Transcription Factor and Kinase-mediated Signaling in Atherosclerosis and Vascular Injury

Neeta Adhikari, PhD, Nathan Charles, BSc, Ute Lehmann, BS, and Jennifer L. Hall, PhD

Corresponding author
Jennifer L. Hall, PhD
University of Minnesota, Cardiovascular Division, Mayo Mail Code 508, 420 Delaware Street SE, Minneapolis, MN 55455, USA.
E-mail: jlhall@umn.edu

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Our understanding of the molecular signaling pathways regulating the initiation and progression of atherosclerosis or remodeling in response to injury has begun to cross the boundaries from regulation of well-described canonical pathways to the interplay between these pathways. The focus of this review is to summarize our current understanding of a finite group of transcription factors and kinases involved in vascular injury and atherosclerosis, including nuclear factor-κB (NF-κB), early growth response factor-1 (Egr-1), activator protein-1 (AP-1), hypoxia inducible factor-1α (HIF-1α), homeobox, and T cell factor/lymphoid enhancer factor (Tcf-Lef), as well as the kinases janus kinase/signal transducers and activators of transcription (JAK/STAT), protein kinase C (PKC), p38, Rho, ERK5, JNK, p44/p42, and phosphoinositide 3 (PI3) kinase/AKT.

Introduction

Our current understanding of the signaling pathways that regulate remodeling and adverse events in the context of vascular disease continues to expand. Genomic and proteomic-based strategies in humans and rodent models have been instrumental in discovering genes and proteins involved in the initiation and progression of atherosclerosis and remodeling in response to injury. In addition, multiple genome-wide approaches are underway to identify variants in the human genome that predispose to, or protect from, cardiovascular disease. The International HapMap project which began in 2002 and includes blood samples from people in Nigeria, Japan, China; people with northern and western European ancestry; and people in the United States, as well as the identification of a panel of ancestral informative markers and admixture approaches, will likely provide a giant leap forward in sorting out the interactions of multiple genes and the environment in cardiovascular disease. Pairing this human genetic data with testing for functionality in the laboratory will be critically important to improving our understanding of the molecular pathways involved in vascular remodeling. This creates an intellectually stimulating work environment and opportunity.

The first goal of this review is to provide an overall look at our current understanding of a finite group of transcription factors and kinases involved in atherosclerosis and vascular injury including nuclear factor-κB (NF-κB), early growth response factor-1 (Egr-1), activator protein-1 (AP-1), hypoxia inducible factor-1α (HIF-1α), homeobox, and T cell factor/lymphoid enhance factor (Tcf-Lef), as well as the kinases janus kinase/signal transducers and activators of transcription (JAK/STAT), protein kinase C (PKC), p38, Rho, ERK5, JNK, p44/p42, and phosphoinositide 3 (PI3) kinase/AKT. The second goal is to present the information in a format that allows the readers to think broadly and identify potential points of convergence between pathways. Due to the wealth of publications, we have focused mainly on in vivo studies and regret we were unable to include a review of the phosphates, whose role is also important. We apologize to our colleagues whose primary work we were unable to include in this review. Readers are encouraged to seek out the original papers for a more thorough examination of each target.

Transcription Factors

The bulk of our understanding of transcription factors involved in the initiation and progression of atherosclerosis and the vascular response to injury is from rodent models, whose lipid profile is dissimilar to that of humans. This is a significant limitation recognized by the

field that has spurred several new approaches to understanding the transcription factors involved in human disease and remodeling. One of these approaches is to utilize an unbiased gene expression profiling strategy to look at changes in mRNA expression and/or a SNP chip platform to identify mutations in genes. The CardioGene study [1] was designed with this in mind. The goals of CardioGene are to identify the genetic determinants of restenosis and define the molecular signaling pathways involved in vascular remodeling in response to injury [1]. Another example of an ongoing human project is the Atherosclerosis Risk In Communities study (ARIC), which is designed to estimate patterns and trends of coronary heart disease incidence, fatality, and mortality in four communities. DNA was collected in these patients along with a host of phenotypic measurements. These human studies will provide an opportunity to identify mutations in genes that alter gene expression and influence initiation and progression of vascular disease. Utilizing DNA, RNA, and multiple sensitive phenotypic markers together will allow us to pinpoint critical transcription factors that may serve as regulatory nodes in the process of disease. The advent of new bioinformatics programs and application technology permits the mining of this data to identify transcription factors based on the search and rescue of common DNA binding elements in promoter regions of genes.

