

Protein Kinase B/Akt regulation in diabetic kidney disease

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1. ABSTRACT

Many reviews have been written on protein kinase B/Akt focusing on its history dating back from the isolation of the Akt8 transforming murine leukemia virus by Staal in 1977, to the co-discovery of the Akt1 gene by the three groups in 1991 (reviewed in 7). There are currently over 22,000 publications in the PubMed database with “Akt” as a keyword - these publications describe a wealth of diverse data on the physiological functions of Akt isoforms. Many of these publications describe roles of Akt ranging from its requirement for cellular processes such as glucose uptake, cell survival and angiogenesis to roles in diseases such as cancer and ischaemia (22). This review will focus on the evidence for Akt signaling in different kidney cells during diabetes, or diabetic nephropathy (DN).

2. INTRODUCTION

Akt belongs to subfamily of serine/threonine protein kinases called AGC protein kinases and is involved in numerous signaling circuits. Akt is a downstream effector of the phosphatidylinositol 3-kinase (PI3K) pathway, where generation of PtdIns-3,4,5-P₃ recruits Akt to the plasma membrane (2, 9, 24). Full activation of Akt requires phosphorylation on Thr308 by PDK1 (1) and Ser473 by the TORC2 complex (51), in addition to DNA-dependent protein kinase (DNA-PK) in genotoxic stressed cells (23). Downstream targets of Akt control multiple cellular processes such as glucose uptake, cell proliferation, gene transcription, protein synthesis and cell survival (7, 19, 60, 61).

The mammalian Akt gene family consists of three highly homologous genes coding for the three Akt

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isoforms termed Akt1, Akt2 and Akt3 (5, 8, 12, 15, 32, 43, 45). The three Akt proteins are very similar in sequence and structure and are thought to be activated by a common mechanism (7). The distinct phenotype of transgenic mice lacking individual Akt isoforms however, suggests that there are well-defined physiological roles for each Akt protein in mammals. Both Akt1 and Akt2 are widely expressed in mammalian tissues (66). Mice lacking Akt1 display growth retardation and increased (~40%) neonatal mortality (11, 65). Akt2 homozygous knockout mice develop a metabolic disorder, exhibiting a mild type 2 diabetes-like phenotype due to insulin resistance (13). Akt3 is predominantly expressed in the brain and was shown to be important in brain development and size (21, 57). More complex double and even triple knockout strategies have been employed to determine the minimal Akt “unit of activity” required for cellular and embryonic survival. Yang and colleagues identified that Akt1^{-/-}; Akt3^{-/-} mice died in utero at E11-12. Interestingly, Akt1^{-/-}; Akt3^{+/-} mice developed severe defects in thymus and heart and died several days after birth, whereas Akt1^{+/-}; Akt3^{-/-} survived normally, suggesting a dominant role for Akt1 (65). These experiments were further extended by the demonstration that Akt2^{-/-}; Akt3^{-/-} mice survived normally, and indeed a single functional allele of Akt1 was all that was required for mouse development and survival (20). Liu and colleagues used Akt1^{-/-}; Akt2^{-/-} mouse embryo fibroblasts (MEFs) to transfect Akt3 siRNA. The data showed that apoptosis of these cells was only increased when approximately 80 % reduction of Akt3 expression was achieved (39). Together, these data suggest that only a small amount of Akt activity is required for normal cellular survival and mammalian development.

3. AKT SIGNALING IN DIABETIC NEPHROPATHY

Diabetic nephropathy (DN) is a progressive fibrotic disease of the kidney as a result of chronic hyperglycaemia during diabetes. The pathogenesis of DN is characterised by progressive loss of kidney function due to glomerulosclerosis (thickening of the glomerulus due to deposition of ECM) and tubulointerstitial fibrosis leading to scarring of the kidney tubules and impaired reabsorptive capacity (40). The cellular mechanisms of both glomerulosclerosis and tubulointerstitial fibrosis have been extensively reviewed (37, 40). Particular focus has been given to the cellular events driving tubulointerstitial fibrosis, as the degree of fibrosis appears to correlate tightly with the severity of renal disease (47). Epithelial-mesenchymal transition is thought to contribute to renal fibrosis in DN, as cytokines such as TGF-β1 that drive EMT are elevated in the diabetic kidney (6, 59). However, recent evidence has suggested that the source of myofibroblasts in kidney may arise not from renal epithelial cells undergoing EMT, but rather from pericytes (29). *In vitro* and *in vivo*, EMT is characterised by loss of epithelial proteins such as E-cadherin and ZO-1, signifying loss of epithelial tight junctions and barrier integrity, leading to compromised renal tubule function. Additionally, EMT features increased expression of fibroblast proteins such as α-smooth muscle actin and vimentin, which leads to

