Blood vessels form large tubular networks that pervade all tissues in the body. These hierarchically branched networks of arteries, capillaries, and veins are lined by endothelial cells (ECs), which integrate functionally into organs to support growth, function, and repair.1-3 As all cells in the body thrive on nutrients and oxygen supplied by these networks, proper vessel maintenance is essential for tissue homeostasis throughout life. However, during lifetime, vessels often become dysfunctional and unable to sustain adequate tissue perfusion, thereby causing tissue ischemia. Abnormal vessel growth and function are hallmarks of various disorders, and they contribute to disease progression. For instance, insufficient vessel growth or enhanced vessel regression cause ischemic heart and brain disease and can also lead to neurodegeneration or hypertension.3,4 On the other hand, excessive vascular growth and remodeling promote cancer, age-related macular degeneration, and inflammatory diseases.3,4 Many of the above-mentioned vessel-related pa-
thologies are common in the elderly and the incidence of vascular disease correlates with aging, even in the absence of risk factors. Current concepts suggest that aging individuals acquire changes in vascular structure and function that provide the basis for future vascular disease.5–8

Aging is not solely a passive degenerative process but is in fact actively regulated by genetic pathways. This realization has fueled interest in understanding the molecular basis of aging, as manipulating these pathways could offer therapeutic opportunities to combat age-related disorders.9,10 In the past decade, research on aging has identified genes and pathways that affect longevity. Among these are the Forkhead box “O” (FOXO) family of forkhead transcription factors as well as nicotinamide adenine dinucleotide (NAD+)–dependent protein deacetylases termed sirtuins that have been described to prolong lifespan in worms and flies.5,11–13 Whereas the lifespan-extending effects of sirtuins have been challenged in recent studies,14 it remains undisputed that FOXOs and sirtuins coordinate a wide range of cellular responses that are frequently deregulated during aging including metabolism, stress resistance, cell cycle progression, and programmed cell death.15–17 Studies in the vascular system point to the vascular endothelium as a pivotal target tissue for the physiological functions of FOXO and sirtuin family members, where they control multiple facets of vessel development and function. In this review, we summarize recent insights into the biology of FOXOs and sirtuins in the vessel wall and discuss their relevance for age-related vascular changes. We will first provide a general overview of how blood vessels grow, mature, and age that will serve as a conceptual framework for the second part, in which we highlight key functions and prototypic principles of FOXO and sirtuin signaling.

Life of a Blood Vessel

Vessel Development and Growth

The first blood vessels in life form by the differentiation of mesoderm-derived endothelial precursor cells (angioblasts) that assemble into a primitive vascular plexus (vasculogenesis).1–3 Continuous endothelial proliferation, migration, and sprouting expand and remodel this primitive plexus into a highly organized and ramified network (angiogenesis) that differentiates into arteries and veins (arterio-venous differentiation)1–3 (Figure 1A). Nascent blood vessels recruit mural cells (pericytes and vascular smooth muscle cells) that enwrap EC tubules to provide stability and to enable dynamic tissue perfusion (Figure 1A). Although angiogenesis is the major mode of vessel growth, the vasculature can also expand its size and complexity by other mechanisms. The enlargement and remodeling of preexisting collateral vessels between arterial networks (arteriogenesis) is essential for revascularization of ischemic tissues.1,3,4 Moreover, the vascular network can be extended through the splitting of preexisting vessels in a process called intussusception.1,3,4 Although debated, circulatory and/or vascular wall resident (endothelial) precursor cells may also contribute to vessel formation.1,3,4 In the healthy adult, ECs are mostly quiescent and angiogenesis occurs only in specific organs (eg, cycling ovary, placenta during pregnancy). However, ECs retain a remarkable plasticity to switch from a quiescent to a highly angiogenic phenotype to promote tissue (re)vascularization. Thus, angiogenesis remains an important instigator for tissue growth and repair throughout life.

Vessel Maturation and Maintenance

Newly formed vessels must become mature and stable to support tissue homeostasis. Vessel maturation involves a gradual shift from actively growing vessels toward a quiescent, fully functional tubular network.1–3 Maturation of nascent vessels entails the suppression of endothelial proliferation, the adoption of survival properties, as well as the establishment of junctional barriers. Vessels also must recruit mural cell (MCs), generate extracellular matrix (ECM), and specialize their ECs, MCs, and matrix to tissue-specific requirements. In mature vessels, long-lived ECs form a tightly aligned and flattened monolayer with cobblestone-like appearance that enables efficient tissue perfusion.

Vessel Remodeling and Regression

Vessels do not only sprout, branch, and elongate but also regress physiologically (pruning) to match perfusion with tissue metabolic demands3 (Figure 1A). Blood flow plays a key role in determining whether vessels regress or persist. In newly formed sprouts, the onset of blood flow stabilizes vascular connections by activating genes that keep vessels quiescent, dilated, and antithrombogenic.3 In contrast, disturbed or abrogated flow causes endothelial retraction and apoptosis. As a consequence, vessels regress, leaving behind empty basement membrane sleeves. The removal of angiogenic or survival factors also contributes to vascular regression, particularly in immature vessels.3 Abnormally enhanced regression causes vascular rarefaction, a characteristic feature of neurodegenerative and hypertensive disorders.3

Vessel Aging

A hallmark of aging in many organs is a gradual decline in tissue cellularity and function, leading to an impaired capacity to maintain tissue homeostasis, especially under challenging environmental conditions.10 Aging blood vessels, in
particular arteries, also experience a well-documented series of structural and functional alterations that impede their tissue nourishing functions. With advancing age, EC function declines, resulting in an attenuation of endothelium-dependent dilatation that promotes vascular stiffness. Age-related changes also limit the plasticity of ECs to switch from a quiescent to a rapidly proliferating and migrating phenotype after injury or ischemia. Endothelial barriers become leaky and vascular smooth muscle cells penetrate into the subendothelial space, where they divide and produce ECM proteins that cause intimal thickening. As a result, arteries become stiffer, thicker, and less able to function. The development of atherosclerotic lesions in such aged vessels further aggravates vessel narrowing, thereby causing hypertension, vascular obstruction, and tissue ischemia in certain vascular beds.

