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Abstract—Akt is a serine/threonine protein kinase that is activated by a number of growth factors and cytokines in a phosphatidylinositol-3 kinase–dependent manner. Although antiapoptotic activity of Akt is well known, it also regulates other aspects of cellular functions, including migration, glucose metabolism, and protein synthesis. In this review, Akt signaling in endothelial cells and its critical roles in the regulation of vascular homeostasis and angiogenesis will be discussed. **(*Circ Res*. 2002;90:1243-1250.)**

Key Words: Akt ■ endothelial cells ■ angiogenesis ■ statins ■ endothelial nitric oxide synthase

Since the identification of several classes of receptor tyrosine kinases and their ligands as crucial mediators of vascular development, considerable progress has been made toward understanding the process of angiogenesis at sites of tissue growth and/or repair.[1,2] A number of clinical trials are currently evaluating angiogenic ligands for their ability to induce neovascularization in ischemic tissues,[3,4] and the intracellular signaling pathways that mediate the proangiogenic effects of these growth factors are being extensively investigated. This review specifically focuses on the role of phosphatidylinositol-3 kinase (PI3K)–Akt signaling axis in endothelial cells because it is activated by many angiogenic growth factors and it regulates downstream target molecules that are potentially involved in blood vessel growth and homeostasis.

PI3K-Akt Signaling Axis: Upstream Activators and Downstream Targets

Akt was originally identified as a cellular counterpart of the oncogene derived from murine AKT8 retrovirus.[5,6] The same gene product was independently isolated as a protein kinase related to protein kinase A and C and was therefore named as protein kinase B (PKB) or RAC (related to protein kinase A and C).[7,8] Mammalian genomes contain three *Akt* genes, *Akt1/PKBα*, *Akt2/PKBβ*, and *Akt3/PKBγ*, whereas *Drosophila melanogaster* and *Caenorhabditis elegans* contain one and two *Akt* genes, respectively.[9,10] These genes encode proteins containing a pleckstrin homology (PH) domain in the amino terminus, a central kinase domain, and a carboxy terminal regulatory domain. All 3 mammalian *Akt* genes are widely expressed in various tissues but Akt1 is most abundant in brain, heart, and lung, whereas *Akt2* is predominantly expressed in skeletal muscle and embryonic brown fat, and *Akt3* is predominantly expressed in brain, kidney, and embryonic heart.[7,11–13] In unstimulated cells, Akt protein exists in cytoplasm and the two regulatory phosphorylation sites at threonine at 308 and serine at 473 are in an unphosphorylated state. On growth factor stimulation, the PH domain binds to the lipid products of PI3K, and Akt is recruited to plasma membrane. Akt is then sequentially phosphorylated at T308 and S473 by upstream kinases referred to as 3-phosphoinositide–dependent protein kinase 1 (PDK1) and PDK2, respectively, to yield a fully activated kinase (Figure 1).[14,15] PDK1 has been isolated and characterized,[15] but the identity of PDK2 is still controversial. Several candidate molecules have been suggested to be a potential S473-kinase including integrin-linked kinase (ILK), MAP kinase–activated protein kinase 2 (MK2), PDK1 (conversion of substrate specificity in association with protein kinase C–related kinase-2 [PRK2]) and Akt itself (autophosphorylation).[16–19] Fully activated Akt becomes available to phosphorylate its downstream substrates and a portion of these molecules detach from the plasma membrane and translocate to various subcellular locations including nucleus.[20] Akt is then dephosphorylated and inactivated by protein phosphatases such as protein phosphatase 2A (PP2A).[21]

