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MINIREVIEW

Emerging Role of Akt Kinase/Protein Kinase B Signaling in Pathophysiology of Diabetes and Its Complications

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Summary

In addition to a number of deleterious effects on cellular integrity and functions, diabetic metabolic milieu has been implicated in a rapidly growing number of alterations in signal transduction. In this review we focus on Akt kinase physiology, its alterations in diabetes mellitus (DM), and on the emerging role of this signaling system in the pathophysiology of diabetic microvascular complications. Studies focusing on Akt in diabetes suggest both decrease and increase of Akt activity in DM. Alterations of Akt activity have been found in various tissues and cells in diabetes depending on experimental and clinical contexts. There is convincing evidence suggesting defective Akt signaling in the development of insulin resistance. Similar defects, as in insulin-sensitive tissues, have been reported in endothelia of DM Type 2 models, possibly contributing to the development of endothelial dysfunction under these conditions. In contrast, Akt activity is increased in some tissues and vascular beds affected by complications in DM Type 1. Identification of the role of this phenomenon in DM-induced growth and hemodynamic alterations in affected vascular beds remains one of the major challenges for future research in this area. Future studies should include the evaluation of therapeutical benefits of pharmacological modulators of Akt activity.

Key words
Diabetes mellitus • Insulin • Akt kinase • Diabetic vascular complications

Introduction

Recent progress in understanding the physiology and pathophysiology of a wide spectrum of cellular signaling pathways and their elaborate cross-talks and interactions has opened new trajectories in most areas of biomedical research, including diabetes mellitus (DM) and its vascular complications. In addition to a number of deleterious effects on cellular integrity and functions, diabetic metabolic milieu has been implicated in a rapidly growing number of alterations in signal transduction. Substantial attention given to these mechanisms is not surprising considering the potential therapeutic impact of specific pharmacological modulations of these pathways (Ishii *et al.* 1996). In this review we will focus on alterations of Akt kinase signaling in DM, and on the emerging role of this signaling system in the pathophysiology of diabetic microvascular complications.

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Akt physiology

Akt protein was isolated as a gene product of the Akt gene. It is a serine/threonine kinase with the pleckstrin homology domain (PH) in its NH_2 terminal region and catalytic domain closely related to protein kinase C (PKC) and protein kinase A (PKA) family members (Staal 1987). These similarities explain alternative terminology used for Akt, i.e. protein kinase B (PKB) or RAC (related to A and C) protein kinase. Mammalian genomes contain three Akt genes, Akt1, Akt2, and Akt3 that encode three widely expressed isoforms of Akt kinase. Tissue and organ expression of individual isoforms is shown in Table 1.

Multistep process of Akt/PKB activation

Akt can be activated by a wide variety of growth stimuli such as growth factors and cytokines (Table 2). The pathway of Akt activation is a process involving membrane translocation and phosphorylation (Bellacosa *et al.* 1998). The typical route of Akt activation is mediated *via* tyrosine kinase receptors such as receptors for insulin or certain growth factors (Fig. 1). Upon stimulation, agonists activate receptor kinase functions resulting in stimulation of tyrosyl phosphorylation of some substrates, such as insulin receptor substrate 1 (IRS1) (Sun *et al*. 1991), IRS2 and Shc proteins (Sun *et al.* 1995). Tyrosyl phosphorylation of IRSs provides binding sites for specific proteins containing SH_2 domains including the 85 kDa regulatory subunit of phospatidylinositol 3-kinase (PI3K), a crucial molecule in this signaling pathway (Burgering and Coffer 1995, Toker and Cantley 1997).

Table 1. Expression profile of Akt family members

	High expression levels	**Low/ moderate expression level**
Akt 1	Brain, heart, testis, thymus	Kidney, liver, spleen
Akt 2	Brown fat, cerebellum (Purkinje cells), heart, skeletal muscle	Brain, kidney, lung spleen, testis
Akt 3	Brain, testis	Heart, kidney, liver lung, skeletal muscle, spleen

Table 2. Vasoactive factors that modulate signaling via PI3K-Akt pathway

Factor	**Reference**
vascular endothelial growth factor*	(Fulton *et al.* 1999)
angiopoietin-1	(Kim *et al.* 2000)
insulin-like growth factor-I	(Camper-Kirby *et al.* 2001)
sphingosine-1-phosphate (S1P)	(Marletta 2001)
hepatocyte growth factor	(Xiao *et al.* 2001)
decorin	(Schonherr *et al.* 2001)
fluid shear stress*	(Dimmeler *et al.* 1998)
estrogen	(Camper-Kirby *et al.* 2001)
reactive oxygen species*	(Thomas *et al.* 2002)
corticosteroids	(Limbourg *et al.* 2002)
angiotensin II*	(Gorin *et al.* 2001)
leptin*	(Vecchione *et al.* 2002)
transforming growth factor-ß*	(Chen *et al.* 2001a)