Nuclear factor-κB (NF-κB)

Nuclear factor-κB is commonly used to denote a family of transcription factors that include p65, c-Rel, rel-B, p50, and p52. The most abundant combination is the p65p50 heterodimer. NF-κB is a key regulator of inflammation and immune responses and is thought to be involved in multiple steps in the progression of atherosclerosis, including initiation of monocyte adhesion, foam cell formation, inflammation, cell death, and migration and proliferation [2••]. NF-κB has been implicated as a common regulatory node induced by multiple upstream factors including low-density lipoprotein (LDL) and 12/15-lipoxygenase, which in turn increase the expression of both cellular adhesion molecules and the number of tethered monocytes to the endothelium [3,4]. A significant component of oxidized LDL, 13-hydroperoxyoctadecadienoic acid, also stimulates NF-κB in vascular smooth muscle cells [5]. NF-κB binds to and induces the expression of several adhesion molecules and cytokines, including P-selectin, E-selectin, intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), interleukin (IL)-6 and IL-10, tumor necrosis factor, IL-1β, IL-12, interferon-γ, and monoycte chemotactic protein-1 [2••].

Nuclear factor-κB has been shown to be active in human lesions [6,7], as well as large and small animal models [8–10], and is localized in endothelial cells, macrophages, and smooth muscle cells within the lesion [6]. A new generation of studies has shown that statins significantly diminish NF-κB activation in human vascular endothelial cells [11].

To date, there are no human data linking a polymorphism in the NF-κB family of transcription factors with risk of developing or altering the progression of vascular disease. A host of in vitro studies in vascular smooth muscle cells, endothelial cells, monocytes, and macrophages have been conducted in which NF-κB has been implicated in vascular remodeling [2••].

In addition to its role in the regulation and control of both pro- and anti-inflammatory signaling pathways, NF-κB promotes endothelial cell survival [2••,12,13]. These data resonate with work showing that NF-κB is essential for T and B cell survival [2••].

Genetic studies have shown that compromising NF-κB activation (50% reduction) specifically in macrophages with a macrophage-restricted deletion of IκB kinase 2 results in more severe atherosclerosis in mice [14]. In contrast, inhibition of NF-κB activity in hematopoietic cells (which was accomplished by reconstituting $LDLR^{-/-}$ mice with bone marrow deficient in the NF-κB1 gene encoding for p50) resulted in smaller atherosclerotic lesions with an inflammatory phenotype [15]. A pharmacologic approach in which pyrrolidine dithiocarbamate (a well-known inhibitor of NF-κB) was administered to apolipoprotein E (apoE)/LDLR double-knockout mice attenuated atherogenesis [16]. In sum, the data suggest an important role for NF-κB in the context of remodeling. It is likely that both temporal and spatial distribution of NF-κB within the lesion is important and that a more subtle rearrangement of NF-κB localization or timing of expression may be an important factor in governing the response to injury or progression of atherosclerosis.

Hypoxia inducible factor-1α (HIF-1α)

Hypoxia inducible factor-1α also likely plays a role in atherosclerosis and vascular injury; however, our understanding is limited. HIF-1α is perhaps best known for its ability to stimulate proangiogenic genes, including VEGF. However, recent work by our laboratory and others suggests that HIF-1α may also stimulate expression of tyrosine kinase inhibitors that inhibit angiogenesis, including Sprouty1 [17].