Akt is a critical regulator of PI3K-mediated cell survival.[22,23] A large number of studies have demonstrated in various cell types that constitutive activation of Akt signaling is sufficient to block cell death induced by a variety of apoptotic stimuli and that transduction of dominant-negative Akt inhibits growth factor–induced cell survival.[24–26] The prosurvival function of Akt has also been demonstrated in the context of the intact organism. Mutation of *Drosophila* Akt leads to embryonic lethality due to massive apoptosis during embryogenesis,[27] and Akt1 mutant mice exhibit increased spontaneous apoptosis in testis and thymus.[28] Several downstream targets of Akt are recognized to be apoptosis-regulatory molecules including Bad, FKHR family of forkhead transcription factors, and IKKα,[29–35] and these findings are consistent with the notion that Akt functions as a survival kinase. However, other downstream effectors of Akt are

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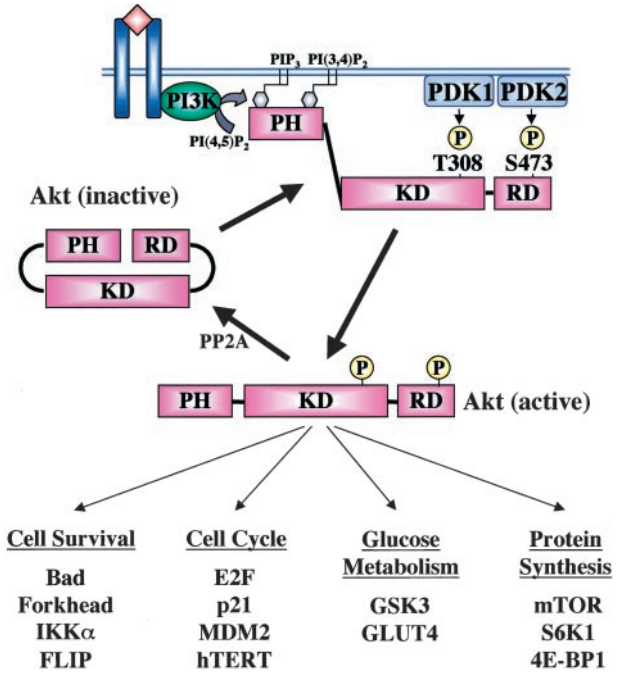

Figure 1. Mechanism of Akt activation and partial list of downstream molecules. Akt is activated by growth factors or cytokines in a PI3K-dependent manner, and phosphorylation of two residues by PDK1 (T308) and PDK2 (S473) is required for its full activation. Downstream target molecules are grouped according to their function. Note that these downstream molecules include both direct Akt substrates and indirect downstream effectors.

involved in different aspects of cellular regulation. For example, (1) Akt enhances glucose uptake by inducing membrane translocation of the glucose transporter GLUT4,[36] (2) Akt promotes glycogen synthesis through the phosphorylation and inactivation of glycogen synthase kinase-3 (GSK-3),[37] (3) Akt regulates cell cycle and cellular senescence, at least in part, through modulating the activities of E2F, p21, MDM2, and human telomerase reverse transcriptase subunit (hTERT),[38–44] and (4) Akt enhances protein synthesis through increasing the phosphorylation of mammalian target of rapamycin (mTOR), eukaryotic initiation factor 4E-binding protein 1 (4E-BP1), and 70-kDa S6 kinase (S6K1),[15,45] although S6K1 may also be directly activated by PDK1 in a PI3K-dependent and Akt-independent fashion (Figure 1).[46,47] Taken together, it is more appropriate to classify Akt as a multifunctional protein kinase rather than a simple regulator of cell survival.

Akt-Dependent Survival Signals in Endothelial Cells

Although originally identified as a factor that induces vascular permeability, vascular endothelial growth factor (VEGF) exhibits multiple biological activities in endothelial cells, including the enhancement of endothelial cell survival.[48] VEGF effects on cell survival have been shown to be mediated by Flk1/VEGFR2-PI3K-Akt pathway.[49,50] Subsequently, it was also shown that several other endothelial cell stimuli including angiopoietin-1 (Ang-1),[51,52] insulin,[53] insulin-like growth factor-I (IGF-I),[54] sphingosine-1-phosphate (S1P),[55,56] hepatocyte growth factor,[57] the small proteoglycan decorin,[58] fluid shear stress,[59] estrogen,[59a] reactive oxygen species,[59b] and corticosteroids[59c] also activate PI3K-Akt signaling, illustrating the central role of this pathway in controlling endothelial cell viability.

