Apoptosis occurs differentially according to glomerular size in diabetic kidney disease

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Abstract

Background. Apoptosis, which is involved in the process of mesangial cell and podocyte loss in diabetic nephropathy, is known to be regulated by protein kinase B/Akt (Akt). A number of studies have therefore investigated the activity of Akt under diabetic conditions, but the results have not been consistent. In this study, we hypothesized that apoptosis may occur differentially and that Akt may be differentially activated according to glomerular size in diabetic kidney disease.

Methods. Fifty male Sprague–Dawley rats were injected intraperitoneally with diluent (C, n = 25) or streptozotocin (DM, n = 25). After 3 months, glomeruli were isolated using sieves with pore sizes of 250, 150, 125 and 75 μm and then classified into large glomeruli (on the 125-μm sieve, LG) and small glomeruli (on the 75-μm sieve, SG) groups. Western blot analyses for phospho-Akt, apoptosis-related molecules (Bax, Bcl-2, active fragments of Caspase-3 and phospho-p53) and cyclin-dependent kinase inhibitors were performed.

Conclusions. The numbers of total cells and podocytes in isolated glomeruli were determined using transmission electron microscopy. Akt phosphorylation was significantly decreased in DM-LG, while it was significantly increased in DM-SG (P < 0.05). The ratio of Bax/Bcl-2 protein expression and active fragments of Caspase-3 and phospho-p53 protein expression were significantly increased in DM-LG compared to DM-SG and C-SG (P < 0.001 and P < 0.01, respectively). In contrast, the expression of p27Kip1 and p21Cip1 was significantly increased in DM-SG compared to DM-LG and C-SG (P < 0.05). The numbers of total glomerular cells and podocytes were significantly decreased in DM-LG (P < 0.05). In conclusion, these data show differential expression of Akt activity and apoptosis-related molecules according to glomerular size in diabetic nephropathy, suggesting that apoptosis may be more operative in more hypertrophic glomeruli, resulting in fewer glomerular cells and podocytes in diabetic nephropathy.

Keywords: Akt; apoptosis; cyclin-dependent kinase inhibitors; diabetic nephropathy; glomerular size

Introduction

Apoptosis removes damaged or unwanted cells and has been implicated in the pathogenesis of numerous diseases such as malignancy, lupus erythematosus and Alzheimer’s disease [1]. In addition, apoptosis has been documented in the course of various renal diseases including diabetic nephropathy [2, 3, 4, 5, 6]. Cell death by apoptosis is believed to be involved in the process of mesangial cell loss in the late stage of diabetic nephropathy [4]. In addition, apoptosis is considered to be one of the underlying causes of podocyte loss, which contributes to the development of albuminuria in diabetic nephropathy [5, 6].

Besides apoptosis, renal hypertrophy is another hallmark of diabetic nephropathy [7, 8]. Kidney size is typically increased in diabetes, even at the time of diagnosis [9]. This is primarily due to glomerular and tubular hypertrophy, although some low-grade proliferation of glomerular cells is present in the early phase [7, 10]. Glomerular hypertrophy is in part the result of glomerular cell hypertrophy and recent studies have suggested that the diabetic milieu per se, hemodynamic changes and local growth factors such as transforming growth factor-β (TGF-β) and angiotensin II (ANG II) are mediators in the pathogenesis of glomerular cell hypertrophy [11, 12].

Protein kinase B/Akt (Akt) is a serine/threonine protein kinase that functions as a critical regulator of several cellular functions, including cellular hypertrophy and apoptosis [13], two characteristic findings in diabetic nephropathy. In addition, previous in vitro studies have demonstrated that high glucose, TGF-β and ANG II, mediators of diabetic nephropathy, are closely linked with the Akt pathway [14, 15, 16, 17, 18]. Owing to these findings, the activity of Akt under diabetic conditions has
been heavily investigated, but the results have not been consistent. Most studies focused on renal hypertrophy in diabetic nephropathy have demonstrated an increase in Akt phosphorylation [15, 19, 20], whereas numerous reports aimed at diabetes-induced apoptosis of glomerular cells have revealed reduced activity of Akt [21, 22]. The reasons for the divergence of changes in Akt activity under diabetic conditions are not clear, but differences between the duration of diabetes or of high-glucose stimulation or the species of animals may contribute to these disparities.

Prior studies have suggested that glomerular hypertrophy in diabetes does not develop in all glomeruli concurrently [23, 24, 25]. Moreover, we have previously shown that nephrin expression is different between relatively small and large glomeruli isolated from early diabetic rats [26]. Based on these findings, it can be surmised that the activity of Akt and the expression of apoptosis-related molecules (Caspase-3, Bax, Bcl-2 and p53) may also be differential in diabetic glomeruli. In this study, differences in the expression of phospho-Akt and apoptosis-related molecules were investigated between relatively small and large glomeruli isolated from 3-month-old diabetic rats. In addition, since accumulating evidence has shown that cyclin-dependent kinase inhibitors (CKIs) play an important role in the process of apoptosis as well as hypertrophy [27, 28, 29, 30, 31, 32], we also examined changes in p21Cip1, p27Kip1 and p57Kip2 expression in less and more hypertrophied glomeruli.