Hypoxia inducible factor-1α is induced in the microvasculature of hypercholesterolemic pigs [18,19]. Recent reviews suggest that blocking HIF-1α may inhibit lesion formation by prohibiting angiogenesis from the vasa vasorum of the injured vessel [20]. A recently identified HIF-1α–responsive gene is Hmox1. Hmox2 encodes the protein heme oxygenase-1, which is activated in response to inflammation and inhibits proliferation [21]. Recent work by Duckers et al. [21] utilizing gene transfer to overexpress Hmox-1 in the pig lesion as well as deletion of the Hmox-1 locus suggests that this HIF-1α responsive target inhibits cellular proliferation and protects against vascular constriction [21].

Early growth response factor-1 (Egr-1)

Early growth response factor-1 is a zinc finger transcription factor recently implicated in atherosclerosis and restenosis [22,23]. Egr-1 is a phosphoprotein expressed in endothelial cells, smooth muscle cells, monocytes, and macrophages [22,24,25]. Shear stress, mechanical stress, hypoxia, and acute tissue injury have all been shown to induce Egr-1 expression through a variety of kinase-mediated pathways, including, but not limited to, ERK, JNK, and p38 signaling [22,24,25]. In the atherosclerotic lesion, Egr-1 appears to be expressed mainly within smooth muscle cells of the fibrous cap as well as macrophages and endothelial cells [26,27]. Expression of Egr-1 is significantly up-regulated in the atherosclerotic vessel compared to normal vessels [27].

Multiple lines of evidence suggest that blocking Egr-1 with antisense oligodeoxynucleotides inhibits smooth muscle cell proliferation in vitro, during neointima formation in rat carotid arteries, and in post-stent placement in pig coronary arteries [28–30]. Santiago et al. [31] recently confirmed these findings in a rat model of balloon injury using an alternative approach to knock down Egr-1 levels with a novel DNA enzyme that cleaved Egr-1 mRNA and demonstrated an inhibition of neointimal formation in a rat model of balloon injury. Taken together at this stage, Egr-1 appears to regulate genes that stimulate lesion formation.

Activator protein-1 (AP-1)

Activator protein-1 (AP-1) is a transcription factor implicated in vascular disease that results from the heterodimerization of Fos and Jun proteins. Mechanical stretch, like that which occurs with angioplasty, has been shown to activate PKC and mitogen-activated protein kinase (MAPK), which in turn leads to c-fos and c-Jun gene expression and activation of AP-1 [32]. Oxidative stress and cytokines stimulate JNK, which in turn regulates c-Jun and hence AP-1. Gene delivery of a vector containing a dominant negative c-Jun inhibited smooth muscle cell proliferation and neointimal hyperplasia in rats [33]. In addition, delivery of an AP-1 decoy oligodeoxynucleotide resulted in a similar response in rats [34]. Use of tissue-specific genetic models will be helpful to provide more evidence of the role for AP-1 in vascular disease.

Homeobox transcription factors (HMF)

Homeobox transcription factors (HMF) are important in the regulation of cell proliferation, migration, and differentiation. HMF proteins play a pivotal role in embryonic as well as in disease state cardiovascular remodeling [35]. Genetically deleting murine HOXA3 (a HOX cluster gene) or PrX1/PrX2 resulted in vascular anomalies [36–38]. The HOX cluster gene HOXB7 mRNA was detected in human atherosclerotic plaques at a higher level that in the normal human artery wall [39]. Overexpression of the homeobox gene Gax inhibited intimal hyperplasia in vivo in injured rat vessels [40,41]. Little is known about the mechanisms by which these proteins regulate cellular proliferation, migration, and differentiation.