* indicates factors implicated in diabetic vascular complications

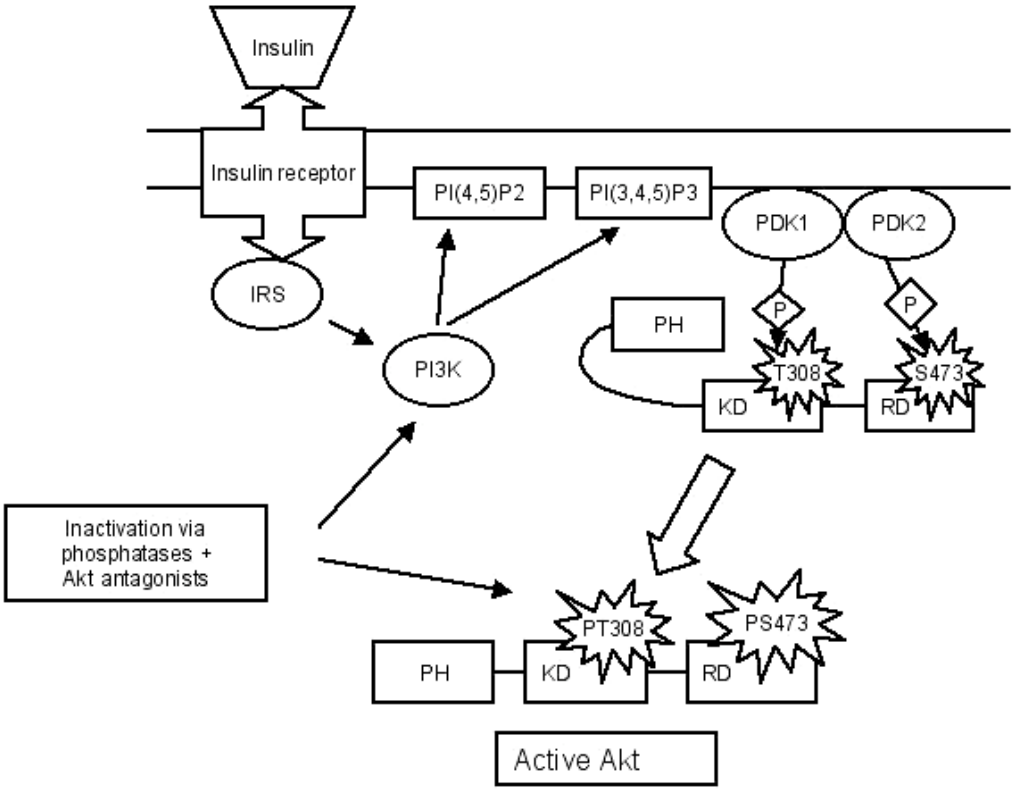

Fig. 1. Mechanism of Akt activation via insulin receptor. Akt is activated by insulin via insulin receptor in PI3-K dependent manner and phosphorylated on two residues by PDK1 (T308) and PKD2 (S473). Insulin receptor substrate (IRS); phosphatidylinositol 3´kinase (PI3-K); 3´-phosphorylates phosphatidylinisitol lipids ($PI(4,5)P_2$, $PI(3,4,5)P_3$); phosphoinositide-dependent protein kinases (PDKs); pleckstrin homology domain (PH); kinase domain (KD); regulatory domain (RD).

Activation of PI3K stimulates its lipid kinase activity *via* activation of p110 catalytic subunit resulting in the addition of phosphate on the D3 position of phosphatidylinositol and production of phosphatidyl-inositol 3-phosphate and other 3-phosphorylated inositides (PI3P). The plecstrin homology (PH) domain of Akt kinases has an affinity for PI3P and the binding of PI3P triggers Akt translocation to the plasma membrane (Bellacosa *et al.* 1998, Bottomley *et al.* 1998). Furthermore, the increasing levels of phosphoinositides function as intracellular second-messenger molecules leading to activation of PI-dependent kinases (PDK1 and PDK2). PDKs activate Akt upon its membrane translocation by phosphorylation on threonine 308 (Thr 308) (Alessi *et al.* 1997) and serine 473 (Ser 473) (Bellacosa *et al.* 1998). Thr 308 phosphorylation is necessary for Akt activation, whereas Ser 473 phosphorylation is required as well for maximal activity (Downward 1998). Activated Akt becomes available for phosphorylation of its downstream targets in various subcellular localizations and nucleus. Inactivation of Akt is accomplished by dephosphorylation by protein phosphatases such as protein phosphatase 2A, or by Akt antagonists (ceramides and PTEN) which block an activation of this enzyme in certain steps of its activation (Ozes *et al.* 2001, Teruel *et al.* 2001).

Downstream targets and physiological consequences of Akt activation

Stimulated Akt has a number of downstream targets (Fig. 2). An exhausting description of all these pathways, effects and pathophysiological consequences is beyond the scope of this review and the reader is referred to recent excellent reviews covering these topics (Testa and Bellacosa 2001, Shiojima and Walsh 2002). In this review, we will focus on those mechanisms that are relevant for pathophysiology of diabetes and its complications.