Molecular Pathways in Aging: Insights From FOXOs and Sirtuins

Aging and the Metabolism Link

The observation that perturbations in specific genetic pathways can either promote or retard the aging process has spurred research into the molecular details of aging and on the mechanisms that link aging with age-related illnesses. Progress in understanding the molecular basis of mammalian aging has been hindered by the complexity of the aging process, the influence of external factors, as well as the lack of biomarkers to reliably determine the extent of aging. Much our knowledge about the molecular regulation of aging stems from the analysis of small, short-lived model organisms such as yeast, worms, and flies. Genetic studies in these model organisms unraveled that many lifespan-extending mutations affect metabolic regulators and control circuits. Examples of such metabolic regulators include the target of rapamycin (TOR), PPARγ coactivator-1α (PGC1α), AMP-dependent kinase (AMPK), FOXO transcription factors, and sirtuins. The importance of metabolic regulation for organismal lifespan is best illustrated by the salutary effects of calorie restriction (CR), the only intervention to date that has consistently been shown to increase life expectancy in species ranging from worms to primates. In this review, we focus on the biology of FOXOs and
sirtuins and their role in ECs during vascular development, maintenance, and aging.

**FOXOs Control Lifespan in Model Organisms**

The first pathway shown to affect aging in model organisms was the insulin/insulin-like growth factor 1 (IGF1) pathway. In the nematode *Caenorhabditis elegans*, mutations that reduce signaling by the insulin IGF1 receptor (DAF2) or its downstream effectors phosphatidylinositol-3-kinase (PI3K) and AKT increase worm lifespan. Activation of PI3K-AKT signaling induces AKT-dependent phosphorylation of the transcription factor DAF16, the *C. elegans* homolog of FOXO (Figure 2). After phosphorylation by AKT, DAF16/FOXO becomes transcriptionally inactive and translocates to the cytoplasm (Figure 2). The increased lifespan of DAF2 mutants depends, at least in part, on this transcription factor as deletion of DAF16 abrogates the longevity phenotype in DAF2 mutant worms. Activation of stress-responsive pathways also leads to a DAF16-dependent lifespan extension, which requires the interaction of DAF16 with the sirtuin deacetylase SIR-2.1 and the adaptor protein 14 to 3–3.20 The lifespan-extending function of DAF16 FOXO is conserved in more complex organisms such as the fruit fly *Drosophila melanogaster*.9,18 Understanding the role of FOXOs in mammalian aging is complicated by the existence of several FOXO isoforms as well as the severe developmental defects in FOXO mutants mice, which preclude the analysis of aging phenotypes.9,18

**Functions of Mammalian FOXOs**

FOXOs belong to the family of forkhead proteins and are characterized by a highly conserved DNA binding domain (forkhead box).15,21 By binding to promoters that contain the FOXO consensus motif TTGTTTAC, FOXOs can act as transcriptional activators and repressors.15,21 Four FOXO isoforms have been identified: FOXO1, FOXO3, FOXO4, and FOXO6. FOXO1, FOXO3, and FOXO4 are ubiquitously expressed; however, their expression levels are dynamic and vary between tissues and developmental stages. On the contrary, expression of FOXO6 is confined to the adult brain. Studies in FOXO loss-of-function mutant mice revealed decisive regulatory roles of FOXOs in a great variety of developmental and physiological programs (Table 1). Global genetic inactivation of FOXO1 in mice causes embryonic lethality, as emerging blood vessels are unable to grow properly.22,23 FOXO3 knockout mice are viable but display age-dependent infertility due to ovarian follicle activation.22,24 In contrast, FOXO4 knockout mice exhibit no apparent phenotype under normal conditions.22 FOXOs govern these diverse functions by transcriptionally integrating signals from key developmental pathways that control tissue differentiation, growth, and maintenance.15,21 Among the prototypical FOXO targets are genes that have pivotal roles in

![Figure 2. Regulation of FOXO transcription factors by the PI3K-AKT pathway. FOXOs control various cellular responses, ranging from apoptosis, DNA repair, and metabolism to ROS detoxification and cell proliferation, by driving the expression of FOXO target genes. FOXOs are key downstream effectors of the PI3K-AKT pathway that blocks FOXO target gene expression. On stimulation of PI3K AKT signaling by growth factors, AKT phosphorylates FOXOs on 3 conserved residues, which leads to their cytoplasmic sequestration and inactivation. Asterisk denotes the bidirectional regulation of cell death and DNA repair by FOXOs: depending on the stress signal and posttranslational modification, FOXOs can shift their transcriptional responses away from apoptosis and toward DNA repair.](http://circres.ahajournals.org/)