T cell factor/lymphoid enhancer factor (Tcf/Lef)

T cell factor (TCF) and lymphoid enhancer factor-1 (LEF-1) transcription factors belong to a family of high mobility group domain proteins and include LEF-1, TCF-1, TCF-3, and TCF-4. This family of transcription factors is best known for their role in cancer biology, as they regulate expression of cyclin D1 and c-myc genes involved in cell proliferation and cell death [42]. Interestingly, recent work from the field of genetics suggests that a variant in the gene that encodes Tcf-4 (TCF7L2) confers risk of type 2 diabetes [43]. These factors are known to play an important role in the Wnt/Wg signaling pathway, which controls proliferation, apoptosis, and migration. TCF factors become potent transactivators upon interaction with the WNT signaling molecule beta-catenin. Reports from our laboratory have demonstrated the involvement of the beta-catenin/TCF pathway in vascular remodeling [44–46]. We demonstrated significant temporal expression of beta-catenin in the intimal lesion following vascular injury in rats [46]. Modeling this in vitro, we demonstrated that stabilization of beta-catenin activated Tcf-4, resulting in transactivation of cyclin D1, increased vascular smooth muscle cell proliferation, and inhibition of apoptosis [46]. The potential relevancy of this Tcf/Lef pathway to lipid binding and atherosclerosis was demonstrated with data showing that LRP6, an LDL receptor-related protein, significantly potentiated Tcf/Lef transactivation [45]. Interestingly, our laboratory also demonstrated cross talk between the Tcf-4 signaling pathway and NF-κB signaling, suggesting another role for Tcf-4 in immune- and inflammatory-mediated signaling in both atherosclerosis and restenosis.

Kinases

Janus kinsase/signal transducers and activators of transcription (JAK/STAT)

The janus kinases (JAK) and signal transducers and activators of transcription (STAT) factors have been implicated in AT1-mediated vascular smooth muscle cell growth [47–49] as well as interaction of the apolipoproteins with adenosine triphosphate–binding cassette protein A1 (ABCA1), thereby regulating cholesterol metabolism and trafficking (Fig. 1) [50]. In addition, mechanical stretch activates STAT3. A review on the role of JAK/STAT signaling in the vasculature was recently published [51]. This pathway represents a series of four kinases (JAK1, JAK2, JAK3, and TYK2) linked to a family of transcription factors (STAT, with seven family members identified to date). Activation of JAK signaling occurs through the multimerization of two JAKs permitting trans-phosphorylation [51]. The JAKs then phosphorylate a conserved tyrosine residue near the C-terminus that permits dimer-

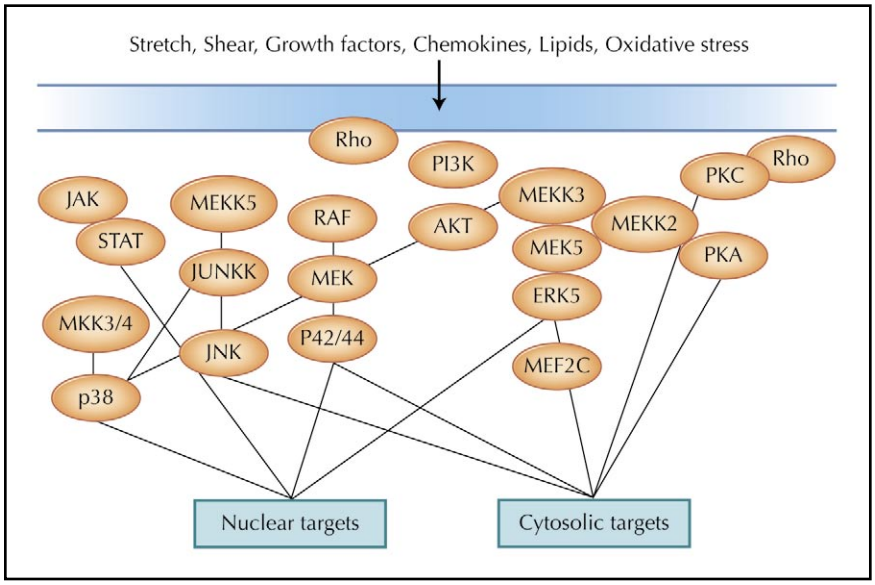

Figure 1. Model of stretch, shear, growth factors, chemokines, lipids, and oxidative stress.