Metabolic functions

Akt is an important mediator of biological functions of insulin. One of major effects of this hormone is the enhancement of glucose uptake in muscle, adipocytes, liver, and other tissues. Therefore, it is not surprising that Akt signaling has major impact on glucose metabolism. Earlier studies recognized that PI3K is

responsible, at least in part, for insulin stimulation of GLUT4, the major insulin-regulated glucose transporter, from intracellular vesicles to the plasma membrane in insulin-sensitive cells (Frevert *et al*. 1998, Okada *et al*. 1994). Therefore, the role of Akt in this process has also been evaluated. It has been suggested that Akt involvement in glucose metabolism occurs on two principal levels. Upon insulin stimulation Akt associates with GLUT4 transporters (Calera *et al.* 1998) and glucose uptake is mediated by Akt-induced translocation of vesicles containing GLUT4 from intracellular stores to the plasma membrane (Kohn *et al.* 1996, Hajduch *et al.* 1998). In this cellular location, GLUT4 mediate glucose uptake. It should be noted that despite proven capability of constitutively active Akt to stimulate glucose uptake in adipocytes (Cross *et al.* 1995), inhibition of Akt through the use of dominant negative mutants does not completely block insulin effect on glucose transport (Franke *et al.* 1997). This phenomenon suggests involvement of multiple kinases. Indeed, atypical members of the protein kinase C family (ζ and λ) have been proposed as alternative mediators of insulin-induced glucose transport (Kotani *et al.* 1998). Furthermore, in response to insulin, Akt promotes glycogen synthesis via serine phosphorylation and inactivation of glycogen-synthase kinase-3 (GSK-3). The enzyme glycogen synthase is inhibited when covalently phosphorylated on serines by GSK-3 (Cross *et al.* 1995, Lawrence and Roach 1997).

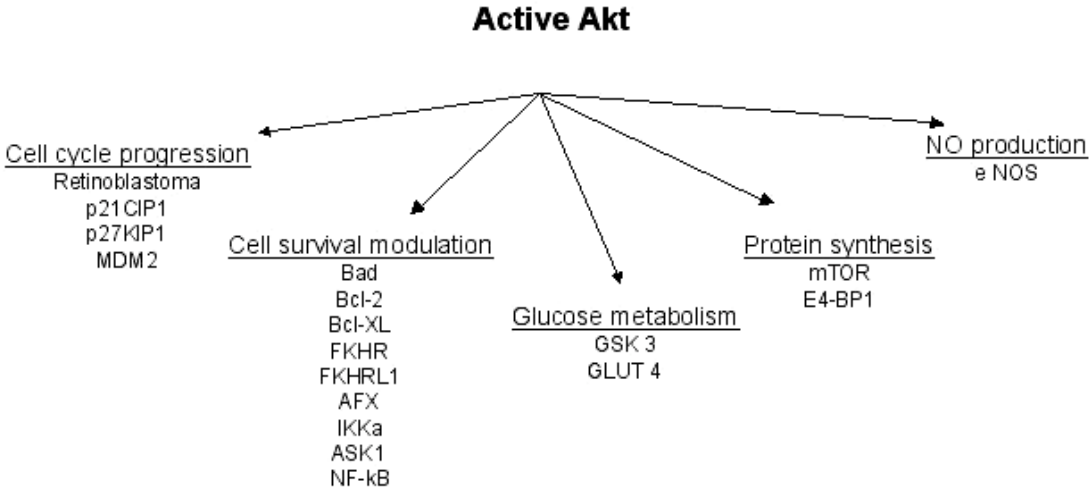

Fig. 2. Akt downstream target molecules
Downstream target Akt substrates are grouped according to their function. See text for abbreviations and more information about these molecules.

Involvement of Akt in prosurvival and antiapoptotic mechanisms

This aspect of Akt physiology and pathophysiology is one of the most intensively studied with respect to its role in a number of areas of biomedical research spanning from oncology to cardiovascular medicine. Akt is critical for cell survival triggered by growth factors, extracellular matrix, and other stimuli. For example, dominant negative alleles of Akt reduce the ability of growth factors and other stimuli to maintain cell survival, whereas overexpression of wild type or activated Akt can rescue cells from apoptosis induced by various stress signals (Kauffmann-Zeh *et al.* 1997, Khwaja *et al.* 1997). Promotion of cell survival by Akt is accomplished on two levels.

First, Akt is involved by transcriptional regulation of prosurvival and antiapoptotic genes. Akt promotes cell survival by directly phosphorylating transcription factors that control the expression of pro- and antiapoptotic genes. Akt appears to both negatively regulate factors that promote the expression of death genes and positively regulate factors that induce survival genes. An example of the former is the forkhead family of transcription factors (FKHR, FKHRL1, AFX) that contain consensus Akt phosphorylation sequences, which can be effectively phosphorylated by Akt *in vitro* (Biggs *et al.* 1999, Rena *et al.* 1999). In addition to negatively regulating forkhead activity, Akt appears to positively regulate at least two prosurvival transcription factors. Transcription factor NF-κB is involved in the regulation

of cell proliferation, apoptosis, and survival by a wide range of cytokines and growth factors (Orlowski and Baldwin 2002). NF-κB is regulated through its association with an inhibitory cofactor I-κB, which sequesters NF-κB in the cytoplasm. Phosphorylation of I-κB by upstream kinases, known as IKKs, promotes its degradation allowing NF-κB to translocate to the nucleus and induce target genes. Akt has been shown to interact with and activate IKKs (Romashkova and Makarov 1999) and contribute to IKK-mediated destruction of I-κB and activation of NF-κB (Ozes et al. 1999, Romashkova and Makarov 1999). Finally, Akt may play a role in regulating the expression of c-FLIP, a caspase-8 homologue that acts as a dominant negative inhibitor of TNF receptor family-induced apoptosis (Panka 2001).