altered cell shape and increased motility (4, 31). TGF-β1 is the primary cytokine that drives EMT *in vitro* and *in vivo*, and utilises both canonical and non-canonical signaling pathways to mediate its effects. TGF-β1-mediated activation of Smad2/3 phosphorylation leads to a range of gene expression changes such as decreased E-cadherin expression and increased alpha-smooth muscle actin that characterise EMT (58, 59). In addition, TGF-β1 activates other pathways such as the PI3K→Akt pathway, and this non-canonical cascade has also been implicated in renal damage during diabetes and other fibrotic conditions. Evidence for the involvement of Akt in diabetic kidney disease and other fibrotic conditions of the kidney will be discussed below.

Akt has been implicated in diabetic nephropathy using a wide range of cell and animal models. Early reports identified that extracellular matrix production, a hallmark of glomerulosclerosis, is regulated by Akt. Krepinsky and colleagues demonstrated that both mechanical stretch and high glucose-induced collagen 1 production in mesangial cells required Akt activity (35). Akt activity may also be required for high-glucose induced increases in TGF-β1 expression in diabetic tubular epithelial cells (36). One of the earliest events in DN is glomerular thickening as a result of mesangial cell hypertrophy. Several publications have identified that Akt signaling contributes to mesangial cell hypertrophy in diabetic kidney disease. Nagai and colleagues identified that growth arrest specific gene-6 (Gas6) signaling through its Axl receptor drives mesangial cell hypertrophy in type 1 diabetic rodent models in an Akt-dependent manner (44). Consistently, hyperglycaemia decreased the expression of the lipid phosphatase PTEN, an inhibitor of Akt activation, leading to hypertrophy of mesangial cells (42). These authors also showed that TGF-β1 treatment of mesangial cells also decreased PTEN expression with concomitant increases in Akt activation (42). Importantly, Kato and colleagues showed that TGFβ1 increased the levels miR-216a and miR-217 in mesangial cells. These miRNAs target PTEN, reducing its expression leading to Akt activation (33). Recent findings from Zhang *et al* demonstrate that micro RNA-21 (miR-21), which targets PTEN, also protects from glomerular mesangial cell proliferation in DN (68). Thus, the regulation of miRNAs by high glucose and TGF-β1 further support the role of Akt signaling in early cellular hypertrophy associated with diabetic nephropathy.

Diabetic nephropathy is associated with loss of renal cells, particularly glomerular podocytes which form the glomerular filtration barrier (3, 55). Akt signaling has been implicated in this process. Levels of Akt phosphorylation are elevated in the kidney tubules of the Goto Kakizaki type 2 diabetic rat (34) and streptozotocin-treated type 1 diabetic mice (46). Chuang and co-workers identified that advanced glycation end-products generated as a result of chronic hyperglycaemia increased apoptosis *in vitro* (14).

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Murine podocytes exposed to AGE-modified BSA were more apoptotic, and also displayed decreased Akt phosphorylation (14). This decrease in Akt activity led to the “liberation” of FOXO4 from the inhibitory constraint mediated by Akt phosphorylation, leading to FOXO4 nuclear translocation and increased expression of the pro-apoptotic Bim protein (14). *In vivo*, the *db/db* experimental mouse model of DN displayed early diabetic nephropathy at 12 wk which was accompanied by decreased Akt phosphorylation in the glomeruli (56). Podocytes isolated from *db/db* mice did not display Akt phosphorylation in response to insulin, whereas *db/+* mice did. Podocyte viability was reduced in *db/db* mice, and this was linked to reduced Akt-mediated survival signaling (56). These two reports implicate changes in Akt signaling as a key event in podocyte loss during early diabetic nephropathy. A recent report from Rane and colleagues focussed on renal tubular epithelial cell apoptosis in response to high glucose, an event regulated by p38MAPK activation (50). Rane *et al* showed that p38MAPK induced apoptosis of renal proximal tubular epithelial cells (RPTCs) could be inhibited by expression of constitutively active Akt. Consistently, PI3Kinase inhibitors or siRNA targeting Akt led to p38MAPK activation in the absence of high glucose (46). Others have suggested that IGF1→Akt signaling may be important for mesangial cell survival in diabetic nephropathy (53).