ization of STATs. The link between the AT1-mediated G protein–coupled receptor and tyrosine kinase activity is not understood. Work by Schieffer et al. [49] provided evidence that activation of the JAK/STAT pathway by angiotensin II required superoxide anions generated by the NADPH oxidase system [49] (for a more thorough review on NADPH oxidase signaling linked to kinases the reader is encouraged to read several excellent reviews [52–54]). Angiotensin II has been shown to stimulate inflammatory signaling cascades (IL-6 being one of the best described) through the JAK/STAT pathway [49]. Both angiotensin II and IL-6 have been co-localized in atherosclerotic lesions [55]. In addition to IL-6, activation of STAT3 also induces IL-10 and IL-27 [56]. Interestingly IL-10 has been suggested to be a protective cytokine.

JAK2 has also been implicated in apolipoprotein interactions with ABCA1, which is required for removing cellular cholesterol [50]. These studies were conducted with the JAK inhibitor AG490. Finally, mechanical stretch has been shown to induce both tyrosine- and serine-dependent phosphorylation of STAT3 [56].

To our knowledge, genetic studies to define the role of the JAK/STAT family in atherosclerosis and vascular remodeling have not been done and will likely be the next step in solidifying the role of this family of kinases and transcription factors in vascular disease. Utilizing a genetic approach to identify the role of JAK/STAT in different cell types will be of great interest, particularly in macrophages, endothelial cells, and VSMC.

Protein kinase C (PKC)

Protein kinase C comprises a superfamily of serine/threonine kinases that are activated by phosphorylation and subsequent binding to the second messenger diacylglycerol. To date, 12 isoenzymes have been identified that play vital roles in a variety of cellular functions. Different PKC isoform-null mice have exhibited impairment of cellular functions, especially in the central nervous, immune, and cardiovascular systems [57,58]. Recently, Andrassy et al. [59] showed that PKCβII-null mice showed decreased intimal hyperplasia in response to injury. Similar findings were seen in wild-type mice treated with the PKCβ inhibitor ruboxistaurin [59]. These studies also showed that PKCβII was involved in vascular smooth muscle cell activation in part through ERK and EGR-1 [59]. Leitges et al. [60] showed that PKC δ-null mice showed reduced arteriosclerosis as compared with the wild-type littermates in a vein graft model. The mechanisms responsible for this are unclear because vascular smooth muscle cells from aortas of PKCδ$^{-/-}$ mice were significantly more resistant to apoptosis (induced by multiple stimuli) compared with controls, whereas mitogenic potential was unchanged [60]. Generation of tissue-specific PKC knockout models will be important in furthering our understanding of the role of PKC in vascular disease [61] and broadening our focus to include inflammatory cells as well as endothelial and smooth muscle cells.

p38

The p38 family consists of four isoforms: α, β, γ, and δ. P38α and β are widely expressed and in general are activated in response to oxidative stress, inflammatory cytokines, and growth factors. Balloon injury in a rabbit and rat results in phosphorylation of p38 within minutes that remains elevated out to 28 days [61,62]. Two distinct pharmacologic compounds have been used to compromise p38 activity in the setting of vascular injury [61,62] and have resulted in decreased lesion formation, inhibition of vascular smooth muscle cell proliferation, and attenuation of lipopolysaccharide-mediated IL-1β mRNA expression [61,62]. Targeted deletion of both p38α and β has been performed, resulting in defective blood vessel development in the p38α-null mouse [63] and no obvious defects in the p38β model [64]. Again, tissue-

specific knockout models of p38α and β will be helpful in furthering our understanding of the role of p38 in inflammation and vascular remodeling.

Rho

The Rho family of small GTPases consists of RhoA, RhoB, and RhoC. For a more thorough review the reader is directed toward a recent review by Seasholtz and Brown [65]. RhoA is the most widely studied of the family and most of the work strongly supports a role for RhoA in vascular smooth muscle cell migration, growth, and lesion formation [3,65–77]. Work done in vivo with both gene transfer of a dominant negative Rho kinase following balloon injury in pigs [73] and pharmacologic inhibitors of Rho kinase [65,68–72] suggests that blocking Rho kinase inhibits lesion formation. Mallat et al. [74] also demonstrated that long-term inhibition of Rho kinase with the inhibitor Y-27632 led to decreased lesion formation in $LDLR^{-/-}$ mice.

