HHS Public Access

Author manuscript

Vascul Pharmacol. Author manuscript; available in PMC 2016 November 01.

Published in final edited form as:

Vascul Pharmacol. 2015 November ; 74: 38–48. doi:10.1016/j.vph.2015.05.008.

The Critical Role of Akt in Cardiovascular Function

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Abstract

Akt kinase, a member of AGC kinases, is important in many cellular functions including proliferation, migration, cell growth and metabolism. There are three known Akt isoforms which play critical and diverse roles in the cardiovascular system. Akt activity is regulated by its upstream regulatory pathways at transcriptional and post-translational levels. beta-catenin/Tcf-4, GLI1 and Stat-3 are some of few known transcriptional regulators of AKT gene. Threonine 308 and serine 473 are the two critical phosphorylation sites of Akt1. Translocation of Akt to the cell membrane facilitates PDK1 phosphorylation of the threonine site. The serine site is phosphorylated by mTORC2. Ack1, Src, PTK6, TBK1, IKBKE and IKKε are some of the non-canonical pathways which affect the Akt activity. Protein-protein interactions of Akt to actin and Hsp90 increase the Akt activity while Akt binding to other proteins such as CTMP and TRB3 reduces the Akt activity. The action of Akt on its downstream targets determines its function in cardiovascular processes such as cell survival, growth, proliferation, angiogenesis, vasorelaxation, and cell metabolism. Akt promotes cell survival via caspase-9, YAP, Bcl-2, and Bcl-x activities. Inhibition of FoxO proteins by Akt also increases cell survival by transcriptional mechanisms. Akt stimulates cell growth and proliferation through mTORC1. Akt also increases VEGF secretion and mediates eNOS phosphorylation, vasorelaxation and angiogenesis. Akt can increase cellular metabolism through its downstream targets GSK3 and GLUT4. The alterations of Akt signaling play an important role in many cardiovascular pathological processes such as atherosclerosis, cardiac hypertrophy, and vascular remodeling. Several Akt inhibitors have been developed and tested as anti-tumor agents. They could be potential novel therapeutics for the cardiovascular diseases.

Graphical Abstract

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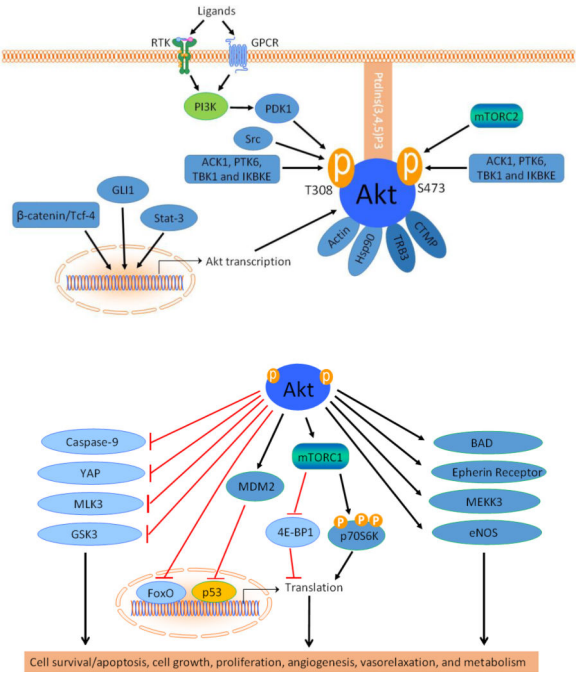

Keywords

Akt; vascular remodeling; Signal transduction

Introduction

Akt family kinases (also known as protein kinase B /PKB) are serine/threonine kinases that belongs to the general class of AGC kinases (AMP/GMP kinase and PKC subfamily of proteins) which has 518 members in humans [1]. While there are Akt homologs from fly to humans, structure and function of mammalian Akt is highly conserved [2–6]. General pathway of Akt is called phosphoinositide-3-kinase–protein kinase B/Akt (PI3K-PKB/Akt) pathway or PI3k/AKT/mTOR pathway, named after upstream and downstream proteins involved. It was discovered by three different groups at the same time. Two of those discoveries were based on PKA or PKC homology based approaches and the other is through retroviral cloning [7–9]. The name Akt stems from the retroviral strain Akt-8 which was used for the cloning experiments. There are three Akt isoforms, Akt1, Akt2 and Akt3 (also known as PKBα, PKBβ, and PKBγ, respectively). Akt1 and Akt3 are ubiquitously expressed while Akt2 is expressed in the insulin-responsive tissues such as brown fat, skeletal muscle and liver [10]. The upstream and downstream targets of these Akt isoforms are quite similar. But there seems to be functional differences between these isoforms in different cell context. They seem to be specific in their interactions with other proteins. For example, onco-protein TCL1b forms oligomers with Akt1, not Akt2 or Akt3 [11]. These differences can be observed in the cell cycle regulation. Akt2 accumulates in the cytoplasm during mitosis [12] and in the nucleus during muscle differentiation [13]. Differences in Akt isoforms are also evident in disease development. Akt2 is indicated in many different tumors [14,15]. Ectopic expression of Akt2 has been shown to induce metastasis and invasion in

human breast cancer cells and induce malignancy in mouse fibroblasts [12,16]. In human thoracic aortic dissection (TAD) and aortic aneurysm and dissection (AAD) Akt2 phosphorylation level is higher while Akt1 phosphorylation levels remain the same between disease and healthy conditions [17]. These differences are also evident in Akt isoform knockout mice. Akt2 deficient mice shows a diabetic phenotype which is not observed in Akt-1 knockout mice [18]. Importance of Akt3 in brain development has also been shown in Akt3 knockout mice. This isoform specific cell development causes smaller brain sizes in Akt3 knockout mice, but not in Akt1 knockout mice [19]. In addition, Akt1 promotes endothelial neoplasms while Akt3 acts in an opposite manner [20]. The reduction of phosphorylation of endothelial nitric oxide synthase (eNOS) by Akt1 knockout can be compensated by Akt2, though phosphorylation of angiogenic substrate by Akt1 seems to be essential for angiogenesis [21]. Since they are involved in signal pathways related to cell proliferation, cell growth, survival and aspects of intermediary metabolism, Akt has been the center of focus for many studies attempting to therapeutically control these aspects.