Crosstalk between different signal transduction cascades downstream of TGF-beta1 is a feature of diabetic nephropathy. Ghosh Choudray and co-workers showed that TGF-beta1 increased fibronectin production in mesangial cells in an Akt-dependent manner (27). Other reports indicate that Akt can enhance Smad3-mediated collagen I expression in mesangial cells treated with TGF-beta1 (49), a process that may also involve the small GTP binding protein Rac1 (28). In contrast, two reports demonstrated that Akt inhibited Smad3 mediated transcription by direct binding and sequestration of Smad3 in the cytosol (16, 48). Akt kinase activity was not required for this inhibition, and the association of Akt and Smad3 was stimulated by insulin but inhibited by TGF-beta1 (16, 48). Similarly, Seong and co-workers demonstrated that proteins such as Smad3 could interact with PDK1, (the Thr308 kinase of Akt), an association that was also facilitated by insulin stimulation but inhibited by TGF-beta1 (52). In contrast to Akt, binding of Smad proteins to PDK1 (the Thr308 Akt kinase) increased its kinase activity (52). Akt has also been implicated in actin disassembly and mesangial cell dysfunction in mesangial cells. In response to connective tissue growth factor (CTGF), a secreted mediator of many TGF-beta1 effects, mesangial cells undergo a rapid change in actin cytoskeleton structure, a process regulated by Akt→p27Kip1 signaling (17). Furthermore, in the diabetic milieu, mesangial cell Akt activation in response to CTGF is blunted, suggesting that chronic hyperglycaemia may alter Akt signaling *in vivo* (25). Furlong and colleagues also noted that incubation of high-glucose treated mesangial cells with an inhibitor of PKCbeta could

restore cellular responses to CTGF (25). Work from the Krepinsky laboratory has identified EGFR→PLCgamma→PKCbeta→Akt pathway that is activated by hyperglycaemia to drive extracellular matrix production (62, 63). Thus, complex signal transduction pathways downstream of TGF-beta1 and other factors integrate Akt signaling with other molecules such as Smad3 and p38MAPK to regulate kidney cell function.

Akt has also been implicated in TGF-beta1-induced damage to renal epithelial cells during diabetic nephropathy. Both PI3kinase and Akt activation are required for TGF-beta1 induced epithelial-mesenchymal transition (EMT) in NRK52E tubular epithelial cells (34). Recent evidence from the cancer field suggests that Akt isoforms regulate miRNA production and EMT. Iliopoulos and co-workers identified that knockdown of Akt1, but not Akt2 decreased miR-200 abundance, promoting EMT in MCF10A breast cancer cells (30). Zeng *et al* showed that biliverdin reductase may serve as an upstream activator of PI3K and Akt to drive EMT in response to hypoxia in HK-2 renal epithelial cells (67). Pharmacological inhibition of integrin-linked kinase (ILK) inhibits Akt phosphorylation and attenuates TGF-beta1-induced EMT in renal tubular epithelial cells (38). Together, these data suggest that Akt signaling is an important regulator of TGF-beta1 mediated cellular events during diabetic nephropathy.

Since inappropriate Akt activation has been reported in many models of diabetic nephropathy, strategies to intervene pharmacologically to alleviate DN in human patients have been explored. In particular, the nutrient sensing mTOR (target of rapamycin) has been the subject of several reports in this area. mTOR is activated by the small GTPase Rheb, which is inhibited by tuberous sclerosis complex1/2 (TSC1/2), which act as a GAP protein to inactivate Rheb (18, 26). TSC1/2 activity is inhibited by Akt, leading to Rheb, and mTOR activation. Song and colleagues present data showing that Akt-mediated inhibition of Smad3 activation requires mTOR activity in prostate epithelium cells (54). Nagai and colleagues identified that Gas6-mediated mesangial cell hypertrophy required both Akt and mTOR activity (Nagai K *et al*, 1999). Treatment of diabetic rats with the mTOR inhibitor rapamycin (sirolimus) for 4 wk decreased mesangial matrix production and attenuated the severity of DN (41, 64). Other pharmacological strategies that affect Akt activity have also been shown to reduce indices of damage in DN in various models. Troglitazone (an insulin-sensitizer) was shown to attenuate high-glucose induced EMT in renal proximal tubule cells, a process that involved Akt signaling (36). Bussolati and colleagues showed that statins could rescue ox-LDL induced podocyte apoptosis, a process requiring statin-mediated Akt activation (10). Thus, inhibition of Akt and its downstream targets such as mTOR may provide future therapeutic benefit for the treatment of diabetic nephropathy.