Second, Akt promotes survival by direct phosphorylation of key regulators of the apoptotic cascade. The most widely studied example of this type of regulation involves BAD, a member of the Bcl-2 family, which promotes apoptosis by binding to and antagonizing the actions of prosurvival members of the family such as Bcl-2 and Bcl-XL. Akt can phosphorylate BAD and this modification promotes the sequestration of BAD in the cytosol, thus preventing BAD from interacting with Bcl-2 or Bcl-XL (del Peso *et al.* 1997). Signaling *via* stress-activated protein kinases, such as JNK, is critically involved in the induction of apoptosis following exposure of cells to physical stress stimuli (Davis 2000). Akt may interfere with JNK signaling and thereby inhibit apoptosis by inhibitory phosphorylation of ASK1, a kinase that transduces stress signals to the JNK and p38 MAP kinase pathways (Kim *et al.* 2001).

Cell cycle

Cell proliferation is a nuclear event divided into different phases called the cell cycle (Shankland *et al.* 2000). Non-dividing cells are in the G_0 phase and enter the cell cycle at G_1, followed by the S phase, where DNA replication occurs. Cells then progress through G_2, and enter mitosis (M phase), which is followed by cell division. Transition from one cell cycle phase to another is a coordinated, sequential, and synchronized process. Cell cycle progression is controlled by positive [cyclins and cyclin-dependent kinases (CDK)] and negative (CDK inhibitors) cell cycle regulatory proteins. CDK inhibitors, such as $p21^{CIP}$ and $p27^{KIP}$ negatively regulate cell cycle progression by inhibiting cyclin-CDK complexes, resulting in cell cycle arrest. Proliferation requires normal progression through the cell cycle, hypertrophy occurs when cells engage the cell cycle but cannot progress beyond late G_1 (G_1/S arrest), apoptosis is associated with exit from the cell cycle, which typically occurs in G_1. These processes may thus share common pathways and may explain why certain cell populations undergo proliferation and apoptosis, whereas proliferation and hypertrophy are independent events. The first direct evidence supporting the role of Akt in this area was provided by a study showing that overexpression of Akt2 accelerated cell cycle progression and caused transformation in murine fibroblasts (Cheng *et al.* 1997). More recent findings indicated that Akt promotes cell cycle progression by several mechanisms that include inhibitory phosphorylations and reduced transcription of CDK inhibitors $p21^{CIP1}$ and $p27^{KIP1}$ (Medema *et al.* 2000, Shin *et al.* 2002, Zhou *et al.* 2001), as well as increased cyclin D transcription and translation.

Effect of Akt on protein synthesis

The effects of Akt stimulation on protein synthesis seem to be closely related to growth and mitogenesis. These effects are achieved on the level of translational regulation. Eukaryotic initiation factor 4E (eIF4E) and its binding protein 4E-BP1 are important translational regulatory factors. In resting cells, eIF4E is held inactive in a complex with 4E-BP1 and is released by the phosphorylation of the latter. Several lines of evidence in various cell types suggested that 4E-BP1 is phosphorylated by the mammalian target of rapamycin (mTOR, FRAP), a key element in this process. Upon insulin and possibly growth factor stimulation, mTOR is phosphorylated (activated) by Akt (Scott *et al.* 1998).

Role of Akt/PKB in vascular biology

A large number of factors that play various roles in vascular, in particular, endothelial biology activate PI3K-Akt signaling (Table 2), illustrating the central role of this pathway in controlling vascular function and viability.

Phosphorylation of endothelial nitric oxide synthase (eNOS)

In this context, one of the most important effects of Akt stimulation in the vascular system is its involvement in endothelial NO production. Akt kinase has been identified as kinase responsible for Ca^{2+}-independent as well as Ca^{2+}-dependent activation eNOS (Dimmeler *et al.* 1999, Fulton *et al.* 1999). Akt activates eNOS by Ser-1177/1179 phosphorylation that facilitates

association of the enzyme with calmodulin reducing its inhibitory interaction with caveolin-1 (CAV-1). Modulation of Akt activity has impact on vascular tone *in vivo* (Luo *et al.* 2000). Most important factors that stimulate endothelial NO production *via* Akt are VEGF (Fulton *et al*. 1999) and insulin (Zeng *et al.* 2000, Zeng and Quon 1996), both well established NO-dependent vasodilators. In addition, other factors relevant to the pathophysiology of diabetes and its complications, such as leptin (Vecchione *et al*. 2002) and TGF-ß (Chen *et al.* 2001a) stimulate NO production *via* Akt. Akt also interacts with and is enhanced by heat shock protein 90 (Hsp90), one of many co-factors that enhance eNOS enzymatic activity (Sato *et al.* 2000).