Materials and methods

Animals

All animal studies were conducted using a protocol approved by the committee for the care and use of laboratory animals of Yonsei University College of Medicine. Fifty male Sprague–Dawley rats, weighing 250–280 g, were used. Twenty-five rats were injected intraperitoneally with diluent [control (C)] and the other 25 with 65 mg/kg streptozotocin (STZ) [diabetes mellitus (DM)]. Blood glucose levels were measured 3 days after STZ injection to confirm the development of diabetes. The rats were given free access to water and standard laboratory chow during the 3-month study period. All rats were sacrificed after 3 months.

Body weights were checked biweekly and kidney weights were measured at the time of sacrifice. Serum glucose was measured biweekly and 24-h urinary albumin at the time of sacrifice. Blood glucose was measured using a glucometer and 24-h urinary albumin excretion was determined by enzymelinked immunosorbent assay (Nachfitt I; Exocell, Inc., Philadelphia, PA).

Glomerular isolation

Glomeruli were isolated using sieves with pore sizes of 250, 150, 125 and 75 μm. We classified glomeruli into large glomeruli (on the 125-μm sieve, LG) and small glomeruli (on the 75-μm sieve, SG). The C glomeruli, glomeruli on the sieve with a pore size of 125 μm from the two to three C rats, were pooled because there were few glomeruli on the 125-μm sieve from the individual samples of the C rats. We also determined the proportion of encapsulated and decapsulated glomeruli on both the 125-μm and 75-μm sieves. Since the juxtamedullary glomeruli are known to be larger than superficial and midcortical glomeruli in C and DM rats, we tried to use only the superficial and midcortical tissues for glomerular isolation. In addition, glomeruli were collected under an inverted microscope to minimize tubular contamination.

Western blot analysis

Counted glomeruli were lysed in sodium dodecyl sulfate (SDS) sample buffer (2% SDS, 10 mM Tris–HCl, pH 6.8, 10% (vol/vol) glycercoll, treated with Laemmli sample buffer, heated at 100°C for 5 min and then electrophoresed in an 8–12% acrylamide denaturing SDS–polyacrylamide gel. Proteins were then transferred to a Hybond-ECL membrane using a Hoeffer semidyblotting apparatus (Hoeffer Instruments, San Francisco, CA). The membrane was incubated in blocking Buffer A [1× phosphate-buffered saline (PBS), 0.1% Tween-20 and 8% nonfat milk] for 1 h at room temperature, followed by an overnight incubation at 4°C in a 1:2,000 dilution of polyclonal antibodies to phospho-Akt (Ser473), total Akt, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 (Cell Signaling, Beverly, MA), Bax, Bcl-2, p21Cip1, p27Kip1, p57Kip2, cyclin D1 or β-actin (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The membrane was then washed once for 15 min and twice for 5 min in 1× PBS with 0.1% Tween-20. Next, the membrane was incubated in Buffer A containing a 1:1,000 dilution of horseradish peroxidase–linked goat anti-rabbit IgG (Amersham Life Science, Inc., Arlington Heights, IL). The washes were repeated and the membrane was developed with a chemiluminescent agent (ECL; Amersham Life Science, Inc.). Band densities were measured using TINA image software (Raytest, Straubenhardt, Germany).

Morphometric measurement of glomerular volume

Glomerular volumes (V) of isolated glomeruli were calculated as previously described [33]. Photographs of 50 decapsulated glomeruli were taken using a digital camera at the time of sieving and the surface areas were traced using a computer-assisted color image analyzer (Image-Pro Ver. 2.0; Media Cybernetics, Silver Spring, MD). V was calculated using the equation: $V = 4/3\pi (Area/\pi)^{3/2}$.

Double immunofluorescence staining

Freshly sieved glomeruli were fixed in parafomaldehyde at 4°C, washed with Hank’s Balanced Salt Solution (HBSS) for 5 min and treated with 0.5% Triton-X solution for 15 min at room temperature. Thereafter, glomeruli were washed with HBSS for 5 min, incubated in HBSS solution containing 0.3% hydrogen peroxide and 0.1% sodium azide for 20 min at room temperature, washed again with HBSS for 5 min and blocked with 10% donkey serum for 1 h at room temperature. Primary polyclonal antibodies to active fragments of Caspase-3 (Cell Signaling), p27Kip1 or p21Cip1 (Santa Cruz Biotechnology) were diluted in 1:100 with antibody diluent (DAKO, Glostrup, Denmark) and was applied overnight at 4°C. After washing, Cy3 (red)-conjugated anti-donkey IgG antibody (Jackson ImmunoResearch Laboratories, West Grove, PA) was added for 1 h at room temperature. A 1:200 dilution of polyclonal Wilms’ tumor (WT)-1 antibody (Santa Cruz Biotechnology) was then applied for 3 h at room temperature, followed by Cy2 (green)-conjugated anti-donkey IgG antibody (Jackson ImmunoResearch Laboratories).