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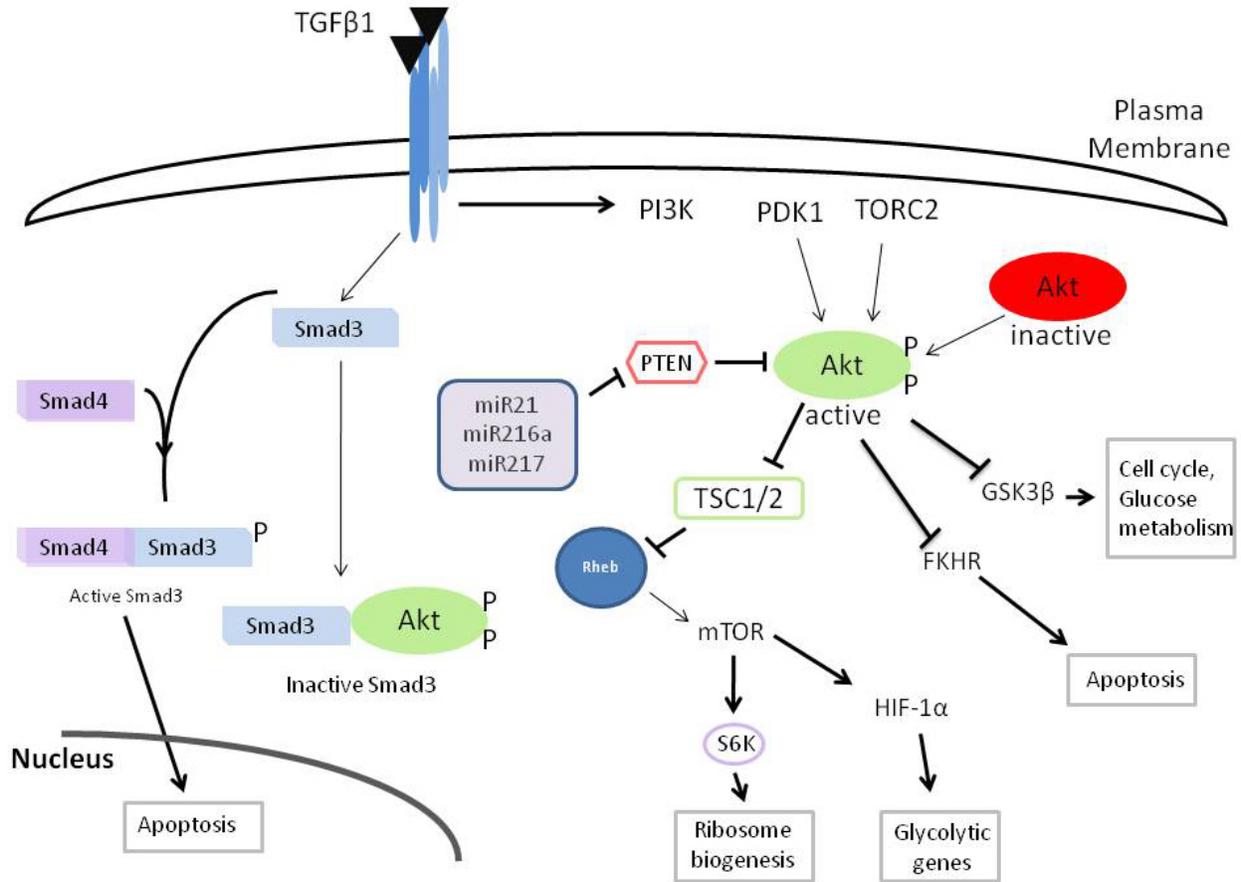


Figure 1. Summary of Akt signaling in kidney cell types. Activation of Akt downstream of the TGF-beta1 receptor triggers multiple signaling cascades impinging on Smad signaling, mTOR regulation and other pathways that regulate multiple processes in diverse kidney cells. Regulation of miRNAs provide additional complexity to the system.

4. CONCLUDING REMARKS

A summary of current knowledge of Akt signaling in the kidney is given in Figure 1. The complexity of these signaling networks emphasises the diverse control of kidney cell processes, be it podocytes, mesangial cells or tubular epithelial cells, regulated by Akt. Future experiments will provide further fascinating insights into the role of the Akt kinase family in kidney physiology and disease.

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Key Words: Akt, Diabetic Nephropathy, Fibrosis, Epithelial Cell, Review

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