**Table 1. Phenotypes of FOXO Mutant Mice**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Knockout Phenotype</th>
<th>Reference</th>
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<tr>
<td>FOXO1</td>
<td>Lethal at E10.5 due to deregulated vascular development: malformed aorta and irregular intersomitic vessels, lack of properly formed branches of the internal carotid artery and vasculature in the yolk sac</td>
<td>Hosaka et al,2004; Furuyama et al,2004</td>
</tr>
<tr>
<td>FOXO3</td>
<td>Viable, age-dependent infertility due to global ovarian follicle activation and early oocyte depletion</td>
<td>Hosaka et al,2004; Castrillon et al,2003</td>
</tr>
<tr>
<td>FOXO4</td>
<td>Enhancement of postnatal vessel formation in models of ischemia-induced vessel growth</td>
<td>Potente et al,2005</td>
</tr>
<tr>
<td>FOXO1, FOXO3, FOXO4</td>
<td>Viable, indistinguishable from wild-type littermates</td>
<td>Hosaka et al,2004</td>
</tr>
<tr>
<td>FOXO1, FOXO3, FOXO4</td>
<td>Premature mortality due to large hemangiomas in the uterus, liver, skeletal muscle</td>
<td>Paik et al,2007</td>
</tr>
<tr>
<td>FOXO1, FOXO3, FOXO4</td>
<td>Lymphoblastic thymic lymphomas</td>
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cell cycle progression (p21, p27), reactive oxygen species (ROS) detoxification (MnSOD), programmed cell death (FAS, BIM), and glucose metabolism (G6PC)\(^{15,21}\) (Figure 2). The transcriptional output of FOXOs is controlled by a range of posttranslational modifications (PTMs) that alter transcriptional activity, DNA binding, subcellular localization, and protein stability. As discussed above, FOXOs are crucial effectors of the PI3K-AKT signaling branch and are inactivated on AKT-mediated phosphorylation\(^{15,21,25}\) (Figure 2). Besides AKT, other kinases can phosphorylate and inactivate FOXOs, including SGK, CK1, DYRK1a, CDK2, ERK, and IKK\(^{15,21,25}\). In contrast, phosphorylation of FOXOs by JNK and the energy-sensor kinase AMPK facilitates FOXO activation\(^{15,21,25}\). FOXOs are also regulated by reversible acetylation. Depending on the FOXO target gene promoter, acetylation can either promote or diminish FOXO transcriptional activity\(^{15,21,25,26}\). Acetylation can also increase FOXO protein stability by protecting FOXOs from poly-ubiquitination and degradation. Accordingly, deacetylation of FOXOs by SIRT1 or class II histone deacetylases affects FOXO target gene expression, subcellular localization, and protein stability.\(^{15,21,25,28}\) Besides SIRT1, other sirtuins similarly regulate FOXO activity, including SIRT2 and SIRT3.\(^{29–31}\) FOXO factors also become methylated and monoubiquitinated/polyubiquitinated, which leads to their signal-dependent activation or inhibition.\(^{21}\) In addition to PTMs, protein-protein interactions of FOXOs with SMADs and β-catenin play important roles in specifying FOXO transcriptional responses.\(^{15,21,25}\)

**Sirtuins: Sustainers of Tissue Homeostasis**

Sirtuins are NAD\(^+\)-dependent deacetylases, which modulate a wide range of biological processes, spanning from DNA repair and oxidative stress responses to energy metabolism.\(^{16,17,32}\) A connection between sirtuins and aging was described more than a decade ago on the basis of studies in model organisms. Overexpression of the sirtuin member Silent Information Regulator 2 (SIR2) was reported to extend lifespan in yeast.\(^{32}\) Subsequent studies in *C. elegans* and in *D. melanogaster* revealed that overexpression of SIR2 orthologues can also promote longevity in these species.\(^{32}\) However, recent conflicting results about the influence of SIR2 on lifespan extension in *C. elegans* and *Drosophila* have challenged the view of SIR2 as a lifespan determinant and kick-started a debate on aspects of sirtuin biology associated with organismal aging.\(^{14,33–35}\) Beyond controversy, sirtuins play critical roles in mounting adaptive responses to diverse forms of cellular stress that fuel tissue degeneration and age-associated diseases.\(^{16,17,32,35}\) Along these lines, mice overexpressing SIRT1, the closest SIR2 orthologue, are protected against age-associated diseases (cancer, diabetes) and reveal an improved health span but do not live longer.\(^{36}\) These findings suggest that mammalian sirtuins have key homeostatic functions that delay age-related tissue degeneration but may not control the aging process per se.

In mammals, 7 sirtuins exist (SIRT1–SIRT7).\(^{16,17,32}\) Despite sharing a conserved catalytic domain, sirtuins have different biological functions and subcellular localizations. SIRT1, SIRT6, and SIRT7 are nuclear proteins, whereas SIRT2 is found primarily in the cytosol.\(^{16}\) SIRT3, SIRT4, and SIRT5 are mitochondrial sirtuins.\(^{16}\) Of note, sirtuins can shuttle between different subcellular compartments. In mammals, 7 sirtuins exist (SIRT1–SIRT7).\(^{16,17,32}\) Despite sharing a conserved catalytic domain, sirtuins have different biological functions and subcellular localizations. SIRT1, SIRT6, and SIRT7 are nuclear proteins, whereas SIRT2 is found primarily in the cytosol.\(^{16}\) SIRT3, SIRT4, and SIRT5 are mitochondrial sirtuins.\(^{16}\) Of note, sirtuins can shuttle between different subcellular compartments. SIRT1, for example, is localized in the cytoplasm of certain tissues but has also been detected in mitochondria.\(^{38}\) Most sirtuins exert their biological function through the deacetylation of acetylated proteins, which comprise histone and nonhistone proteins\(^{16,17,32}\) (Figure 3A). The deacetylase reaction requires the cofactor NAD\(^+\) and involves the transfer of an acetyl group from an acetylated protein substrate to the ADP-ribose moiety of NAD\(^+\). This reaction leads to a deacetylated protein, 2′-acetyl-ADP ribose and nicotinamide (NAM), a noncompetitive inhibitor of sirtuin activity\(^{39}\) (Figure 3A). Many of the physiological functions of sirtuins result from the adaptation of cell signaling and gene expression to the energetic state of the cell, which is sensed through NAD\(^+\) levels.\(^{16,17,32}\) NAD\(^+\) serves as an electron carrier in cellular metabolism (glycolysis, mitochondrial oxidative phosphorylation), and the levels of NAD\(^+\) are elevated in situations of energy distress (Figure 3B). Because sirtuins require NAD\(^+\) for their catalytic activity, their activity is higher in situations of nutrient scarcity such as CR.\(^{16,17,32}\)
FOXOs Are Essential Regulators of Vascular Growth

