meantime, minimizing exposures to injurious stimuli through appropriate lifestyle choices are an important avenue of mitigating risk and enhancing angiogenesis. In some cases, these also may have aging-related mechanisms that contribute. For example, smoking and obesity correlate with shorter telomeres, whereas moderate exercise appears to protect from telomere-dependent cellular aging.164–166 In fact, exercise has been shown to enhance virtually all of the steps impaired in angiogenesis in the elderly through a variety of mechanisms, providing one more rationale to encourage continued exercise training in patients generally and in the aged in particular (Table).

Conclusions

A variety of systemic and intrinsic changes seen with aging limit vascular homeostasis and angiogenesis in the elderly. These changes should be considered when designing therapeutic strategies for cardiovascular diseases. Lower basal capillary density in aged tissues and diminished vascular reactivity predispose tissues to ischemia, and reduced responsiveness to angiogenic stimuli and proliferative capacity of endothelial cells pose limitations to tissue recovery and repair. Current treatment strategies focus on mitigating the effects of established vascular risk factors, but ongoing research may yield approaches specifically targeting pathways intrinsic to aging with an impact on angiogenesis.

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Disclosures

None.

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