Growth factor activation of angiogenesis is dependent on proper endothelial cell–extracellular matrix attachment,[60] and in the absence of matrix attachment, cells undergo apoptotic cell death through a process termed anoikis (a Greek word for "homelessness").[61] VEGF activation of Akt in endothelial cells is dependent on matrix attachment, and constitutively active Akt blocks cell detachment–induced apoptosis.[50] These findings suggest that matrix attachment is required for growth factors to activate Akt and maintain endothelial cell viability. Cell attachment is mediated mainly through the engagement of extracellular matrix with integrin molecules. When integrins bind to extracellular matrix they become clustered and associate with the actin cytoskeleton through adaptor/signaling molecules, which further promotes integrin clustering and the assembly of actin filaments and leads to the formation of focal adhesion and activation of intracellular signaling.[62] The α_v integrin combinations have been most extensively investigated in terms of their roles in angiogenesis.[63] Endothelial cells stimulated with angiogenic growth factors or those in newly formed vessels express high levels of $\alpha_v\beta_3$ integrin, and antagonists against $\alpha_v\beta_3$ or $\alpha_v\beta_5$ integrin block the growth factor-induced angiogenesis. It has also been shown that $\alpha_v\beta_3$ integrin associates with VEGF and platelet-derived growth factor (PDGF) receptors and potentiates VEGF or PDGF signaling, respectively.[63] Because several integrin signaling molecules including focal adhesion kinase (FAK), ILK, and Shc have been implicated in Akt activation,[61] downregulation of Akt activity induced by cell detachment is likely due to the decrease in integrin-dependent Akt activation. Caspase-mediated cleavage of Akt is also implicated in the downregulation of Akt protein level during long-term suspension culture.[64] Collectively, these findings suggest that integrin signaling induced by cell attachment (outside-in signal) is an important regulator of growth factor–dependent endothelial cell survival and angiogenesis through PI3K-Akt pathways. Furthermore, VEGF-induction of inside-out signals has also been shown to activate integrins,[65] suggesting that integrin and growth factor signaling are cooperative and synergistic with regard to activation of Akt signaling (Figure 2).

Currently, relatively little is known about the downstream mediators of Akt-dependent survival pathway in endothelial cells, although several candidate molecules has been identified including survivin,[51] FLICE-inhibitory protein (FLIP),[66] and MEKK3.[67] Thus, possible combinations of these and other unidentified Akt target molecules may control endothelial cell survival depending on the context of pro- and antiapoptotic stimuli encountered in the cellular environment.

Regulation of Endothelial Nitric Oxide Synthase (eNOS) Activity by Akt

In addition to its antiapoptotic effects, VEGF induces hypotension in the intact organism, nitric oxide (NO)–dependent vasodilation in isolated coronary arteries, and NO release in

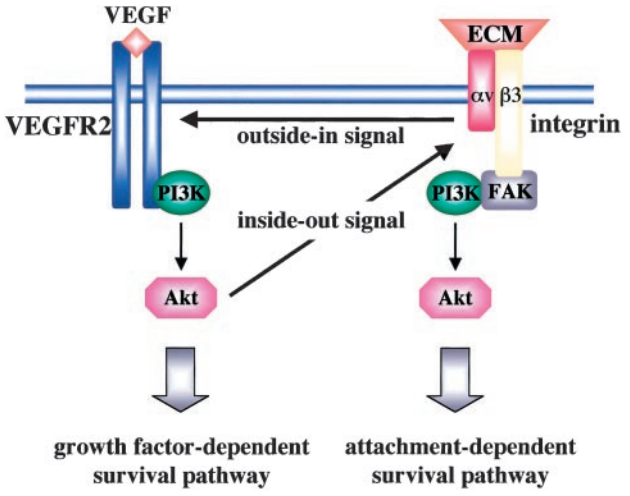

Figure 2. Growth factor– and cell attachment–dependent survival is mediated by PI3K/Akt signaling in endothelial cells. Integrin-dependent signals are prerequisite for growth factor–mediated activation of Akt. Akt-mediated crosstalk between these two signaling systems synergistically promotes endothelial cell survival.