Vascular networks arose in evolution as organisinal size and metabolic activity increased in species and outgrew the capacity to supply all cells with oxygen and nutrients by diffusion. It is therefore not surprising that the vasculature constitutes the first organ in mammalian development, whose adequate function is essential for growth and morphogenesis. FOXO1 plays an essential role for vascular development. FOXO1 knockout mice show no apparent defects until embryonic day E9.0, and endothelial differentiation from angioblasts and the formation of an initial vascular labyrinth (vasculogenesis) appear normal. However, subsequent angiogenic remodeling into a hierarchically organized network is severely deregulated in FOXO1 mutants, leaving behind a primitive, dysfunctional vascular plexus that causes embryonic lethality at E10.5. At that stage, FOXO1 is predominantly expressed in the endothelial lining suggesting an essential cell-autonomous function of FOXO1 in ECs. Consistent with such a model, FOXO1 overexpression in surrogate assays of angiogenesis inhibits endothelial migration and sprouting, whereas FOXO1 knockout increases angiogenic behavior of ECs. Similarly, ECs derived from FOXO1-deficient embryonic stem cells display aberrant morphological responses after vascular endothelial growth factor (VEGF) stimulation. Collectively, these studies suggest that FOXO1 acts as a critical negative regulator of endothelial angiogenic behavior, whose activity is required to coordinate vessel morphogenesis.

The observation that FOXO1 restricts endothelial angiogenic behavior might, at first sight, appear paradoxical, as genetic inactivation of FOXO1 does not enhance vessel formation but instead impairs vascular development. However, it is crucial to point out that the growth of a functional vessel network requires the coordination of proangiogenic and antiangiogenic activities of ECs. The current understanding of the sprouting process rests on the realization that the populations of ECs that compose sprouting vessels are heterogeneous in their angiogenic behavior and signaling: When stimulated by proangiogenic signals (e.g., VEGF) only a subset of ECs acquires an explorative phenotype. These so-called "tip cells" are located at the leading end of the vessel sprout and extend numerous filopodia toward the source of the angiogenic signal. (Figure 1B). The cells that follow tip cells, termed "stalk cells," extend less filopodia, and are less motile but proliferate and define the base of the sprout (Figure 1B). Uncoordinated tip and stalk cell behavior causes malformed and dysfunctional blood vessels that often result in embryonic lethality. Although the precise mechanisms through which FOXO1 regulates endothelial angiogenic behavior remain unresolved, it is tempting to speculate that FOXO1 co-determines tip stalk cell specification to enable a balanced vascular response to proangiogenic signals.

Cooperative Regulation of Vessel Growth by FOXOs

Although FOXO3 and FOXO4 are dispensable for embryonic vascular development, both of them enhance the growth-suppressive activity of FOXO1 in adult mice. Successive somatic inactivation of FOXO3 and FOXO4, in addition to FOXO1, results in more pervasive endothelial overproliferation and hemangioma formation in adult mice when compared with the deletion of FOXO1 alone. Further support for a critical role of FOXOs in vascular growth stems from the observation that FOXOs are upregulated in ECs of ischemic muscles and that FOXO3 deficiency enhances vessel density after ischemic stress in mice. The cell responses and target genes controlled by different FOXOs in ECs are overlapping but not entirely redundant. For example, overexpression of a constitutively active FOXO1 or FOXO3 mutant inhibits endothelial sprouting and migration, whereas forced expression of FOXO4 has no discernible effect. Likewise, whereas FOXO1 and FOXO3 cooperate in controlling EC responses, overexpression of FOXO3 cannot restore the abnormal function of FOXO1-deficient ECs. Together, these studies suggest that FOXOs function as key negative regulators of endothelial angiogenic behavior, whose activity is required for vessel development. Within this family, FOXO1 is the most potent regulator of vascular growth, with lesser but physiologically significant contributions from the other FOXO members.