Neovascularization and endothelial cell migration

Role of Akt in angiogenesis is closely related to its effects on cell cycle progression. In previous sections, we reviewed the abundant evidence identifying Akt as a potent pro-survival kinase. Vascular endothelial cells and the vascular compartment in general are not, in this context, exceptional and most of the mechanisms described above are applicable to vascular cells. Angiogenic factors that activate Akt, such as VEGF or angiopoietin-1, can prevent endothelial cell apoptosis (Gerber *et al.* 1998, Kim *et al.* 2000). The ability of endothelial cells to migrate and form capillary-like structures is essential for angiogenesis *in vivo*. VEGF enhances endothelial cell migration and capillary-like structure formation *in vitro* and these activities of VEGF are PI3K-Akt-dependent (Kureishi *et al.* 2000). Akt-induced eNOS activation also contributes (Dimmeler *et al.* 1999, Fulton *et al.* 1999). Conversely, oxidized LDL inhibits endothelial cell migration toward VEGF by promoting the dephosphorylation of Akt (Chavakis *et al.* 2001). Other components of the PI3K-PKB/Akt pathway involved in angiogenesis include PTEN, which induces the secretion of thrombospondin-1, a negative regulator of angiogenesis (Wen *et al.* 2001).

Akt pathophysiology in Type 2 diabetes

Role of Akt in the pathophysiology of insulin resistance

Considering the major role of PI3K-Akt pathway in insulin signaling, it is not surprising that this system has been investigated as a possible site of insulin resistance (IR), the hallmark of Type 2 DM. Numerous studies in various experimental settings, exploring insulin-sensitive cell types and tissues, have suggested that this assumption is correct. To our knowledge, the role of Akt in the development of IR has been first suggested by Krook *et al.* (1997). In these studies, insulin-stimulated Akt phosphorylation was impaired in the skeletal muscle of insulin-resistant Goto-Kakizaki rats and in muscle biopsies from Type 2 diabetic patients. Impaired activation of Akt in response to insulin has been then described in insulin-resistant human (Rondinone *et al.* 1999) and rodent adipocytes (Carvalho *et al.* 2000), as well as in rodent skeletal myocytes (Song *et al.* 1999). Defects in GLUT4 translocation and expression were associated with the defective Akt phosphorylation (Carvalho *et al.* 2000, Tremblay *et al.* 2001).

Defects in Akt activation may be, in part, secondary to factors characteristic for diabetic milieu. Ability of insulin to activate Akt is impaired by increased glucose levels. Akt phosphorylation in insulin-resistant hyperglycemic Goto-Kakizaki rats can be normalized by phlorizin treatment (Krook *et al.* 1997) that normalizes hyperglycemia in experimental diabetes by inhibition of tubular reabsorption of glucose. This finding also suggests that Akt is a site of secondary insulin resistance as observed in Type 1 diabetic patients and glucose toxicity in general. Other factors may also be responsible. Glucosamine, product of hexosamine pathway, intensively studied in the pathophysiology of diabetic complications (Schleicher and Weigert 2000), has also been shown to diminish Akt activation in endothelial cells (Du *et al.* 2001, Nakamura *et al.* 2001), albeit other investigators suggested that inhibitory effects of glucosamine on GLUT4 translocation in the skeletal muscle are associated with defects in insulin signaling up-stream from Akt, but not Akt itself (Kim *et al.* 1999b). Another factor implicated in impaired Akt activation in diabetic tissues is oxidative stress (Tirosh *et al.* 1999). Overproduction of H_2O_2 in adipocytes leads to 90 % reduction of Akt phosphorylation. Finally, tumor necrosis factor-α (TNF-α), an inflammatory cytokine, has been identified as one of possible mediators of IR (Peraldi *et al.* 1997). As recently demonstrated, TNF-α acts on the Akt level by stimulating ceramide, natural antagonist of Akt phosphorylation (Teruel *et al.*2001).

Although most of the studies localize the defects responsible for the development of IR in the insulin receptor signaling cascade, not all authors agree with the involvement of Akt. Studies reported normal Akt activation by insulin despite defects up-stream from Akt. Kim *et al.* (1999a) and Storgaard *et al.* (2001) observed

insulin-induced defects in IRS and PI3K, but normal Akt activation in the skeletal muscle of Type 2 DM patients and subjects with impaired glucose tolerance. In experimental conditions, similar dissociation of stimulated PI3K and Akt activities was reported in adipocytes of male insulin-resistant BtB6 mice (Nadler *et al.* 2001). This phenomenon suggests possible involvement of another kinase capable of activating Akt in response to insulin stimulation. Atypical isoforms of PKC can play a role in this process.

Another line of evidence that does not support the role of Akt in the development of IR suggests defects down-stream from Akt, despite normal Akt activation. Meyer *et al.* (2002) found no differences in insulin-stimulated activities of Akt and up-stream components of insulin receptor signaling in subjects at risk for the development of Type 2 DM and in Type 2 diabetic patients with normalized glucose concentrations, as compared to normal subjects, although glucose disposal was reduced in the diabetic subjects and relatives. Furthermore, baseline activities of Akt corresponded to plasma insulin levels even in insulin-resistant subjects.

In salt-sensitive Dahl rats, a high-salt diet contributes both to pathogenesis of hypertension and IR. Ogihara *et al.* (2002) reported that despite the presence of IR, high salt loading in these rats resulted in enhanced insulin-induced tyrosine phosphorylation of the insulin receptor and IRSs, activation of PI3K, and phosphorylation of Akt in isolated soleus muscles. The same group reported similar findings in rats infused with Ang II, that developed IR despite enhanced activation of PI3K-Akt pathway.