Akt protein structure

Akt has a characteristic pleckstrin homology (PH) domain at the amino terminal (~ 110 amino acids), a middle kinase domain (~260 amino acids) and a carboxy-terminal regulatory domain (~70 amino acids) (Figure 1). Of these, pleckstrin homology domain controls the membrane translocation of Akt. This structure is conserved in diverse species from flies to man. Akt has a higher sequence identity to other AGC family member proteins which made therapeutic strategies and developing specific inhibitors more challenging [22]. The region homology varies among the isoforms. Pleckstrin domain is 80% identical between Akt isoforms, while this being 30% identical to pleckstrin domains of other proteins. The region between PH domain and the catalytic domain is called the linker (LINK). This link is poorly conserved amongst Akt isoforms and lacks similarities to other human proteins. The catalytic domain shares a higher similarity within Akt isoforms (90%) and a significant similarity to other AGC family proteins. Because of the difficulty of obtaining crystal structure of the linker region, the structures of Akt protein is speculative [23].

Upstream regulatory pathways of Akt

In cardiovascular system, Akt is activated by several stimuli such as insulin, platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) [24,25]. In addition, some phosphatase inhibitors also activate Akt [26]. Reactive oxygen species (ROS) have been shown to activate Akt through angiotensin II [27,28]. These growth factors regulate Akt activity through transcriptional or posttranslational mechanisms (Figure 2).

Transcriptional regulation of Akt

Transcriptional regulation of Akt genes remains largely unknown. It has been shown that upregulation of total Akt protein results in an increase in Akt activity, though the expression of a kinase may not be necessarily a reflection of its activity level [29]. The Akt gene promoters contain binding sites for several signaling molecules such as Stat3, β-catenin/

Tcf-4, and GLI1, indicating that Akt gene transcription may be regulated by these molecules.

The 4.2-kb region upstream of the transcription start site of the *AKT1* promoter contains five putative Stat3-binding motifs. However, the promoter is not induced by Stat3 and/or Src. Actually the major Stat3 response elements are located within exon 1 and intron 1 regions of the *AKT1* gene, which is upstream of the AKT1 translation initiation site. Stat-3 interacts with this region and increases the Akt1 gene transcription [30].

The transcription of Akt1 gene is induced by β-catenin/Tcf-4 [29]. Nine putative b-catenin/Tcf/Lef-binding sites (TBE) in the AKT1 gene have been found that show a high degree of homology to the core consensus sequence AGATCAAAGGG [29]. Four of these TBEs are located upstream of the transcriptional start, whereas five TBEs are situated in Exon 1. Moreover, the promoter region of the AKT2 gene contains six potential TBEs [29], and seven complete or partial TBEs have been identified within 1340 nucleotides of the promoter region of the AKT3 gene [29]. The inhibitory effect of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and indomethacin downregulate Akt1 gene transcription through stabilization of β-catenin phosphorylation [31].

GLI1, a zinc finger transcriptional factor of the Hedgehog signaling, increases the transcription of of *AKT1, AKT2*, and *AKT3* genes [32]. *AKT1* promoter possesses two *GLI1* binding sites (BS1 and BS2) located upstream of the transcriptional start site of *AKT1* gene. The homology of each GLI1 binding site to the consensus sequence was 67% for BS1 and 78% for BS2. *AKT2* and *AKT3* promoters also contain three and two GLI1 binding sites, respectively [32].

In addition, one possible AP-1 binding site (ACTCAGT or TGAGTCA) and two potential nuclear factor kappa B (NFκB) binding elements have been detected in the AKT1 promoter region [29]. However, the functions of these binding sites are not clear yet.

Posttranslational regulation of Akt by phosphorylation

Phosphorylation is the most important posttranslational determinant in Akt activity. There are two major phosphorylation sites in Akt protein. Positions of these sites vary slightly among Akt isoforms. These phosphorylation sites are threonine residue in the kinase domain (Akt1 at 308, Akt2 at 309 and Akt3 at 305) and serine residue in the hydrophobic domain (Akt1 at 473, Akt2 at 474 and Akt3 at 472) (Figure 1). Phosphorylations of both sites positively increase the activity of Akt in varying degrees. For example, phosphorylation of threonine 308 (T308) in Akt1 increases the enzymatic activity of Akt by 100 fold and phosphorylation of serine 473 (S473) will increase Akt activity by 10 fold [33]. There is growing evidence of the diverse roles these phosphorylation sites play in Akt activation. Akt activation induced by T308 phosphorylation seems to be important in phosphorylation of substrates PRAS40, TSC2 and TBC1D4 while S473 phosphorylation doesn't seem to have significant role in phosphorylating those substrates [34]. In contrast only S473 phosphorylation shows a positive correlations with substrates like BAD while substrates like GSK3β can be phosphorylated by Akt phosphorylation at either critical sites [35] (Figure3). These differences are manifested in disease development where one phosphorylation site

shows more correlation with certain cancer [36,37]. The activation of Akt by phosphorylation follows the classical AGC family kinases behavior. The catalytic region of Akt which is located at the central region consists of a conserved threonine residue whose phosphorylation partially activates Akt [38,39]. The carboxyl region has a sequence F-X-X-F/Y-S/T-Y/F hydrophobic motif (where X is any amino acid) which is a characteristic of all AGC family kinases. It is known that phosphorylation of the serine and threonine residue in Akt2 causes a conformational change in the kinase region resulting in enzyme activation channel [40]. The activity of Akt is enhanced by the hydrophobic motif interaction of the N-terminal lobe channel [40].

One of the key requirements in activation of Akt is its translocation to the plasma membrane. This translocation requires the PH domain and is affected by wortmannin treatment. PH domain is not required for the activation of serine or threonine residues of Akt. Affinity of PH domains to PtdIns(3,4,5)P3 explains this membrane translocation [41–43]. Once Akt is translocated a protein kinase is capable of phosphorylating the threonine residue (T308). This protein kinase is named 3-phosphoinositide-dependent protein kinase-1 (PDK1) due to its association with PtdIns (3,4,5)P3 [44–46]. Following the activation Akt detaches from the plasma membrane and translocates into the cytosol and nucleus [39,43]. This is called the canonical pathway in which Akt activation is PI3 kinase dependent. Either tyrosine kinase receptors or G-protein coupled receptors can activate PI3K. Based on these, upstream receptor types PI3K is divided in to class IA (for receptor tyrosine kinase) and class 1B (for G-protein coupled receptors) [47]. Either of these two types of receptors recruits PIP2 and then PI3 kinase acts on its remaining OH group to form PIP3. This is negatively regulated by phosphatases like PTEN which reverses the PI3 kinase action [48]. Unlike other AGC kinases co-localization of Akt with PDK1 is necessary for phosphorylation of the threonine residue. Once Akt is translocated to the cell membrane, conditions at the cell membrane help phosphorylate both T308 and S473 [39]. The exact mechanism of how S473 is phosphorylated is poorly known. Initially it was assumed that PDK1 plays a role in the phosphorylation process. However, later research revealed that PDK1 is not essential although it can increase the S473 phosphorylation [49–51]. More recent studies revealed the importance of mammalian target of rapamycin 2 (mTOR2) in the phosphorylation of the critical S473 residue in Akt [52].