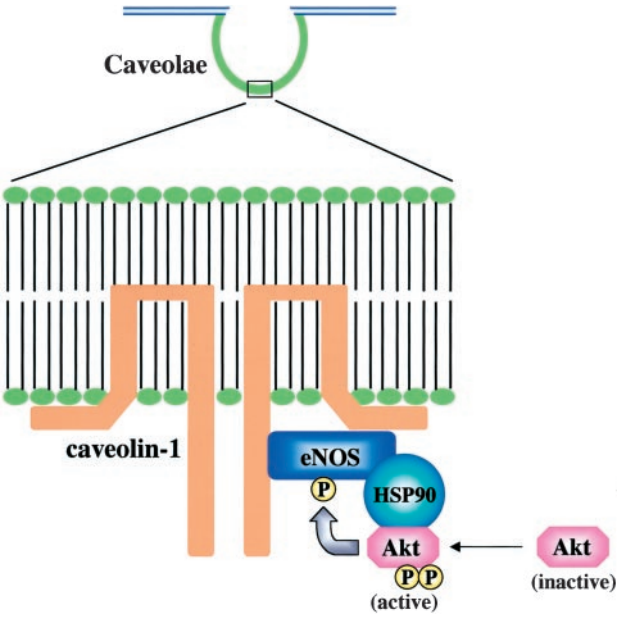

Figure 3. Schematic illustration of the Akt-eNOS interaction at caveolae. Caveolin-1 is localized to caveolae and associates with a number of regulatory molecules including eNOS. Association of eNOS with caveolin-1 negatively regulates eNOS activity, although targeting of eNOS to caveolae is required for proper eNOS function. Activated Akt and eNOS also associate with Hsp90. Hsp90 is believed to function as a scaffold protein for activation of eNOS by Akt-mediated phosphorylation.

isolated vessels and in cultured endothelial cells.[68–70] Early studies demonstrated that VEGF-induced increase in NO release from endothelial cells is attenuated by PI3K inhibitors,[71] and subsequently, it was demonstrated that VEGF stimulates Akt-mediated eNOS phosphorylation at Ser1177 (in human eNOS, equivalent to Ser1179 in bovine eNOS), leading to an increase in eNOS activity.[72,73] It is also reported that production of NO in response to fluid shear stress in cultured endothelial cells is controlled by Akt-dependent phosphorylation of eNOS,[73] although another study has shown that shear stress induces eNOS phosphorylation predominantly through a protein kinase A–dependent, Akt-independent mechanism.[74] Studies in intact animals have shown that overexpression of constitutively active Akt in the vascular endothelium increases resting diameter and blood flow, whereas transduction of dominant-negative Akt attenuates endothelium-dependent vasodilation induced by acetylcholine,[75,75a] demonstrating that Akt functions as a regulator of vasomotor tone in vivo. PI3K/Akt signaling has also been implicated in the control of endothelium-dependent vasorelaxation induced by adrenomedullin,[76] and hyperglycemia has been shown to lead to the glycosylation of the Akt phosphorylation site in eNOS, resulting in an inhibition of eNOS activity.[77]