SIRT1 Regulates Revascularization of Ischemic Tissues

Targeted mutations of sirtuins in mice have shown that few developmental processes are absolutely dependent on sirtuins. A cardinal function of sirtuins is the adaptation of cellular responses to environmental signals to maintain tissue homeostasis. Consequently, their functions become eminent under conditions of stress. One such example is the role of SIRT1 in vascular growth. Genetic inactivation of SIRT1 in ECs of mice is compatible with life and exhibits no severe vascular defects during embryonic development. However, SIRT1 deficiency impairs tissue revascularization in response to ischemia. SIRT1 deficiency causes impaired EC branching and proliferation, leading to reduced blood vessel density. In zebrafish, knockdown of SIRT1 compromises intersomitic vessel formation, resulting in a sparse and misguided vessel network. The abnormal angiogenic behavior of SIRT1-deficient ECs is in part caused by an unrestrained activity of FOXO1. FOXO1 deacetylases endothelial FOXO1, thereby limiting its antiangiogenic activity. Recent studies demonstrate that the Notch pathway is a pivotal target of SIRT1 in...
SIRT6 mediates tip and stalk behavior.55 The NOTCH1 intracellular domain (NICD), released on NOTCH1 receptor activation, is deacetylated by SIRT1, which causes its destabilization and proteasomal degradation. SIRT1-deficient ECs are thus sensitized to Notch signaling and preferentially adopt stalk cell phenotypes. Notably, SIRT1 can inhibit Notch responses also by epigenetic mechanisms.56 SIRT1 associates with the demethylase LSD1 in chromatin-associated complexes that repress Notch target gene expression through histone demethylation and deacetylation. The consequences of SIRT1 activation on Notch signaling may, however, depend on the cellular and environmental context. Studies in aging neuronal cells revealed that SIRT1 can increase Notch activity indirectly through RXR-dependent regulation of ADAM10.57

FOXOs and Sirtuins at the Vascular-Metabolic Interface

The ability of blood vessels to adjust nutrient and oxygen delivery to changing metabolic demands of tissues is vital for development and homeostasis. It is therefore not surprising that metabolism and blood vessels reciprocally affect each other’s function.58 How this vascular-metabolic cross-talk occurs and how their functions are coordinated is not well understood. Emerging evidence indicates that key regulators of cellular bioenergetics play major roles in vessel formation, particularly in situations of ischemia. For example, the metabolic master regulator PGC1α not only boosts oxidative metabolism but also augments ischemia-induced blood vessel growth through an HIF-independent induction of VEGF.59 Other examples of angiogenesis-boosting regulators of metabolism are AMPK, LKB1, and SIRT1.58 AMPK and its upstream activating kinase LKB1 similarly promote ischemia-induced vessel formation by controlling VEGF levels, whereas SIRT1 restrains the antiangiogenic activity of FOXO1 through deacetylation.53,60–62 FOXOs themselves appear to coregulate metabolism and angiogenesis toward stress tolerance and cell protection.15 FOXOs are key effectors of insulin action in metabolic processes, including hepatic glucose production.15 In the liver, FOXO1 promotes transcription of glucose-6-phosphatase (G6PC) and phosphoenolpyruvate carboxykinase (PKC), the rate-limiting enzymes in hepatic glycolysis and gluconeogenesis, respectively.15 In situations of energy distress, FOXO1 is upregulated and more active, thereby contributing to the maintenance of blood glucose levels.15 Under such conditions, the restriction of angiogenesis by FOXOs might help to redirect glucose and nutrient supply to tissues that are indispensable for life.

Intriguingly, many of the above-mentioned “vascular-metabolic regulators” are controlled by acetylation. Indeed, PGC1α, LKB1, and FOXOs are acetylated proteins that are deacetylated by SIRT1 in many tissues including ECs. Metabolism itself codetermines protein acetylation as the metabolic flux through glycolytic pathways and the tricarboxylic acid cycle supplies the acetyl-groups (acetyl-CoA) required for acetylation reactions.63 These observations raise the possibility that changes in lysine acetylation provide an important regulatory mechanism in the cross-talk of angiogenesis and metabolism signaling. By deacetylating nodal regulators of angiogenesis and metabolism, SIRT1 may act as an integrator of vascular-metabolic signaling to support tissue revascularization in situations of nutrient and oxygen scarcity.

FOXOs and Sirtuins in Vascular Maintenance and Homeostasis

In healthy adults, most vessels are quiescent, forming stable conduits with a streamlined inner surface.3,4 Genetic studies in adult mice revealed that FOXOs are essential regulators of vascular quiescence48 (Figure 4). Conditional mutant mice that globally lack FOXO1, FOXO3, and FOXO4 spontaneously develop massive EC overproliferation that leads to widespread hemangiomas, hemorrhage, and premature death of the animals.48 These findings suggest that ECs are exquisitely sensitive to alterations in FOXO signaling and that adequate FOXO activity is required to keep endothelial quiescence. FOXOs control endothelial quiescence by governing the expression of genes with key roles in endothelial growth and morphogenesis including the general tyrosine kinase inhibitor SPROUTY2.48