In addition to the canonical pathways, Akt can be activated by a non-canonical pathway independent of PI3K. Ser/Thr/Tyr kinases can directly activate Akt in this pathway. For example, receptor tyrosine kinases can lead to activation of Akt (at both S473 and T308) through nonreceptor tyrosine kinase Ack1 (ACK/TNK2). This activity can occur even in the presence of PI3 kinase inhibitors [53]. Moreover, certain kinases have the ability to affect the activation status of Akt through phosphorylating other critical tyrosine residues. For example, Src can phosphorylate T315 and T326, leading to increased T308 phosphorylation [54]. The effect on S473 phosphorylation by Src is not known. Protein tyrosine kinase 6 (PTK6) can phosphorylate T215 and T326 residues in low EGF concentrations in the same manner. This can activate both the critical S473 and T308 residues. However this activation is not prominent in higher EGF concentrations, suggesting that PI3K pathway to be the dominant pathway in high concentrations of growth factors [55]. Furthermore, IKBKE or IKKε (I-kappa-B kinase epsilon), TANK-binding kinase 1 (TBK1) and DNA-PKcs have

been shown to directly activate Akt [56–58]. The serine/threonine kinase IKBKE is a non-canonical IKK signal transduction family member. In response to inflammatory factors like lipopolysaccharides (LPS) and phorbol myristate acetate (PMA), active IKBKE directly phosphorylate T308 and S473 as well as p65/RelA, interferon response factors 3 and 7 (IRF3 and IRF7) and STAT1 [59–61]. IKBKE-induced Akt activation is not affected by inhibition of PI3K, knockdown of PDK1 or mTORC2 complex. It is also not dependent on the PH domain of Akt as shown by Akt inhibitor studies [56]. In addition, TANK binding kinase (TBK1) has been shown to phosphorylate Akt at S473 and subsequently induce maximal activation of interferon regulatory factor 3 (IRF3) and expression of IFN-b in the antiviral cellular responses [57].

Posttranslational regulation by protein-protein interaction

There are several proteins that bind and modulate Akt activity. One of the emerging areas in the regulation of Akt activity is the subcellular movement facilitated by non-substrate ligands. Treatment of cells with PDGF increases Akt association with actin skeleton [62]. This is through direct interaction of actin with the PH domain of the Akt. Phosphorylation of both S473 and T308 is important in this process as mutation of those sites to alanine completely abrogates the Akt co-localization to actin. This co-localization of Akt with actin induced by PDGF is enhanced by small GTP-ases Rac1 and Cdc42 [62]. It has been shown that Akt is associated with heatshock protein 90 (Hsp90), which helps keep Akt active through preventing the dephosphorylation of Akt by protein phosphatase 2A (PP2A) [63]. Amino acid residues 229–309 of Akt were found to bind to the region of 327–340 of Hsp90. In similar manner Hsp27 interact with amino acids 117–128 of Akt and help phosphorylate S473 residue [64]. On the other hand, Akt association with other proteins can negatively regulate Akt activity. Carboxyl-terminal modulator protein (CTMP) binds to the carboxyl terminal regulatory domain of Akt1 at the plasma membrane. This interaction reduces phosphorylation of Akt at both T308 and S473 sites [65]. TRB3, a mammalian homolog of Drosophila tribbles, which is expressed in the liver under fasting condition, also bind to the central region of Akt and negatively regulate its activity [66].

Downstream signaling of Akt

Synthetic peptide substrates related to the phosphorylation site of GSK3 paved the way to identifying minimal conditions required for Akt downstream targets. This is given as R-X-R-X-X-S/T-B where X can be any amino acid and B a bulky hydrophobic amino acid. The most effective substrate for Akt is R-P-R-T-S-S-F [67]. The action of Akt on its downstream targets determines its function in cardiovascular physiology [68]. Akt plays important roles in cell survival, growth, proliferation, angiogenesis, vasorelaxation, and cell metabolism. The downstream signaling of Akt is described in these physiological processes (Figure 3).

Cell survival and apoptosis

It is widely accepted that Akt mediates cell survival induced by growth factors. Cell survival is enhanced by blocking apoptosis. The initial event leads to apoptosis is the loss of mitochondrial integrity. Hallmark of loss of mitochondrial integrity is the release of cytochrome C to the cytoplasm. This cytochrome C can bind and activate apoptotic

protease-activating factor (Apaf-1). Apaf-1 and caspase-9 together create a pro-apoptotic signaling cascade. Akt directly phosphorylates caspase-9 and inhibits activation of caspase-9. This negatively regulates cytochrome-c/Apaf-1/caspase-9 pathway [69]

Akt can phosphorylate several other target proteins which regulates apoptotic or pro-apoptotic molecules. BAD, a member of Bcl-2 family, forms a protein complex with Bcl-2 and Bcl-X and inhibits their anti-apoptotic effects. Akt can phosphorylate BAD at Ser136 which enables BAD to bind with 14-3-3 protein. This in turn releases Bcl-2 and Bcl-X to promote more anti-apoptotic behavior [70]. Moreover, Akt phosphorylates murine double minute 2 (MDM2) at S166 and S186, resulting in increased nuclear translocation of MDM2 and ubiquitination and degradation of p53 and subsequently promoting cell cycle transition and survival [71–74]. Furthermore, Akt phosphorylates Yes-associated protein (YAP) at S127 in the cytosol and increases the binding of 14-3-3 protein to phosphorylated YAP. This helps YAP exporting from the nucleus to the cytoplasm, reducing the apoptotic activity of p73 [75].

In addition to directly affecting apoptotic or pro-apoptotic proteins, Akt can increase the cell survival by indirectly regulating the expression of these proteins. Forkhead family of transcriptional factors (FoxO) induces pro-apoptotic Bcl-2 family of proteins or stimulates expression of death receptor ligands such as Fas ligand and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL). It can also enhance the levels of cyclin dependent kinase inhibitors (CDKIs). Phosphorylation of FoxO by Akt inhibits the transcriptional functions of FoxOs and leads to decreased Bcl-2 and increased cell survival [76].