It should be noted that most of the evidence suggesting the defect in Akt activation in IR observed these alterations after stimulation with supraphysiological concentrations of insulin (Krook *et al.* 1998). It is also important to compare baseline levels of Akt phosphorylation and activity or levels stimulated with physiological doses of insulin. In this regard, available evidence suggests similar findings in insulin-resistant animals and humans as compared to healthy controls (Krook *et al.* 1998, Storgaard *et al.* 2001). This lack of baseline differences in Akt activity could be considered as another argument for impaired Akt signaling since IR models and humans display markedly elevated plasma insulin levels. Provided that Akt activation was normal in IR, baseline activities should be enhanced.

Despite the controversies discussed above, recent observations in Akt2 knock-out mice shed a new light on the roles of Akt in the development of IR. They suggest Akt as a possible primary defect, and identify the Akt2 isoform as the most important for insulin metabolic signaling. Akt2 –/– mice displayed mild hyperglycemia, impaired glucose tolerance (as assessed by oral glucose tolerance test), hyperinsulinemia and defects of *in vitro* glucose disposal into the skeletal muscle (Cho *et al.* 2001). Otherwise, Akt2 –/– were perfectly viable and macroscopically normal. In contrast, Akt1 knock-out mice demonstrate predominantly growth abnormalities due to enhanced apoptosis, but not diabetic phenotype (Chen *et al.* 2001a,b).

Akt signaling and vascular complications in Type 2 diabetes mellitus

The crucial question arising from Akt research is whether defects in its activation in insulin sensitive tissues are also present in vascular system and organs or tissues affected by diabetic complications. Associations between IR and Type 2 diabetes on one side, and cardiovascular disorders on the other, have been attributed to insulin effects on vascular smooth muscle growth (King *et al.* 1985), extracellular matrix production (Tamaroglio and Lo 1994), or renal sodium reabsorption (DeFronzo *et al.* 1976).

As summarized above, number of these effects could be mediated by Akt signaling. An assumption underlying this theory is that IR is tissue specific to the muscle and adipose tissue only, whereas vascular and renal tissues remain sensitive to insulin. Theoretically, provided that insulin signaling is normal in these tissues, increased circulating insulin concentrations due to IR may have substantial impact on morphology and function of endothelial and vascular smooth muscle cells and their derivatives in various organs. However, insulin-induced vasodilation is reduced in IR patients (McVeigh *et al.* 1992), suggesting the presence of IR also in endothelial cells, specifically in signaling pathways leading to NO production.

To explain possible dissociation of growth and vasomotor effects of insulin, Jiang *et al.* (1999) studied aortas and microvessels from Zucker lean and obese (Type 2) diabetic rats. They found that vascular insulin-induced phosphorylations and activations of components of insulin signaling from the receptor level down-stream to Akt were blunted in obese IR rats. These reductions resembled the above described defects that appear in

muscle, liver and adipose tissue. In contrast to PI3K-Akt pathway, tyrosine phosphorylation of ERK module of mitogen-activated protein kinase family (MAPK) was normal in obese animals, despite enhanced baseline activities. ERKs are critically involved in transducing proliferative and growth signals implicated in a number of vascular disorders. Thus, in this state of selective insulin resistance in vascular tissues, growth and potentially pro-atherogenic signals of insulin are transmitted by MAPK, and even enhanced due to hyperinsulinemia, whereas vasoprotective NO-mediated effects mediated by PI3K-Akt are impaired.

Interestingly, more recent findings by other investigators suggest baseline unstimulated twofold increase in Akt activity in the kidney of a murine model of Type 2 diabetes (Feliers *et al.* 2001), as well as in renal cortex of obese Zucker rats (Ždychová *et al.* 2003). In the former study, these changes coincided with the onset of hypertrophy and matrix accumulation, structural hallmarks of diabetic nephropathy. An indirect evidence suggests that renal cortical matrix accumulation in Type 2 DM is at least in part attributable to Akt effects on protein translation mediated via 4E-BP1 phosphorylation and mTOR (Bhandari *et al.* 2001). The concept of selective insulin resistance is further supported by Schnyder *et al.* (2002) who demonstrated high glucose-induced defects in Akt-mediated insulin signaling and NO production in endothelial cells, whereas these cascades, as well as insulin-induced sodium reabsorption, were unaffected in renal tubular cells. Theoretically, these findings are in accordance with the putative role of insulin in enhanced sodium reabsorption and development of hypertension in Type 2 DM. In the kidney, defects in endothelial and vascular smooth muscle Akt signaling, as described by Jiang *et al.* (1999), may be overshadowed by large pool of tubular Akt. Hypothetical mechanisms linking metabolic and vascular abnormalities in Type 2 DM and the role of Akt in these processes are shown in Figure 3.