ERK5

The role of ERK5 (or Big MAPK) in atherosclerosis and vascular injury is not well understood. ERK5 is induced by growth factors, shear stress, and oxidative stress [78,79]. The ERK5 signaling pathway is thought to be activated by Mekk3 and Mek5 [78]. Activation of ERK5 has been shown to protect endothelial cells from survival while stimulating migration and proliferation of vascular smooth muscle cell [78]. Interestingly, mice deficient in the upstream modulators of ERK5-Mekk3 or ERK5 exhibited severe defects in vascular development [80,81]. Global deletion of ERK5 resulted in defective blood vessel and cardiac development [80]. Vasculogenesis occurred in these animals but vessels exhibited a disorganized pattern, with many lacking appropriate density of vascular smooth muscle cells [80]. Recent work has shown that ERK5 regulates angiogenesis in part through HIF-1α–dependent regulation [82]. Splice variants of ERK5 have also been identified [83]. However, the regulation and role of these spliced variants is not entirely clear. Hayashi et al. [84] generated a conditional deletion of ERK5 that succumbed to death 2 to 3 weeks after induction of the gene that correlated with leaky and disrupted vessels. An endothelial-specific knockout of ERK5 had similar vascular and heart disruptions to that seen in the ERK5 knockout [84], whereas the mouse harboring cardiac myocyte specific deletion of ERK5 did not exhibit the heart defects [84]. To our knowledge, a genetic model lacking ERK5 in smooth muscle has not been published. An identified downstream transcription factor of ERK5 is myocyte enhancer factor 2c (MEF2c), and mice deficient in this gene harbor severe defects in cardiovascular development and vascular organization [78,85]. Components of the disorganized vascular phenotype in the MEF2c-null animals closely match those seen in the $ERK5^{-/-}$ mouse, further suggesting that ERK5 signals through MEF2c. Other substrates of ERK5 that are less understood include Sap1a (Ets domain transcription factor), SGF (serum and glucocorticoid inducible kinase), connexin43, and Bad [78]. ERK5 stimulation also stimulates transcriptional activation of cyclin D1, similar to p42/p44 MAPKs [78].

JNK

In general, the JNK family of kinases responds to oxidative stress and inflammatory cytokines [86]. JNK protein kinases are encoded by three genes: JNK1, JNK2, and JNK3. JNK1 and JNK2 are expressed ubiquitiously whereas JNK3 expression appears restricted to heart, brain, and testes [86]. Evidence to date suggests that four spliced isoforms exist for both JNK1 and JNK2 [86]. Activation of JNK is mediated through cytokine and/or stress-mediated stimulation of MAPK kinase kinase that activate MKK4 and MKK7, which in turn phosphorylate tyrosine and threonine residues within the activation domain of JNK. The best known factor downstream of JNK is c-Jun, which is part of the AP-1 transcription factor complex [86].

Mice deficient in either JNK1 or JNK2 are immunocompromised with specific defects in T-cell differentiation and cytokine production [87]. A murine model deficient in both JNK1 and JNK2 dies at embryonic day 11 with neural tube defects [88]. Ricci et al. [89] crossed the $JNK2^{-/-}$ mouse with an $ApoE^{-/-}$ mouse and demonstrated a deletion of atherosclerotic burden compared with $ApoE^{-/-}$ mice. Ricci et al. [89] then established mice with a targeted deletion of JNK2 specifically in macrophages and demonstrated a specific defect in foam cell formation that was linked to defective uptake and degradation of modified lipoproteins. Collectively, these genetic models have been instrumental in identifying a role for JNK2 in adaptive immunity and lipid metabolism. In turn, these findings have led to a better understanding of a role for adaptive immunity in the incidence and progression of atherosclerosis. Of all the kinases studied to date in the field of vascular remodeling, the JNKs are one of the best characterized.