Table 2. Phenotypes of Sirtuin Mutant Mice

<table>
<thead>
<tr>
<th>Gene</th>
<th>Knockout Phenotype</th>
<th>Reference</th>
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<tbody>
<tr>
<td>SIRT1</td>
<td>Most mice die perinatally, smaller in size, eye abnormalities, bone, cardiac, and pancreatic defects, infertility</td>
<td>McBhury et al., 2003</td>
</tr>
<tr>
<td>SIRT2</td>
<td>Developmentally normal, cancer prone</td>
<td>Kim et al., 2010</td>
</tr>
<tr>
<td>SIRT3</td>
<td>Developmentally normal</td>
<td>Lombard et al., 2007</td>
</tr>
<tr>
<td>SIRT4</td>
<td>Developmentally normal</td>
<td>Haigis et al., 2010</td>
</tr>
<tr>
<td>SIRT5</td>
<td>Developmentally normal</td>
<td>Nakagawa et al., 2009</td>
</tr>
<tr>
<td>SIRT6</td>
<td>Death at 4 wk, premature aging phenotype (kyphosis, osteopenia, loss of subcutaneous fat, lymphopenia, hypoglycemia)</td>
<td>Mostoslavsky et al., 2006</td>
</tr>
<tr>
<td>SIRT7</td>
<td>Reduced lifespan, premature aging phenotype (kyphosis, loss of subcutaneous fat), degenerative cardiac hypertrophy, and inflammatory cardiomyopathy</td>
<td>Vakhrusheva et al., 2008</td>
</tr>
</tbody>
</table>
In mature vessels, ECs have to form barriers between the blood and the surrounding tissue to control the exchange of fluids and to regulate the entry of immune cells. Essential for this barrier function is the ability of ECs to regulate cell-cell adhesions between themselves and neighboring cells. Several transmembrane adhesive proteins have been identified including vascular endothelial (VE) cadherin at adherens junctions (AJs), and claudin-5 at tight junctions (TJs). The formation, maintenance and remodeling of these intercellular contacts requires a functional interaction between AJs and TJs. Interestingly, this interaction involves FOXO1 which has been shown to repress claudin-5 expression in a β-catenin–dependent manner (Figure 4). This repressive effect is antagonized by VE-cadherin–mediated EC adhesion, which promotes nuclear exclusion of FOXO1 via activation of the PI3K pathway.

ECs must adopt survival properties in quiescent vessels to maintain the integrity of the inner vessel lining. In addition to hemodynamic forces, autocrine and paracrine survival signals from ECs and MCs protect the vessel from environmental stress signals. The PI3K-AKT pathway is a pivotal endogenous survival pathway that inhibits apoptosis in part by blocking the expression of proapoptotic FOXO target genes and vessel destabilization (MMP7, expression of FOXO target genes involved in apoptosis activation of the PI3K-AKT pathway, thereby blocking the expression of proapoptotic FOXO target genes (Figure 4). Endothelial survival cues such as angiopoietin-1 (ANG1), VEGF, or shear stress inactivate FOXOs through PI3K-AKT signaling. SIRT1 can also limit the antiangiogenic effects of FOXO1 in ECs through FOXO1 deacetylation. Depending on the target gene promoter, SIRT1 can promote or repress FOXO-dependent gene expression.

**FOXOs and Sirtuins Are Controlled by Blood Flow**

Blood vessels are constantly subjected to hemodynamic forces induced by pulsatile blood pressure and flow. ECs are in direct contact with the flowing blood and bear most of the hemodynamic forces, which in turn also modulate their functions. For instance, laminar flow upregulates genes that protect vessels from atherosclerosis and keeps them quiescent, dilated and free of clots. One of the key transcription factors that govern the expression of these flow-responsive genes is Krüppel-like factor 2 (KLF2), which induces vasodilatory, anti-inflammatory, and anticoagulant proteins such as endothelial nitric oxide synthase (eNOS) and thrombomodulin. Recent studies suggest that KLF2 is regulated by SIRT1. Treatment of ECs with resveratrol, a polyphenol compound that (indirectly) activates SIRT1, enhances the expression of KLF2 and its target genes by promoting MEK5/MEF2 signaling. Remarkably, SIRT1 expression itself is enhanced by flow resulting in increased SIRT1-mediated deacetylation and activation of eNOS. By synergistically modulating KLF2 and its effector eNOS, SIRT1 is able to protect vessels from inflammatory and procoagulant stress signals (Figure 5).

Emerging evidence indicates that FOXOs are an integral component of the signaling network that transduces the endothelial response to flow. Studies in cultured ECs revealed that laminar flow prompts PI3K-AKT-dependent inactivation of FOXO1. AMPK has also been implicated in flow-mediated inhibition of FOXO1. Whether this inhibitory effect requires the direct phosphorylation of FOXO1 by AMPK must be determined.

**Sirtuins and the Response to Hypoxia**

In resting vessels, ECs acquire barrier functions and rarely migrate or proliferate to enable efficient tissue perfusion. This phenotype with cobblestone-like appearance has been termed “phalanx” phenotype because of its resemblance to the ancient Greek military formation. The phalanx phenotype is regulated by prolyl hydroxylase domain protein 2 (PHD2), an oxygen sensor that controls signaling by hypoxia-inducible factors (HIFs). Three HIF isoforms exist (HIF-1α, HIF-2α, FIH), which orchestrate adaptive responses to changes in oxygen tension by regulating the expression of genes involved in cell survival, metabolism, and angiogenesis. In normoxia, HIFs are hydroxylated by PHDs in an oxygen-dependent manner, which primes HIFs for proteasomal degradation. Under hypoxic conditions PHDs become progressively inactive allowing HIFs to escape degradation. Partial inactivation of PHD2 streamlines the abnormal endothelium in tumor vessels that otherwise impairs perfusion. This endothelial normalization toward the phalanx phenotype depends, in part, on the HIF-2α–mediated induction of the VEGF decoy receptor VEGFR1 (inhibiting EC sprouting) and the junctional protein VE-cadherin (stabilizing vessels). Intriguingly, PHD2 and HIF-2α are regulated by SIRT1, which activates HIF-2α through deacetylation, while promoting PHD2 degradation, together resulting in enhanced HIF-2α signaling (Figure 6). Since SIRT1 expression and activity increase during hypoxia in an HIF-dependent manner, SIRT1 activation might favor an endothelial phenotypic shift toward a phalanx-like phenotype in hypoxic conditions. Whether such a cooperative regulation of HIF signaling is operational in ECs remains to be elucidated. However, given the well-established function of SIRT1 as a sensor of cellular metabolism, it is tempting to speculate that SIRT1 integrates metabolic and hypoxia signaling to...
coordinate the vascular response to oxygen- and nutrient-deprivation.