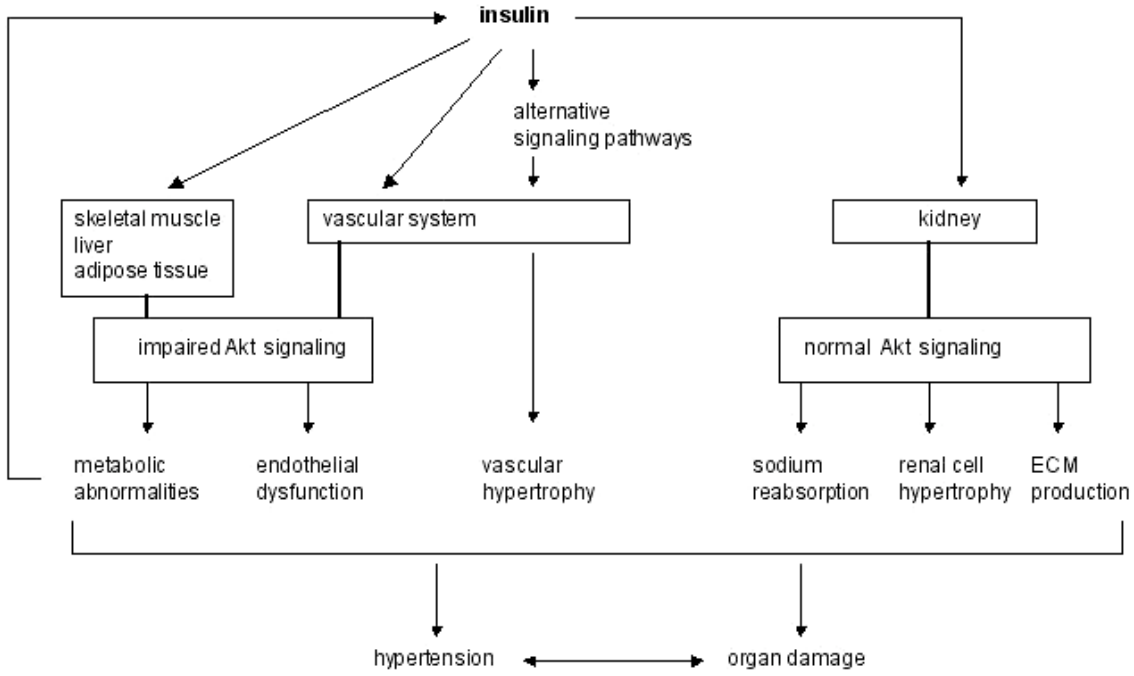

Fig. 3. Hypothetical mechanisms linking metabolic and vascular abnormalities in Type 2 diabetes

Akt pathophysiology in Type 1 diabetes

Akt and insulitis

Autoimmune destruction of beta-cells in islets of Langerhans underlies the development of Type 1 DM. As summarized above, Akt transduces major cell survival signals. In agreement with these effects, overexpression of Akt in murine beta-cells in islets of Langerhans increases both beta-cell size and total islet mass, accompanied by improved glucose tolerance, fasting as well as fed insulin, and resistance to experimental diabetes compared to wild-type mice (Bernal-Mizrachi *et al.* 2001, Tuttle *et al.* 2001). Furthermore, pharmacological activation of Akt by simvastatin and vanadate has been shown to improve viability of beta-cells *in vitro* (Contreras *et al.* 2002). Therefore,

pharmacological modulation of Akt activity is one of possible targets in future treatment of early stages of Type 1 DM that may prevent or delay the destruction of beta-cells.

Akt and vascular complications in Type 1 diabetes mellitus

Unlike Type 2 DM, insulin signaling in Type 1 DM is supposed to be normal or nearly normal with some reduction caused by the effects of hyperglycemia. The absolute lack of insulin may result in lower Akt activities and enhancement of apoptosis, as observed, for example, in endothelial cells of diabetic retinal capillaries (Mizutani *et al.* 1996). On the other hand, administration of exogenous insulin to treat the disease may lead to variable periods of hyperinsulinemia and Akt activation. Therefore, the prediction of Akt activity in individual vascular beds and its impact on the local morphology and function is hard to predict. An increasing number of studies have been addressing this potentially important issue.

Gerhardinger *et al.* (2001) found that streptozotocin (STZ)-diabetic rats with a low-dose insulin treatment tended to have enhanced retinal Akt phosphorylation (123 %, p=0.07) as compared to non-diabetic animals. These authors suggest that, in addition to insulin, Akt activity in DM1 is also under the influence of local factors and speculate about a possibility that Akt is activated by stress-activated kinases, such as p38 MAP kinase, to counteract deleterious effects of DM-induced local stresses. In another study (Joussen *et al.* 2002), baseline Akt activity was increased by 54.3 % in the diabetic retinas when compared to the non-diabetic controls. Treatment with angiopoietin-1 prevented and reversed retinal vascular changes in both new and established DM. These beneficial effects were associated with normalization of mRNA and protein levels of well established factors involved in the pathophysiology of retinopathy, such as VEGF and intercellular adhesion molecule-1. Furthermore, these changes coincided with 40 % reductions in retinal Akt and eNOS activities, studied as mediators of VEGF bioactivity. Thus, despite similar findings in the two mentioned studies indicating enhanced retinal Akt in Type 1 DM, these studies suggest opposite roles for Akt in the development of retinopathy.

Similar to the eye, kidney is a conglomerate of various cell types. Thirone *et al. (*2002) investigated renal growth hormone (GH) signaling in the STZ-diabetic rats and non-diabetic controls. GH has been repeatedly investigated as one of factors responsible for diabetic renal hypertrophy and hyperperfusion (Christiansen *et al.* 1981) and later development of kidney disease. The authors found enhanced GH-induced Akt and MAPK activation as compared to control rats. Furthermore, these alterations were ameliorated by GH antagonist.