The activity of eNOS is also regulated by subcellular localization and/or protein-protein interactions. Of note, eNOS has been shown to be localized in a specific domain of plasma membrane called caveolae and to interact with caveolin-1 through caveolin-1 scaffolding domain, which inhibits eNOS activity.[78–81] Although originally implicated in transmembrane trafficking of macromolecules, the finding that caveolae contain a variety of signaling molecules and caveolin-1 directly interacts with those caveolae-associated proteins have suggested that caveolae and caveolin-1 are involved in the compartmentalization and integration of signal transduction pathways at the cell membrane. Consistent with the inhibitory role of caveolin-1 on eNOS activity, administration of caveolin-1 scaffolding domain fused to cellular internalization sequences in vivo attenuates eNOS activity,[82] and acetylcholine-induced vasorelaxation and NO production are enhanced in caveolin-1-deficient mice.[83] The targeting of eNOS to caveolae, however, seems to be required for efficient and proper activation of eNOS on stimulation, because conditions that inhibit the localization of eNOS in caveolae also attenuate eNOS activity.[84,85] It has also been shown that eNOS interacts with heat shock protein 90 (Hsp90) on stimulation with VEGF or shear stress, and this interaction enhances eNOS activity.[86] Interestingly, Akt also interacts with Hsp90 on stimulation and this interaction enhances Akt enzymatic activity,[87] suggesting that Hsp90 may serve as a scaffold protein for the efficient phosphorylation of eNOS by Akt at caveolae (Figure 3).[88,89,89a]

Regulation of Endothelial Cell Migration by Akt

The ability of endothelial cells to migrate and form capillary-like structures is essential for angiogenesis in vivo.[1] VEGF enhances endothelial cell migration and capillary-like structure formation in vitro and these activities of VEGF are PI3K-Akt–dependent.[90–92] S1P has also been shown to enhance endothelial cell migration and capillary formation in vitro through the activation of the endothelial differentiation gene (EDG) family of G protein–coupled receptors and PI3K-Akt–dependent pathways.[56,93,94] Conversely, oxidized LDL inhibits endothelial cell migration toward VEGF by promoting the dephosphorylation of Akt.[95]

Studies in other cell types have also implicated PI3K and Akt in the control of directional cell migration and the sensing of chemoattractant gradients by the cell. It has been shown that Akt transiently localizes to the leading edge membrane of migratory cells in a PI3K-dependent manner,[96,97] and gene ablation studies in mice have demonstrated that PI3Kγ is required for chemotaxis and chemoattractant-dependent activation of Akt in macrophages and neutrophils.[98–100] Akt has been shown to be required for chemotaxis in *Dictyostelium* cells as well.[96]

Cellular movement requires the reorganization of actin cytoskeleton and distinct patterns of actin reorganization are required as cells establish leading edge and then generate contractile force to migrate forward.[101] Previous studies have implicated the Rho family of small G proteins as one of the major regulators of actin reorganization. Among Rho family members, Rho, Rac, and Cdc42 are most widely studied and each regulates specific aspects of cytoskeletal reorganization. Rho stimulates cytoplasmic stress fiber formation and actomyosin-based contractility, Rac induces membrane ruffling and extension of lamellipodia, and Cdc42 induces the extension of membrane protrusions (filopodia) and is also involved in chemoattractant gradient sensing.[102,103] In endothelial cells, it has been shown that VEGF-induced cell migration is dependent on Rho family GTPases.[104,105] However, the relationship between Akt and Rho family of G proteins is complicated and controversial. On one hand, Akt was shown to negatively regulate Rac1 activity by phosphorylating Rac1 and inhibiting its GTP-binding activity.[106] In contrast, a recent study has demonstrated that Akt phosphorylates S1P receptor EDG-1 and induces Rac activation and cell migration in endothelial cells.[107] Other reports show that Rac and Cdc42 are situated upstream of Akt and that they promote Akt signaling.[108–110] Consistent with these findings, Akt has been shown to be required for cell motility induced by Rac or Cdc42 in fibroblasts.[108]