Akt-mediated cell survival can also be through MAP kinase pathway and JNK pathway. These two pathways are commonly known as stress activated protein kinase pathway (SAPK). Mixed lineage kinase 3 (MLK3) is a mitogen-activated protein kinase kinase kinase (MAPKKK) that activates c-jun N-terminal kinase (JNK) which activates apoptosis. MLK3 can interact with Akt, and insulin regulates this interaction. Akt phosphorylates MLK3 at S674, resulting in inhibition of MLK3-related cell death [77]. Moreover, Akt phosphorylates the apoptosis signalregulating kinase 1 (ASK1) at S83, leading to cell survival and inhibition of apoptosis [78]. Gain and loss of function of Akt has been shown to increase and decrease the survival of cardiomyocytes, respectively [79].

Cell growth and proliferation

The regulation of cell growth and proliferation by Akt requires the interplay between the mammalian target of rapamycin complex 1 (mTORC1) and the tuberous sclerosis complex 1/2 (TSC1 and TSC2) or the proline-rich Akt substrate of 40 kDa (PRAS40). TSC2 is an inhibitor of Ras-related small G protein Rheb, an activator of mTORC1. Phosphorylation of TSC1 by Akt inhibits TSC2 activity, leading to activation of mTORC1 [80]. Akt phosphorylates PRAS40 at T246 which facilitates PRAS40 phosphorylation of S183 by mTORC1. PRAS40 is known to negatively regulate mTORC1 [81,82]. Phosphorylation of PRAS40 by Akt and by mTORC1 per se results in the dissociation of PRAS40 from mTORC1 and the relief of an inhibitory constraint on mTORC1 activity [83]. This leads to phosphorylation of various mTORC1 substrates which are involved in cell proliferation

including p70 s6 kinase (p70S6K1) and eukaryotic initiation factor 4E (eIF4E) binding protein 1 (4E-BP1) [84–86].

In addition to cell growth and survival, cell proliferation can be stimulated by Akt through cell-cycle regulation. Growth factors cause Akt to increase the transcription of c-Myc and reduce the degradation of c-Myc by Mad1 [87]. C-Myc prevents cell cycle arrest [88]. Akt directly inactivates GSK3β, increasing Cyclin D1 which then inhibits Forkhead family transcription factors and the tumor suppressor tuberin (TSC2), reducing p27Kip levels [89]. This positively regulates G1/S cell cycle progression.

Angiogenesis

Akt mediates angiogenesis through stimulating the secretion of vascular endothelial growth factor (VEGF) [90,91]. Akt acts on TSC1–TSC2/Rheb/mTORC1 pathway in endothelial cells and increases the protein level of hypoxia inducible factor 1α (HIF-1α) which stimulates VEGF release. Akt has been shown to increase HIF-1α protein translation by activating the translational regulatory proteins p70 S6 kinase and eIF-4E [92–94]. However, sustained Akt activation leads to the formation of functionally abnormal blood vessels [95].

Endothelial cell migration is another essential function in angiogenesis. VEGF-induced endothelial migration is through Akt-PI3K pathway [96–98]. PDK1 activity is essential for this migration [99]. Ephrins and their receptor tyrosine kinases play an important role in the migration of vascular cells. Ephrin receptors can be phosphorylated by Akt even in the absence of ligands, causing cell migration [100,101].

Proper attachment to extracellular matrix proteins such as integrins helps endothelial cell attachment. Binding to integrin $\alpha_v\beta_3$ is required for VEGF to activate Akt that blocks anoikis [102,103].

Akt-dependent endothelial survival pathway also promotes angiogenesis. Angiopoietin-1 (Ang-1) acting via Tie 2 receptor has been shown to induce Akt phosphorylation which up-regulates survivin to reduce apoptosis in endothelial cells [104]. Akt can enable endothelial cells to be resistant to Fas-mediated apoptosis by expressing the FLICE-inhibitory protein (FLIP) [105]. Constitutively active Akt has been shown to phosphorylate MEKK3 that reduces MKK3/6-and p38 MAPK-activated apoptosis [106].

The HMG-CoA reductase inhibitor statins seems to act with Akt pathway to bring pleiotropic effects. Statins have been shown to promote the proliferation and differentiation of endothelial progenitor cells (EPCs) through PI3K/Akt pathway [107].

Akt can directly phosphorylate eNOS [108]. eNOS plays a predominant role in angiogenesis and vascular permeability induced by growth factors and angiotensin II [109] [110]. Akt directly phosphorylates eNOS at S1197, enhancing its enzymatic activity and altering the sensitivity of the enzyme to Ca^{2+} [108,111]. Akt interacts with Hsp90, a protein that associates with and activates eNOS. Hsp90 serves a scaffolding function to facilitate Akt-mediated phosphorylation of eNOS in the calveolae [112–114].

Vasorelaxation

Akt has been shown to regulate vasomotor tone in vivo [115,116]. Since NO is an important regulator of vasomotor tone, effects of Akt on eNOS phosphorylation and activation can significantly enhance vasorelaxation. Over-expression of Akt causes a significant increase in resting vessel diameter and blood flow while inhibition of Akt attenuates endothelium-dependent vasodilatation in response to acetylcholine [115]. Therefore, dysregulation of Akt activity can lead to development of endothelial dysfunction in hypertension [117].

Metabolism

Akt can increase metabolism in cells by increasing either the uptake of nutrients or the cellular glucose and lipid metabolism. Hexose uptake and GLUT4 translocation can be increased by Akt2 activity [118]. Akt is also associated with sterol-regulatory element-binding proteins (SREBPs), a transcriptional regulator in lipid metabolism, mTORC1 and GSK3 pathway [119]. Akt might be playing a crucial role in cholesterol import and synthesis, fatty acid synthesis or SREBP production [119]. Akt is one of the major signal molecules in insulin-related signal transduction. After tyrosine phosphorylation of insulin receptors, insulin receptor substrate (IRS) family members of proteins are phosphorylated. This activates PI3K pathway and phosphorylates Akt, which leads to inactivation of GSK3 and phosphorylation of the Forkhead transcription factor. This signal leads to increases in the glucose metabolism, glycogen, lipid and protein synthesis, and other specific gene expressions [120,121].