Unlike the evidence in diabetic retina and kidney, studies in diabetic hearts have suggested reductions in Akt activity. In the myocardium of STZ-diabetic rats the administration of insulin resulted in enhanced activity of most of the components of its signaling cascade including the fivefold increase in Akt phosphorylation on Thr-308 as compared to non-diabetic controls (Laviola *et al.* 2001). However, baseline Ser-473 Akt phosphorylation, albeit not Thr-308, and Akt activity determined by the *in vitro* assay, was reduced in STZ-diabetic rats (33 % of controls). Unlike the control rats, insulin-induced phosphorylation of glycogen synthase kinase (GSK)-3, the major cellular substrate of Akt, was absent in diabetes. These changes were ameliorated by more physiological administration of insulin in diabetic rats treated with islet transplantation. In another study (Dobrzynski *et al.* 2002), STZ-diabetic rats lacking insulin demonstrated baseline reductions in cardiac Akt activity. Treatment with *in vivo* adrenomedullin gene delivery that ameliorated DM-induced structural and functional cardiac changes also normalized Akt signaling. Interestingly, the latter observation, in particular, corresponds to well-established clinical roles of insulin treatment in improving outcomes of myocardial infarction in diabetic patients.

So far we have related Akt pathophysiology in both types of DM to the effects of insulin. However, it is important to note that Akt activity may be modulated by other growth and vasoactive factor that have been implicated in the pathophysiology of diabetic organ complications (Table 2). These phenomena may also explain cell/organ specificity of Akt defects.

Several lines of indirect evidence also implicate Akt in diabetic complications. NF-κB activation has been implicated in the pathophysiology of microangiopathy and linked to ROS signaling (Nishikawa *et al.* 2000). However, considering the capability of Akt, one of physiological activators of NF-κB, the role of Akt in this process cannot be excluded. Cell cycle regulation is impaired in tissues affected by microvascular complications (Wolf 2000). Interestingly, the relations between Akt, as a powerful modulator of cell cycle, and molecules directly involved in cell cycle progression

remain largely unknown. These topics represent possible directions for future research.

Akt as a potential target of treatment

There is increasing evidence that Akt activity can be modulated by several groups of agents widely used in clinical medicine, including the treatment of diabetes and associated conditions.

HMCoA-inhibitors, statins, have been used for treatment of hypercholesterolemia, and exert well established cardiovascular and renal protective effects. More recently, so called pleiotropic beneficial effects, not necessarily associated with their lipid-lowering actions, have been intensively studied. Statins act, at least in part, *via* NO-dependent, proangiogenic and antithrombotic mechanisms. (Dimmeler *et al.* 2001, Endres et al. 1998, Eto *et al.* 2002, Treasure *et al.* 1995). There is convincing evidence implicating Akt in these processes (Dimmeler *et al.* 2001, Eto *et al.* 2002). Interestingly, statin-induced Akt activation is further supported by the data from large clinical studies, indicating that patients treated with statins had lower risk of developing Type 2 diabetes, suggesting thus the improvement of Akt signaling also in clinical settings.

Thiazolidinediones (TZD), another group of metabolically active, antidiabetic, and potentially vasoprotective compounds, act as agonists of peroxisome proliferator-activated receptors gamma (PPARγ). In insulin-sensitive tissues, stimulation of PPARγ enhances Akt phosphorylation that has been described in human skeletal muscle biopsies and is linked to beneficial effects of these agents on insulin sensitivity (Hori *et al.* 2002, Jiang *et al.* 2002, Meyer *et al.* 2002b). Studies focusing on cardiovascular vascular system have revealed strikingly complex relationships between PPARγ and PI3K-Akt signaling. In endothelial cells, prolonged TZD treatment increased NO production *via* Akt-mediated phosphorylation of eNOS (Cho *et al.* 2004). In contrast, TZD have been shown to inhibit leptin-induced endothelial cell migration mediated *via* Akt. This was associated with PTEN up-regulation and could be implicated in beneficial vascular effects of these agents (Goetze *et al.* 2002). Furthermore, several reports have suggested that PPARγ could be a target and a new effector molecule of Akt. For example, growth factor-induced PPAR gamma up-regulation in vascular smooth muscle is Akt-dependent (Fu *et al.* 2001). With respect to differential activation of Akt in various tissues in diabetes discussed above, it is conceivable that the effects of TZD on Akt may also be cell-specific. Available evidence, particularly in cardiovascular system, is rather controversial, and will require further investigations.

Conclusion

Studies focusing on Akt suggest both decreases and increases in Akt activity in DM. These alterations are highly variable depending on type of DM as well as cell type. There is persuasive evidence suggesting defects in Akt signaling in the development of insulin resistance, i.e. in the pathophysiology of DM Type 2. The role of alterations in Akt signaling in the development of diabetic vascular complications have only recently been investigated. Similar defects as in insulin-sensitive tissues have been reported in endothelium in DM Type 2 models, possibly contributing to the development of endothelial dysfunction under these conditions. In contrast, Akt activity is increased in some tissues and vascular beds affected by complications in DM Type 1. Identification of the role of this phenomenon in diabetes mellitus-induced growth and hemodynamic alterations in affected vascular beds remains one of major future challenges for research in this area. With respect to the availability of pharmacological modulators of Akt activity, these studies should include the evaluation of therapeutical benefit of these compounds.

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