Another possible downstream effector of Akt that regulates cell motility is p21-activated protein kinase (PAK). PAK was originally identified as a Rac1-binding protein that specifically interacts with GTP-bound form of Rac.[111] Subsequently, it was shown that PAK is activated by Rac or Cdc42 and that it regulates polarized cytoskeletal reorganization.[111] Recently it was shown in *Dictyostelium* cells that Akt regulates cell polarity and chemotaxis through the regulatory phosphorylation of PAK,[112] suggesting a direct functional link between Akt and PAK in the regulation of cytoskeletal reorganization. In mammalian fibroblasts, it was also shown that Akt stimulates PAK1 activation and dominant-negative Akt inhibits Ras-induced activation of PAK1.[113] However, the Akt phosphorylation site in *Dictyostelium* PAK is not conserved in mammalian PAK1, suggesting an indirect activation of mammalian PAK1 by Akt. Nonetheless, PAK family of protein kinases are attractive candidates for Akt effectors in the regulation of endothelial cell migration, and may be a convergence point of signals from Rac/Cdc42 and Akt.

Statins and Akt Signaling

The 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, or statins, are widely prescribed for the treatment of hypercholesterolemia, and several clinical trials have demonstrated that statins are effective for both primary and secondary prevention of coronary artery diseases.[114] It has also been shown that statins rapidly improve vasomotor responses of atherosclerotic coronary arteries both in humans and in animal models,[115–117] and studies in normocholesterolemic animals revealed that statins protect against stroke and myocardial ischemia/reperfusion injury possibly through NO-dependent mechanisms.[118,119] These findings are consistent with the notion that cardioprotective effects of statins are partly independent of their serum lipid-lowering effects and may be due to the activation of eNOS in vascular endothelium.

Recent research has revealed a link between statins and Akt. Statins have been shown to rapidly promote the activation of Akt in endothelial cells leading to eNOS phosphorylation and increased NO production.[91] Low statin concentrations have been shown to protect endothelial cells from serum deprivation–induced apoptosis and promote capillary-like structure formation on matrigel in an Akt-dependent manner, whereas higher concentrations are toxic.[91] Consistent with their Akt-activating function, treatment with clinically-relevant doses of statins enhances angiogenesis in the ischemic hindlimbs of normocholesterolemic animals through an eNOS-dependent mechanism.[91,120] It has also been shown that the activation of angiogenesis by statins is biphasic: low doses promote vessel formation, whereas high doses inhibit angiogenesis.[121] More recently, it was shown that statins enhance the mobilization of endothelial progenitor cells (EPCs) from bone marrow to newly forming blood vessels in a PI3K-Akt–dependent manner,[122,123] suggesting another mechanism of Akt-dependent proangiogenic effects of statins. Moreover, it has been shown that statins promote EPC mobilization in patients with stable coronary heart diseases.[124] Although there are numerous lines of evidence to suggest that statins promote endothelial cell function and angiogenesis, there is no evidence in clinical studies linking statin treatment to increase in cancer risk.[125]

Activation of Akt by statins is blocked by treatment with wortmannin or LY294002,[91] suggesting that statin activation of Akt is mediated by PI3K. However, the mechanisms by which statins activate PI3K are unknown at present. In this regard, statins have been shown to decrease caveolin-1–eNOS interaction and enhance the formation of eNOS-Hsp90-Akt complex in endothelial cells,[89] although it is not clear whether these effects of statins are secondary to Akt activation or not. It should also be noted that endothelial cells are relatively unique in this response because activation of Akt by statins is not observed in cardiac or smooth muscle cells,[91] suggesting an endothelial cell–specific pathway of PI3K-Akt activation. A recent report has shown that low, clinically relevant doses of statin activate endothelial Ras and promote Akt and eNOS phosphorylation.[126] It was also reported that higher statin doses are toxic to endothelial cells although they promote an increase in eNOS protein expression. Presumably, the toxicity results from an inhibition of protein prenylation,[127] and this may explain the antiangiogenic effects observed in studies performed with higher statin concentrations.[128,129]

In addition to their proangiogenic effects, statins have also been shown to exhibit antithrombotic actions in humans, which appears to be independent of their serum cholesterol-lowering effects.[130] Recent studies have shown that PI3K-Akt pathway inhibits the expression of tissue factor,[131,132] which is the primary cellular initiator of blood coagulation and whose expression is induced in endothelial cells and macrophages by a number of stimuli, including interleukin-1β and tumor necrosis factor-α.[133] Although VEGF activates both tissue factor expression and PI3K-Akt signaling, administration of inhibitors of PI3K-Akt signaling further enhances VEGF-induced tissue factor expression.[131,132] Taken together, these data suggest that statins may inhibit blood coagulation, at least in part, through a selective activation of PI3K-Akt signaling in endothelial cells, leading to an inhibition of tissue factor expression.