Akt in cardiovascular pathologies

Atherosclerosis

Oxidized LDL has been shown to reduce Akt phosphorylation causing the inhibition of endothelial cell migration [122]. In atherosclerosis, instability and rupture of the plaque can lead to myocardial infarction, which can be caused through apoptosis of vascular smooth muscle cells. Akt seems to have a role in smooth muscle apoptosis via insulin-like growth factor 1 (IGF1) receptor signaling. Reduction of IGF1R signaling and dysregulation of phosphorylation of Akt, FoxO3a and GSK3 leads to apoptosis of vascular smooth muscle cells [123]. Inhibition of Akt in vascular smooth muscle cells can lead to significant increase in p-JNK and p-c-jun, pro-apoptotic proteins. This works in opposite manner in immune cells [124]. For example, lack of Akt2 in macrophages shows a decrease in pro-inflammatory genes and a reduction of cell migration. Loss of Akt2 suppresses macrophages undergoing M1 polarization. All these events on macrophages help reduce atherosclerosis [125]. Peroxisome proliferator-activated receptors (PPARs) have been shown to protect vasculature from pathological alterations like atherosclerosis. This is achieved through inhibition of VEGF-induced Akt phosphorylation pathway [126].

Cardiac hypertrophy

Cardiac hypertrophy can be defined at cellular level as increased cardiomyocyte cell volume [127,128]. Normal growth, growth induced by physical conditioning and growth induced by pathologic stimuli are the three major types of cardiac hypertrophy [129]. Cardiac hypertrophy caused by normal growth or physical conditioning such as exercise is called

adaptive cardiac hypertrophy [130]. Maladaptive hypertrophy can be either caused as a response to excessive hemodynamic workload or by genetic mutations. This can eventually lead to heart failure [131–133].

Growth factors and exercise can activate a 110-kDa lipid kinase, PI3K subgroup Iα [134,135]. Another class of PI3K, p110γ, is activated by G-protein coupled receptors (GPCR) due to biomechanical stress and neuro-hormonal mediators [136,137]. Both of these pathways can lead to Akt phosphorylation and causing inhibition of GSK3β signaling, which eventually leads to protein synthesis and transcriptional activation causing cardiac hypertrophy [129].

Over-expression of Akt has been shown to be maladaptive for the heart. This ill-effect seems to be rescued by PI3K, suggesting the importance of PI3K in cardiovascular treatments [138]. Akt over-expression seems to enlarge the cell size and increase the contractility of cardiomyocytes in Akt transgenic mouse model [139].

Constitutively active Akt can increase the angiogenesis in heart. Initially this can contribute to adaptive cardiac hypertrophy. At a later stage this can lead to cardiac hypertrophy and heart failure [140]. However, this is a dilemma since exercise is known to increase the cardiovascular health through increased Akt activity [141]. There must be a fine balance between the healthy and maladaptive Akt activation levels and duration which is yet to be investigated.

Vascular remodeling

Vascular remodeling process consists of changes of cell growth, cell death, cell migration and production or degradation of extracellular matrix [142]. Akt plays a role in the pathogenesis of vascular remodeling. Akt substrate GSK3β is a crucial protein in smooth muscle proliferation where inhibition of GSK3β by Akt-induced phosphorylation increases smooth muscle proliferation [143]. Increased cell survival is also achieved through Akt signaling [144]. In restenosis and atherosclerosis, increased proliferation of vascular smooth muscle cells is mediated through Akt pathway [145]. Increased proliferation and survival of vascular smooth muscle cells contribute to medial thickening. Akt also affects the activity of adventitia in the vasculature. After arterial injury, Akt activity is significantly higher in the adventitia and contributes the increased proliferation of adventitial fibroblasts [146].

Several mitogens such as platelet-derived growth factor (PDGF), transforming growth factor-beta (TGFβ), endothelin and thrombospondin-1 stimulate Akt phosphorylation causing the polyubiquitination and proteasomal degradation of cAMP response element binding protein (CREB) in pulmonary artery smooth muscle cells and resulting in medial smooth muscle cell proliferation, hypertrophy and extracellular matrix production [147]. In addition, mTORC2 also induces the activation and phosphorylation of Akt which leads to mTORC1 and S6K pathway in pulmonary vascular remodeling [148–150]. Akt1 rather than Akt2 is the important signaling molecule for the development and progression of pulmonary vascular remodeling and pulmonary hypertension [151].

Akt inhibitors

Several Akt inhibitors are currently being developed and tested as anti-tumor agents. But only few of them have been tested in cardiovascular diseases. There are several strategies to inhibit the activation of Akt by its upstream signaling. Akt upstream inhibitors, allosteric inhibitors, pseudo-substrates, pleckstrin homology (PH) domain inhibitors and ATP binding pocket inhibitors are some of the major inhibitors.

Upstream inhibitors

Many upstream molecules of Akt pathway can be inhibited to reduce their downstream activation of Akt. Wortamanin, PX-866, and LY294002 are well-known potent pan-PI3K inhibitors [152–154]. HS-173/PIK75, TGX-221, CZC24832 and CAL-101 are selective PI3K inhibitors for PI3K p110 catalytic subunits α, β, γ and δ respectively [155–158]. PDK inhibitors OS-03012, BX-795, BX-912 and PHT-427 have been used successfully to reduce proliferation and increase cell apoptosis in various cancer models [159–161]. Rapamycin is one of the first successful mTORC inhibitors discovered [162]. Rapamycin forms a complex with FK-binding protein 12 (FKBP12) and inhibits mTOR. Several rapamycin derivatives including temsirolimus, everolimus and deforolimus have been developed to be more potent mTORC1 inhibitors [163]. Pan-mTOR inhibitors AZD8055, KU-0063794, and PP242 can be more effective alternative since they inhibit both up-stream mTORC2 and the downstream mTORC1 [164–166].

Dehydroepiandrosterone (DHEA), a widely used steroid, is another Akt upstream inhibitor tested for treatment of vascular remodeling by inhibiting smooth muscle proliferation and activating vascular smooth muscle cell apoptosis. DHEA inhibits Akt pathway through an as-yet unidentified GPCR. That leads to increased GSK3 activity causing suppression of smooth muscle proliferation and activation of vascular smooth muscle cell apoptosis. In addition, DHEA directly acts on Kv (4-aminopyridine-sensitive [4-AP]) and BKCa (iberiotox-insensitive [IbTx]) channels, causing reduction of smooth muscle [Ca^{2+}], cell proliferation, and vascular remodeling [167,168].

DNA-PK inhibitors NU7441and KU0060648 can be used to inhibit Akt activation [169–171]. These inhibitors have been studied as anti-cancer agents.