Determination of total glomerular cells and podocyte numbers

Total glomerular cells and podocyte numbers in isolated glomeruli were determined using the exhaustive count method as previously described [34]. Briefly, isolated glomeruli were fixed in 50 mM sodium cacodylate buffer (pH 7.4) containing 2% glutaraldehyde for 30 min at 32°C, post-fixed in 1% OsO4 for 2 h at 4°C and dehydrated by treatment with a graded series of ethanol (5 min each in 50, 60, 70, 80, 90, 95 and twice in 100%). Next, isolated glomeruli were treated with propylene oxide and embedded in Epon according to standard procedures. The glomeruli were cut into sections with a thickness of 3 μm using an Ultratome R ultratome (Leica) and then stained with toluidine blue. Two adjacent toluidine blue-stained sections were observed in pairs under transmission electron microscopy at a magnification of ×2000 and the nuclei present in the top section but not in the bottom section were counted and summed. Ten glomeruli in five rats from each group and 13–15 semithin sections from the midglomerular area were examined.

Statistical analysis

All values are expressed as the mean ± SEM. Statistical analysis was performed using the statistical package SPSS for Windows version 11.0 (SPSS, Inc., Chicago, IL). Results were analyzed using the Kruskal–Wallis nonparametric test for multiple comparisons. Significant differences by the Kruskal–Wallis test were further confirmed by the Mann–Whitney U-test. P values of <0.05 were considered to be statistically significant.

Results

Animal data

All animals gained weight over the experimental period, but weight gain was higher in C rats compared to DM rats.
Apoptosis and glomerular size in DM

The ratio of kidney weight to body weight in DM rats (1.07 ± 0.06%) was significantly higher than in C rats (0.49 ± 0.03%) (P < 0.01). The mean blood glucose levels of C and DM rats were 114 ± 5 mg/dL and 475 ± 15 mg/dL, respectively (P < 0.001). Compared to the C group (0.42 ± 0.04 mg/day), 24-h urinary albumin excretion was significantly higher in the DM group (2.34 ± 0.22 mg/day) (P < 0.01) (Table 1).

**Glomerular volume**

We first examined the volume of glomeruli found on the 125-μm (large glomeruli, LG) and 75-μm sieves (small glomeruli, SG). The mean volumes of DM-LG (1.68 ± 0.06 × 10^6 μm^3) and C-LG (1.51 ± 0.08 × 10^6 μm^3) were significantly higher than those of the corresponding DM-SG (0.98 ± 0.04 × 10^6 μm^3) and C-SG (0.91 ± 0.03 × 10^6 μm^3) (P < 0.01) (Figure 1). The proportions of decapsulated glomeruli on the 125-μm sieve in C and DM rats were 89.3 ± 4.5% and 90.7 ± 5.2%, respectively, and on the 75-μm sieve were 91.1 ± 3.2% and 91.9 ± 2.9%, respectively.

**Phospho-Akt, Bax, Bcl-2, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 protein expression in less and more hypertrophied glomeruli**

We secondly determined the changes in glomerular phospho-Akt, Bax, Bcl-2, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 protein expression according to the size of the glomeruli. Glomerular phospho-Akt protein expression was significantly decreased in DM-LG relative to the C-SG (P < 0.01) (Figure 3). On the other hand, there were no significant differences in phospho-Akt, Bax, Bcl-2, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 protein expression between C-SG and C-LG.

**CKIs and cyclin D1 protein expression in less and more hypertrophied glomeruli**

Figure 5 shows a representative western blot with the lysates of C-SG, C-LG, DM-SG and DM-LG at 3 months after streptozotocin injection. Glomerular p27^{kip1} and p21^{cip1} protein expression were significantly increased in the DM-SG group compared to the DM-LG and C groups. Densitometric quantitation revealed 98, 159, 137 and 115% increases in Bax, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 protein expression, respectively, in DM-LG compared to C-SG (P < 0.05 or P < 0.01). In contrast, the expression of Bcl-2 protein was significantly decreased in DM-LG relative to the C-SG (P < 0.01) (Figure 3). On the other hand, there were no significant differences in phospho-Akt, Bax, Bcl-2, active fragments of Caspase-3, phospho-p53 and phospho-Smad3 protein expression between C-SG and C-LG.