Integrated Regulation of Growth and Angiogenesis by Akt

In addition to its role in angiogenesis, Akt has also been implicated as a general regulator of tissue and organ growth. Studies in *Drosophila* have demonstrated that components of the insulin/IGF signaling pathway including Akt are involved in the regulation of organ growth and body size in response to nutritional input.[134,135] The role of this pathway in vertebrate growth control is indicated by the growth retardation observed after targeted disruption of various components of this pathway in mice.[28,136,137] Importantly, it has recently been shown that the targeted disruption of Akt1 gene in mice results in general growth retardation.[28,137]

In higher animals, organ growth is accompanied by the recruitment of new blood vessels. The dual role of Akt signaling in angiogenesis and tissue growth suggests that both processes can be coordinately regulated by this signaling step during organ enlargement. Consistent with this notion, it has been demonstrated that exercise training, a well-known stimulator of muscle hypertrophy, enhances VEGF expression and increases vessel density in skeletal muscles, and that exercise-induced increase in vessel density is blocked by a VEGF-neutralizing antibody.[138] Likewise, cardiac muscle cell-specific deletion of VEGF gene results in thin ventricular wall with fewer coronary vessels.[139] These results indicate that angiogenesis associated with physiological muscle tissue growth is dependent on paracrine VEGF secretion. Based on the notion that Akt positively regulates organ growth, we have examined the hypothesis that Akt may be involved in VEGF secretion associated with muscle hypertrophy. Indeed, overexpression of Akt in skeletal muscles in vivo induces skeletal muscle hypertrophy, local VEGF production, and angiogenesis.[140] Collectively, these findings suggest that Akt signaling in both muscle cells and endothelial cells coordinately regulate overall growth of muscle tissues in vertebrates. This concept may also be applicable to other organs as well.

Conclusions

PI3K-Akt signaling axis is activated by a variety of stimuli in endothelial cells and regulates multiple critical steps in angiogenesis, including endothelial cell survival, migration, and capillary-like structure formation. Furthermore, this signaling pathway also regulates cardiovascular homeostasis and vessel integrity at least in part by controlling NO synthesis (Figure 4). Angiogenesis has been implicated in the pathophysiology of a number of diseases, and regulation of angiogenesis, both its increase and decrease, could be an important therapeutic strategy for those disease states. Thus, further dissection of the PI3K-Akt pathway and elucidation of the downstream effector molecules will lead to a better understanding of blood vessel growth and may provide avenues for the development of novel therapeutic interventions.

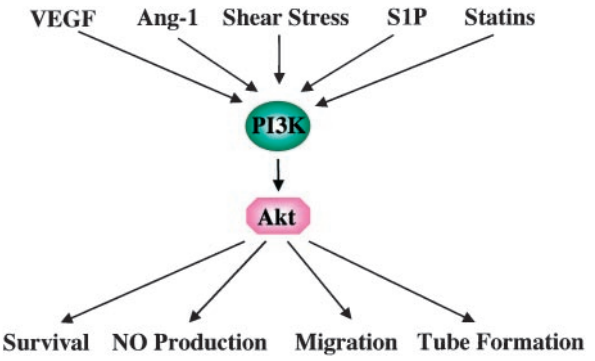

Figure 4. Activation of PI3K-Akt signaling axis in endothelial cells. Various growth factors, mechanical stimuli, and pharmacological interventions activate Akt signaling in endothelial cells, and Akt-dependent pathways control several events critical for cardiovascular homeostasis and angiogenesis.

Acknowledgments

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