Allosteric inhibitors

New strategies are needed to lower the toxic off-target effect of pan-kinase inhibitors. Allosteric inhibitors block the activity of the target kinases by altering the protein conformation through interacting with regions distal to the ATP binding site [172]. Triciribine suppresses the phosphorylation level and kinase activity of Akt. Triciribine can selectively inhibit all Akt isoforms without inhibiting known upstream activators, PDK1 and PI 3-Kinase. MK-2206 is an allosteric inhibitor of Akt, which is now in clinical trials as a cancer drug. However the specific mechanism of action of this is not known. MK-2206 inhibits all isoforms of Akt. IC50 of MK-2206 is 8nM for Akt1, 12nM for Akt2, and 65nM for Akt3 [173,174].

Pseudo-substrates

A 14-mer AKTide-2T peptide with a sequence of ARKRERTYSFGHHA has been identified to be able to bind to the substrate binding domain of Akt1 with a Ki of 12 mM. A hybrid of this sequence and a region of FOXO3 (sequence: VELDPEFEPRARERTYSFGH) has improved its affinity with a Ki of 1.1 mM. Replacing the tyrosine and serine residue to alanine (sequence: VELDPEFEPRARERAYSFGH and VELDPEFEPRARERTYAFGH) has improved their inhibitory effect to Ki of 95 nM and 110 nM, respectively [175].

Pleckstrin homology domain inhibitors

Perifosine is a new class of Akt inhibitors which target the PH domain. It is a synthetic alkylphospholipid which is effective in controlling Akt-mediated cell proliferation [176]. Tirucallic acid and PITENINs (antagonists of PIP3/PH domain binding) are novel classes of PH domain inhibitors [177,178]. However their inhibitory concentration is in μM range, making them less potent for pharmacological interventions.

ATP binding pocket inhibitors

Several small molecules which compete for the ATP binding pocket have been found as potent inhibitors of Akt. Analogs of H89, which are inhibitors of PKA, have been developed as a Akt inhibitors [179]. A-443654 is another example of indazole-pyridines used as an ATP binding pocket inhibitor. However, inhibition of Akt phosphorylation by this inhibitor can create a feedback loop where phosphorylated Akt can increase later [162].GSK690693 is a pan Akt inhibitor which competitively inhibits all isoforms of Akt with IC50 of 2 nM for Akt1, 13 nM for Akt2, and 9 nM for Akt3 [180]. New isoform-selective ATP binding pocket inhibitors are being experimented. For example, A-674563 is a potent and selective Akt1 inhibitor with an IC50 of 14 nM. A-674563 also inhibits activity of PKA and CDK2 with IC50 of 16 and 46 nM, respectively. CCT128930 inhibits Akt2 selectively with IC50 of 6 nM, 28-fold greater selectivity for Akt2 than PKA and other Akt isoforms [181].

Future perspectives

As more evidence of complexity of Akt signal transduction pathway is discovered, it is important to consider these complexities in future research and retrospective analysis of Akt research data. The initial research on Akt with regards to cancer or cardiovascular diseases largely neglected isoform-specific and phosphorylation site-specific effects of Akt. It is important to understand the roles of these different mechanisms in disease context. As more and more cross-talk between Akt and other signal transduction pathways becomes evident, more research should focus on the system biology of these pathways [182]. Moreover, cardiovascular system consists of several types of tissues in a unique arrangement. Interactions between these cell types play an integral role in health and disease. There is growing evidence of Akt signaling in cardiovascular system to be influenced by the neighboring cell types in tissues [183]. Simulation of these conditions will be possible with co-culture of cells other than mono-cultures. Further, novel research areas like long-noncoding RNAs seems to have a control in the signal transduction pathways. Akt and non-coding RNA associations are also evident now [184]. In addition, Akt is affected by epigenetic control and also acts as a mediator for epigenetic control of disease [185,186].

Another ignored area in Akt studies is the non-canonical pathway of its activation. Knowledge and research in these pathways might be able to explain phenomenon which cannot be explained by the classical Akt pathway. Finally, most of the research on Akt is in the field of cancer, though there are some similarities between certain pathological conditions in cardiovascular disease and the hallmarks of cancer. More studies should be done to understand Akt in cardiovascular diseases. There is an urgent need to develop potent tissue specific inhibitors and activators of Akt pathway for research and therapeutic purposes.

Acknowledgement

This work was supported by NIH grants HL088261 and HL115078 to YS and Flight Attendants Medical Research Institute grant 113018_CIA to YS, and by the Department of Veterans Affairs.

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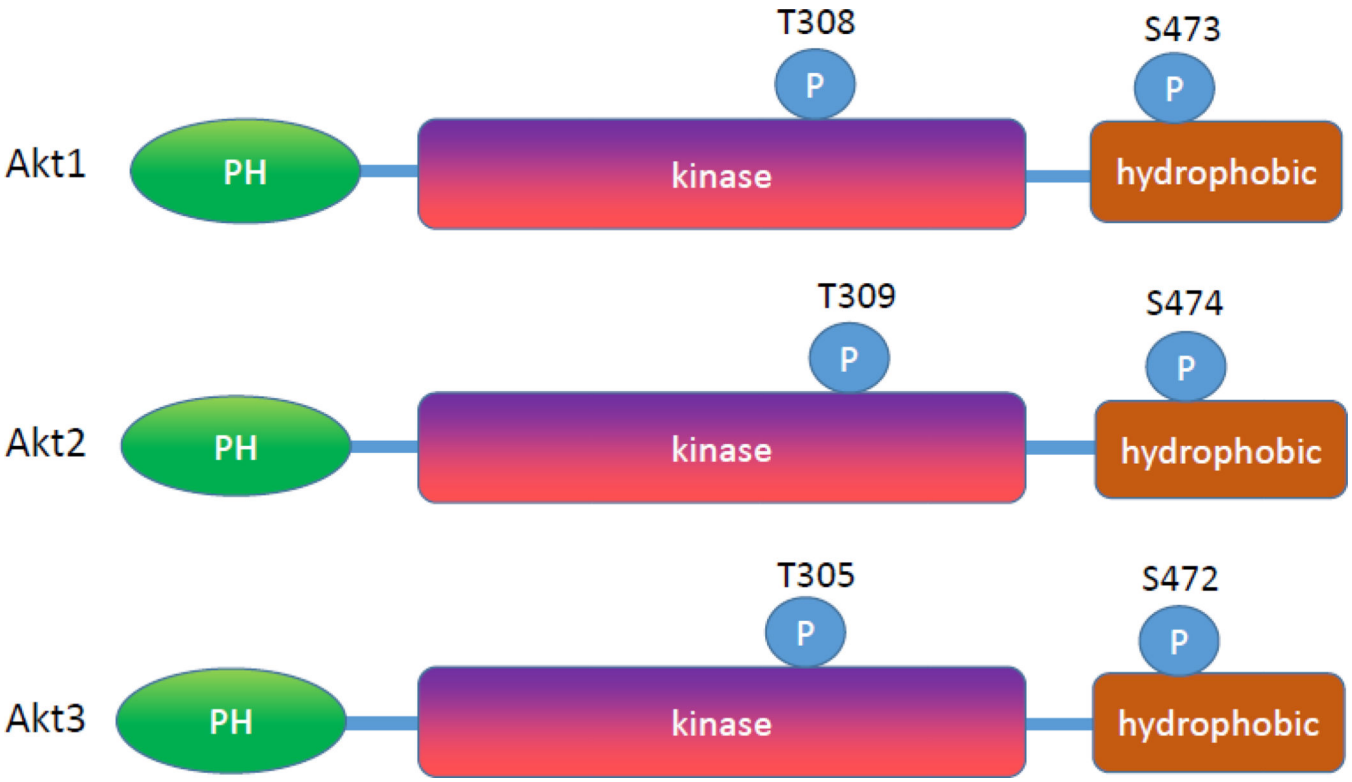