Several other sirtuins have been shown to interfere with HIF signaling76 (Figure 6). For example, the chromatin-associated deacetylase SIRT6 acts as a corepressor of HIF-1α by deacetylating histones at HIF-1α–responsive promoters.82 The mitochondrial-localized sirtuin, SIRT3, attenuates HIF-1α activity indirectly by controlling intracellular ROS levels.83 Unlike HIF-2α, HIF-1α activity is inhibited by SIRT1 in a deacetylation-dependent manner.84 Together, these results suggest a central regulatory function of sirtuins in the cellular response to hypoxia.

FOXOs and Sirtuins in the Aging Vasculature

Aging blood vessels become thicker, stiffer, and less plastic, resulting in a reduced ability to adjust vessel shape and function to changing tissue demands. Hallmarks of vascular aging are endothelial senescence and inflammation that compromise vasomotor function as well as vessel growth and repair. Underlying these hallmarks are increasing oxidative stress, telomere shortening, DNA damage, and mitochondrial dysfunction, which set in motion a detrimental cycle of endothelial damage. Although it is not clear whether FOXOs and sirtuins control vascular aging directly, it is an intriguing possibility that alterations in FOXO and sirtuin signaling pathways contribute to the functional decline of aged blood vessels.

Endothelial Aging Phenotypes Are Regulated by Sirtuins and FOXOs

Senescence, particularly in the endothelial lining, is intimately linked to many of the age-associated phenotypes in the vascular wall. Senescence is a stress response characterized by an inhibition of cell proliferation, which often becomes irreversible and independent of the initiating stress signal.85 Senescence occurs in most cell types after prolonged propagation in culture and is a consequence of telomere attrition after repeated cell divisions.86 In addition to telomere shortening, stressors such as genomic instability, DNA damage, and oxidative stress cause a similar cell cycle arrest and trigger senescence.85 Senescent cells exhibit alterations in morphology and gene expression that extinguish essential cellular functions. In ECs, these alterations evoke a phenotype that boosts inflammation, thrombosis, and atheroscle-
Nitric Oxide Signaling in Aging Vessels Is Regulated by SIRT1

When ECs age, levels of endothelial-derived nitric oxide (NO) diminish. This decline in NO availability is a consequence of an impaired eNOS activity as well as of a progressive NO inactivation through increasing levels of superoxide anions. As a result, aged vessels exhibit compromised vasodilatory properties that cause increased vascular resistance and impaired perfusion. Moreover, reduced NO bioavailability prompts vascular inflammation and atherogenesis and impedes endothelial repair. ECs therefore must be equipped with a molecular machinery to maintain proper eNOS function. Not surprisingly, given the important antisenescent effects of SIRT1 in ECs, SIRT1 is part of this machinery (Figure 5). Indeed, SIRT1 has been shown to deacetylate eNOS, thereby augmenting NO production. Notably, the reverse regulation is also true with NO modulating SIRT1. eNOS-derived NO increases on CR and augments SIRT1 expression indicating that SIRT1 and eNOS form a signaling circuit to preserve EC function.

Regulation of FOXOs and Sirtuins in Aging Blood Vessels

The vital functions of (selected) FOXO and sirtuin members in the vascular wall imply that deregulation of their expression and/or activity could accentuate or even trigger aging phenotypes. An example is the modulation of SIRT1 expression by microRNAs (miRs). miR-217 expression is progressively enhanced in aging ECs, in which it promotes senescence and impairs angiogenesis signaling. These effects are mediated by a miR-217–dependent downregulation of SIRT1 (Figure 5). Another miR that targets SIRT1 is miR-34, which induces apoptosis and endothelial senescence in a SIRT1-dependent manner (Figure 5). SIRT1 expression is also extensively regulated by several transcriptional complexes (HIC1, CtBP, p53, E2F), and its activity is modulated by posttranslational modifications (phosphorylation, sumoylation) and through protein–protein interactions (DBC1, AROS). Whether these mechanisms modulate SIRT1 during aging remains to be elucidated.

Changes in FOXO activity might similarly contribute to endothelial aging. In healthy vessels, FOXO activity must be balanced and tightly controlled. Indeed, ECs must maintain a certain level of FOXO signaling to keep quiescence, while at the same time prevent excessive FOXO activation that causes apoptosis. Stress-induced changes in the extent and dynamics of FOXO signaling might thus shift EC responses toward apoptosis and vessel dysfunction. In this context, it is interesting to note that FOXOs are strongly regulated by oxidative stress, which is considered to be a main driver of the aging process. Oxidative stress can alter FOXO activity through a multitude of PTMs including phosphorylation (by JNK, ERK), acetylation (p300, CBP, SIRT1), and ubiquitylation (MDM2, SKP2). Furthermore, FOXOs can be directly oxidized on cysteine residues under oxidative stress conditions, thereby altering their acetylation status and transcriptional activity.