p42/p44

p42/p44 Kinases have been shown to be activated in response to oxidative stress, growth factors, lipids, oxidative stress, and chemokines. It has been widely shown in vitro that stimulation of p42/p44 promotes proliferation, migration, and cell survival of vascular smooth muscle cell as well as endothelial cells. Activation of p42/p44 is regulated generally through binding of ligands to transmembrane receptors, with intrinsic protein tyrosine kinase activity leading to autophosphorylation and the association of adapter proteins such as Grb2 and Shc, which provide the molecular scaffolding and interactions for Ras, Raf, and MEK. Mice deficient in p44 (ERK1) do not display any defects in vascular development [90]. Deficiency in p42 (ERK2), however, is lethal and harbors placental defects [91]. To better understand the role of

p42 and p44 in vascular remodeling, we will need mice harboring conditional targeted deletions of these kinases. Deletions in the kinases K-Ras, A-Raf, $MEK1^{-/-}$ upstream of p42 and p44 result in lethality [91], whereas deletion of N-Ras, H-ras, and $MEK2^{-/-}$ are not lethal and do not possess any gross vascular defect. New areas of research likely to be relevant to vascular remodeling are oxidized LDL-mediated proliferation of macrophages through a p42/p44-dependent mechanism [92] as well as a link between cytokines NF-κB and ERK via a tumor progression locus 2 (TPL2; MAP3K8)-dependent link [93–95].

Phosphoinositide 3 (PI3) kinase/AKT

Phosphoinositide 3-kinases have been implicated in the regulation of cell proliferation, survival, metabolism, and differentiation. These kinases generate inositol phospholipids that act as second messengers on multiple targets, the best defined of which in vascular biology may be AKT. PI3K activates AKT through recruitment to the plasma membrane and phosphorylation by phosphoinositide-dependent kinase-1 (PDK1). Maximal activation of AKT requires phosphorylation at two regulatory sites (threonine 308/309/305) and serine 473/474/472. To date, three mammalian isoforms have been discovered: Akt1, 2, and 3 [96–98]. AKT is activated by multiple factors, including oxidative stress and growth factors [52]. New data suggest a role for statin therapy in promoting endothelial nitric oxide synthase (eNOS) activity through a PI3kinase/Akt-dependent signaling pathway [99,100].

Mice deficient in Akt1 exhibit enhanced angiogenesis, impaired vascular maturation, and impaired vascular permeability [101], whereas those deficient in Akt2 exhibit a severe diabetic phenotype [102]. Daly et al. [103] recently showed that Akt signals through the forkhead transcription factor [103]. Activation of forkhead has been shown to inhibit neointimal formation [104]. Recent findings suggest that Akt and forkhead may regulate an interface between longevity and tumor suppression [105••]. The surprisingly tumor suppression function of Akt is linked to its ability to activate the E3 ubiquitin ligase HDM2, resulting in degradation of nuclear factor of activated T cells (NFAT), an invasion promoting factor [105••]. More work will be needed to define the role of Akt and forkhead as well as other downstream factors (including NFAT) in vascular disease.

Conclusions

The use of tissue-specific and conditional genetic murine models to understand the role of transcription factors and kinases in vascular remodeling and initiation and progression or regression of disease has helped identify how each of these molecular signaling pathways influences vascular disease. As genome-wide scans become more routine in large-scale cardiovascular studies in humans, these data will be a tremendous resource in identifying new pathways and highlighting novel interactions between pathways. A better understanding of the targets involved will aid in the identification of early biomarkers in the serum and/or genetic screens in patients based on genetic variation that may allow us to move towards preventive medicine–focused approaches to inhibit the initiation and progression of cardiovascular disease.

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This is an excellent review highlighting a role for FOXO transcription factors in longevity and tumor suppression.