Figure 1.

Human Akt protein structure. Akt has a characteristic pleckstrin homology (PH) domain at the amino terminal (~ 110 amino acids), a middle kinase domain (~260 amino acids) and a carboxy-terminal regulatory domain (~70 amino acids).

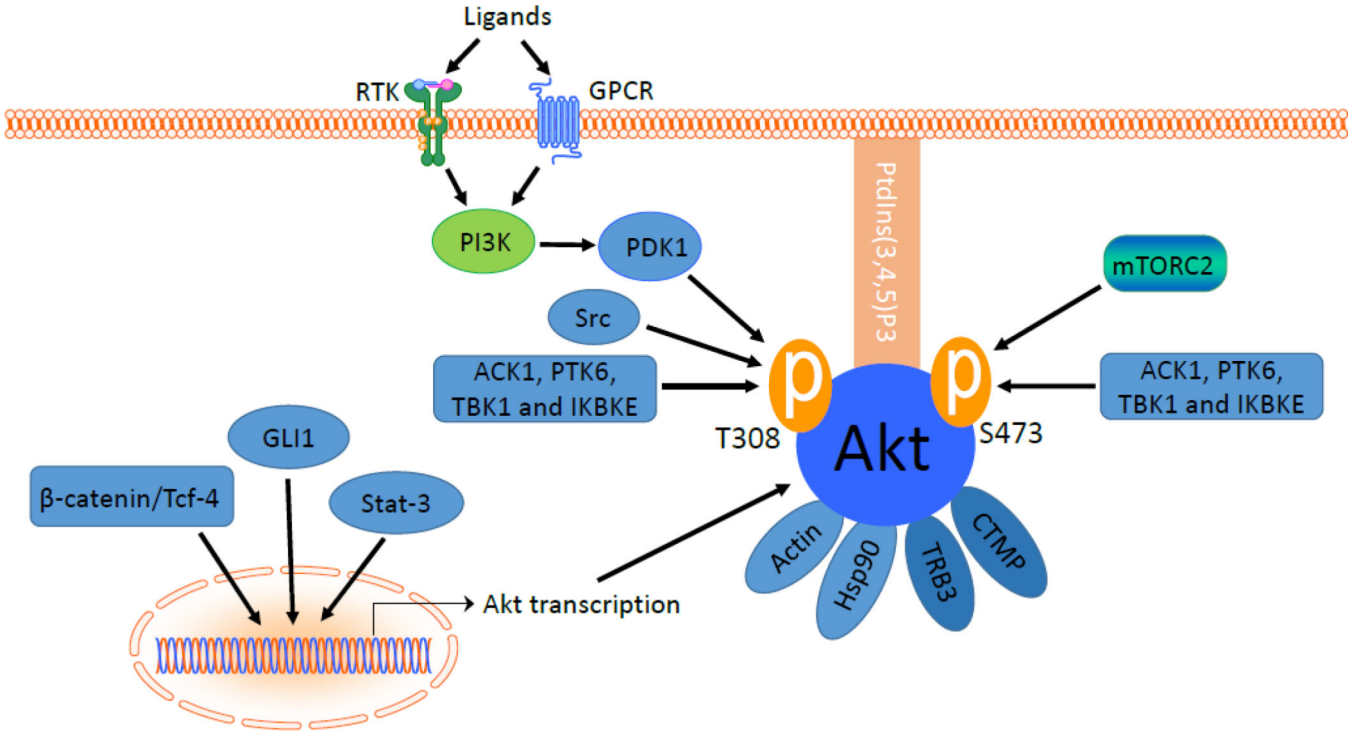

Figure 2.

The upstream signals of Akt. Akt activity is regulated by its upstream regulatory pathways at transcriptional and post-translational levels. beta-catenin/Tcf-4, GLI1 and Stat-3 are known transcriptional regulators of AKT gene. T308 and S473 are the two critical phosphorylation sites of Akt1. Affinity of Akt to PtdIns (3,4,5) P3 is required for membrane translocation. PI3 kinase-dependent phosphorylation of T308 is through PDK1. S473 is phosphorylated by mTORC2. Ack1, Src, PTK6, TBK1, IKBKE and IKKε are some of the non-canonical pathways which affect the Akt activity. Protein-protein interactions of Akt to actin and Hsp90 increase the Akt activity while Akt binding to CTMP and TRB3 reduces the Akt activity.