SIRT1 in Atherosclerotic Disease

Atherosclerosis is a chronic, age-associated disease of the arterial wall that involves complex cell-cell interactions and alterations in cholesterol homeostasis that leads to vessel narrowing and ischemia. Studies in mouse models of atherosclerosis have suggested that SIRT1 acts as a potent antiatherogenic factor. Transgenic overexpression in ECs or pharmacological activation by resveratrol significantly reduced atherosclerotic plaque formation. SIRT1 counteracts atherogenesis by cell-autonomous and nonautonomous...
effects. In ECs, SIRT1-mediated activation of eNOS\textsuperscript{110} and inhibition of proinflammatory NFκB signaling\textsuperscript{120} reduce the susceptibility for atherosclerotic lesion formation\textsuperscript{110,120} (Figure 5). Cell-nonautonomous effects of SIRT1 on atherogenesis include effects on glucose and lipid metabolism. SIRT1 improves glucose metabolism and insulin resistance in type 2 diabetes, another pivotal risk factor for atherosclerosis. SIRT1 increases insulin sensitivity through repression of the tyrosine phosphatase PTP1B\textsuperscript{121–125} and promotes insulin secretion in pancreatic β cells by suppression of UCP2.\textsuperscript{126} Moreover, SIRT1 facilitates cholesterol clearance from the vessel wall by deacetylating the nuclear receptors LXR\textsuperscript{127} and FXR,\textsuperscript{128} 2 key regulators of reverse cholesterol transport. Contrary to these findings, a recent study reported a proatherogenic lipid profile in mice that ubiquitously overexpress SIRT1, which fueled atherosclerotic lesion formation.\textsuperscript{129} Thus, SIRT1 has pleiotropic effects on atherosclerosis and may affect atherogenic plaque formation in a tissue- and context-dependent manner. The potential impact of FOXOs on atherosclerosis remains enigmatic, as their role in this process has not been specifically investigated.

**Dietary Restriction, Vascular Health, and the Connection to Sirtuins and FOXOs**

In addition to genetics, environmental factors play major roles in determining whether vessels remain healthy and plastic or become diseased and age prematurely. A prime example for the environmental influence on vascular health is nutrition. Although overeating calories is an eminent risk factor for almost all vascular diseases, restricting calorie intake is beneficial and prevents many age-related diseases including hypertension, atherosclerosis and ischemic heart disease. CR, a dietary regime of reduced food intake without malnutrition, leads to an increase in life and health span in a broad range of species, including yeast, flies, worms, fish, and rodents.\textsuperscript{18,130} Many of these favorable effects also apply to nonhuman primates and humans. A study in rhesus monkeys revealed that long-term CR delays mortality and the onset of diabetes, cardiovascular disease, cancer, and neurodegeneration.\textsuperscript{131} How CR extends lifespan is far from complete, and numerous questions remain. For instance, the many demonstrated roles of FOXOs in ECs have made it challenging to generate a unifying model of how FOXOs operate. Existing evidence suggests that ECs reiteratively utilize FOXO signaling at various stages of development and even in the adult, to control endothelial growth, morphogenesis, and survival. How these diverse functions are coordinated and how specificity is achieved cannot be easily reconciled at this point. However, an intriguing possibility is that combinations of PTMs specify FOXO responses by determining selective cofactor recruitment and target gene selection.\textsuperscript{25} Defining such a “molecular code” of FOXO regulation will provide insights into how FOXOs respond to a combination of environmental signals they encounter and will aid the understanding of signal integration during vessel growth and maintenance.

The numerous proteins deacetylated by SIRT1 also pose hurdles to the identification of those targets that are relevant for SIRT1’s effects on blood vessels. The ever-growing list of SIRT1-deacetylated proteins suggests that SIRT1 does not exert its function through profound regulation of a single target but rather through modulation of multiple targets that sometimes act at different levels of a pathway. In this sense, SIRT1 appears to act as a “fine tuner” that adjusts the activity of pathways that are inappropriate for a particular environmental setting. Surprisingly, the role of other sirtuins in vascular physiology has not been investigated thus far. As highlighted above, a number of sirtuins have emerged as key regulators of metabolism and stress resistance and are likely to have shared functions in the vasculature as well. Examples include SIRT3, a mitochondrial sirtuin associated with the development of metabolic syndrome,\textsuperscript{133} as well as SIRT6, a chromatin-associated sirtuin, which transmits its signals through histone deacetylation.\textsuperscript{82} Identifying their roles in normal and diseased blood vessels will provide new perspectives on the emerging cross-talk between vessels and metabolism, and we expect rapid progress in this field of research in the coming years.

Based on the above outlined findings, the implications for numerous angiogenesis- and vascular-related pathologies, including ischemic heart and brain disorders, blinding eye disease, and cancer, are apparent, and future studies on the roles of FOXOs and sirtuins in these disease settings are warranted. These studies also must address whether abnormal FOXO and/or sirtuin signaling is a cause or consequence of aging and how such dysregulation is linked to the onset and progression of the above-mentioned age-related maladies. Finally, a better understanding of FOXO and sirtuin biology will undoubtedly aid the development of strategies to target FOXOs and sirtuins therapeutically in the context of proangiogenic and antiangiogenic medicine.

**Conclusions and Perspectives**

Despite advances in understanding the physiological roles of FOXOs and sirtuins in vascular development and disease, our knowledge of how individual FOXOs and sirtuins function is far from complete, and numerous questions remain. For instance, the many demonstrated roles of FOXOs in ECs have been reconciled at this point. However, an intriguing possibility is that combinations of PTMs specify FOXO responses by determining selective cofactor recruitment and target gene selection.\textsuperscript{25} Defining such a “molecular code” of FOXO regulation will provide insights into how FOXOs respond to a combination of environmental signals they encounter and will aid the understanding of signal integration during vessel growth and maintenance.

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