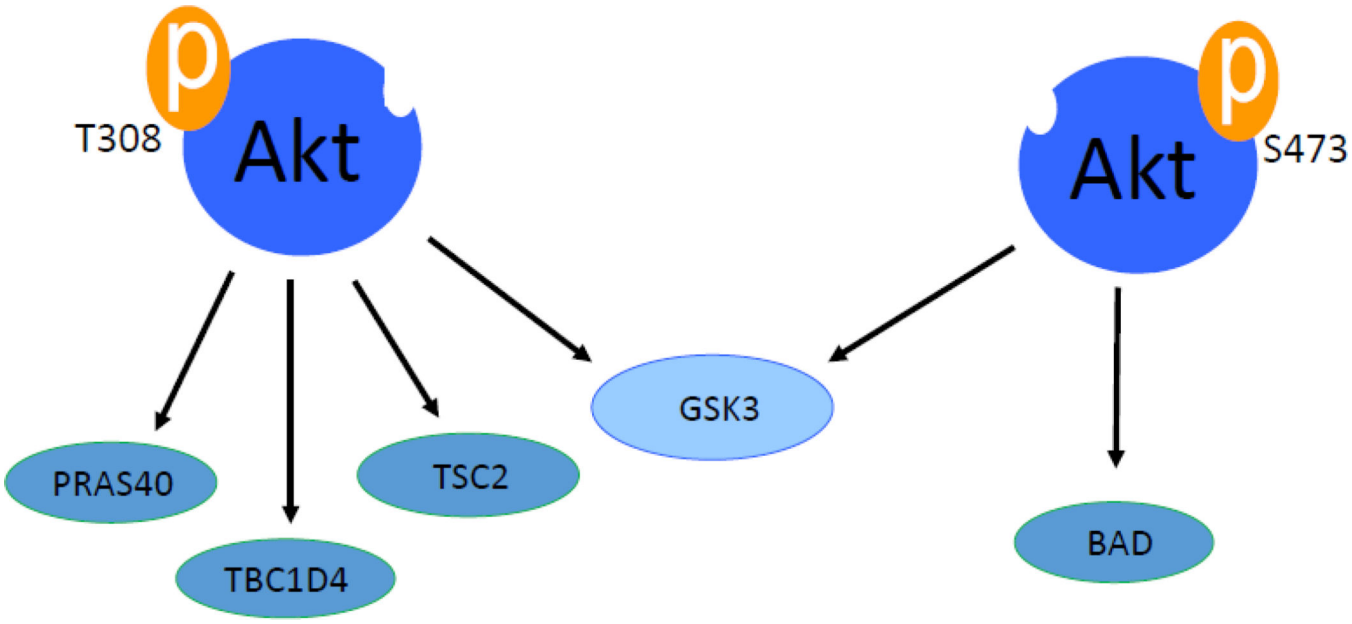

Figure 3.

Two critical phosphorylation sites of Akt has different downstream targets in certain disease conditions. For example Phosphorylation of Akt at T308 significantly increases phosphorylation of PRAS40, TBC1D4 and TSC2 where Phosphorylation of S473 has less effect on these substrates. In similar manner, phosphorylation of S473 significantly increases phosphorylation of BAD. However GSK3 can be phosphorylated by any of the critical phosphorylations on Akt1.

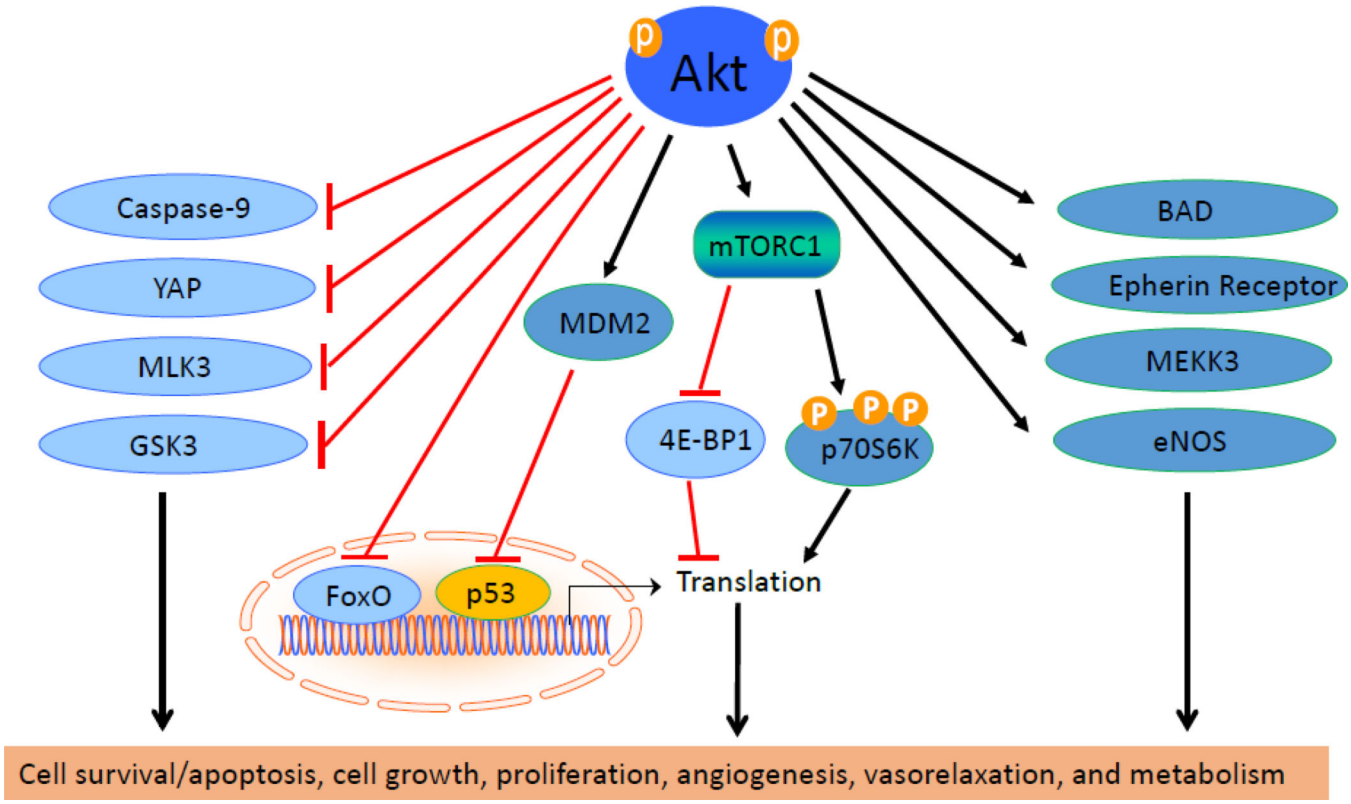

Figure 4.

The downstream signals of Akt. Akt stimulates cell growth and proliferation through mTORC1. Akt phosphorylates BAD, epherin receptor, MEKK3, eNOS and MDM2, and up-regulates the activities of these proteins. Direct phosphorylation of caspase-9, YAP, MLK3, GSK3 and FoxO by Akt results in inhibitions of their enzymatic activities. The action of Akt on its downstream targets determines its function in cardiovascular processes such as cell survival, growth, proliferation, angiogenesis, vasorelaxation, and cell metabolism.