**Table 1. Animal data**

<table>
<thead>
<tr>
<th></th>
<th>C (n = 25)</th>
<th>DM (n = 25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (Bwt)</td>
<td>616 ± 11</td>
<td>319 ± 8*</td>
</tr>
<tr>
<td>Kidney Wt</td>
<td>3.01 ± 0.66</td>
<td>3.42 ± 0.08*</td>
</tr>
<tr>
<td>Kidney Wt/Bwt (%)</td>
<td>0.49 ± 0.03</td>
<td>1.07 ± 0.06*</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>114 ± 5</td>
<td>475 ± 15*</td>
</tr>
<tr>
<td>24-h urinary albumin (mg/day)</td>
<td>0.42 ± 0.04</td>
<td>2.34 ± 0.22*</td>
</tr>
</tbody>
</table>

*P < 0.001 versus C group, #P < 0.05 versus C group, 1P < 0.01 versus C group.
Double immunofluorescence staining for WT-1 and p27Kip1 or p21Cip1 was performed to clarify the type of glomerular cells with increased p27Kip1 or p21Cip1 expression and revealed that podocytes were the main cells responsible for the increases in p27Kip1 and p21Cip1 protein expression in DM-SG (Figure 6).

Fibronectin protein expression in less and more hypertrophied glomeruli

Fibronectin protein expression was significantly increased in DM-SG and DM-LG compared to C glomeruli (P < 0.05). However, there was no difference in fibronectin expression between DM-SG and DM-LG (Figure 7).

Total glomerular cells and podocyte numbers in less and more hypertrophied glomeruli

Total glomerular cells and podocyte numbers were determined from the toluidine blue-stained semithin sections. The numbers of total glomerular cells in DM-SG (687.7 ± 15.0 per glomerulus) and C-LG (682.0 ± 16.9 per glomerulus) tended to be higher than in C-SG (658.8 ± 13.7 per glomerulus). In contrast, there were significantly fewer total glomerular cells in the DM-LG group (604.5 ± 14.5 per glomerulus) compared to the other groups (P < 0.05). Podocyte number was also significantly decreased in DM-LG (140.1 ± 5.1 per glomerulus) relative to the other groups (C-SG, 172.5 ± 6.9 per glomerulus; C-LG 178.3 ± 7.3 per glomerulus; DM-SG, 165.9 ± 8.1 per glomerulus) (P < 0.05) (Figure 8).

Effect of insulin on the expression of phospho-Akt, Bax, Bcl-2, active fragments of Caspase-3 and total glomerular cells and podocyte numbers in less and more hypertrophied glomeruli

In additional experiments using diabetic rats treated with 3–5 U/day of insulin (Ultralente; Eli Lilly, Indianapolis, IN), the increases in active Caspase-3 protein expression and the ratios of Bax/Bcl-2 protein expression and the decrease in Akt phosphorylation were significantly abrogated in LG of these rats (Figure 9). In addition, insulin treatment significantly ameliorated the reduction of total glomerular cells (696.3 ± 13.1 per glomerulus) and podocyte numbers (174.2 ± 6.2 per glomerulus) in DM-LG. These findings suggest that the changes in diabetic glomeruli were not due to STZ per se.
Discussion

In this study, we show that Akt activity is significantly decreased in DM-LG glomeruli along with concomitantly increased protein expression of Bax, active fragments of Caspase-3 and phospho-p53 and decreased Bcl-2 protein expression. In contrast, Akt phosphorylation is significantly increased in DM-SG. In addition, total glomerular cells and podocyte numbers are significantly lower in relatively large diabetic glomeruli. These findings suggest that apoptosis may occur differentially in diabetic glomeruli. In particular, a more operative process of apoptosis resulting in fewer glomerular cells may occur in more hypertrophied diabetic glomeruli.

Glomerular hypertrophy is a hallmark of diabetic nephropathy. Although inflammatory cell infiltration, extracellular matrix (ECM) accumulation and hemodynamic factors are known to play a role in glomerular hypertrophy [35], the changes in glomerular cells themselves are the main reason for the observed hypertrophy. In this study, the glomeruli were classified into two groups of relatively small and large glomeruli. Since encapsulated glomeruli containing Bowman’s capsule are larger than decapsulated glomeruli, the proportions of decapsulated and encapsulated glomeruli were determined. In addition, to rule out the possibility that the difference in ECM accumulation may affect glomerular size, the protein expression of fibronectin was also examined. As results, we observed comparable proportions of decapsulated glomeruli (~90%) from the 125- and 75-μm sieves and found that there was no difference in fibronectin protein expression between relatively small and large glomeruli, suggesting that the difference in glomerular size may be primarily attributed to the variation in glomerular cell hypertrophy.

Glomerular hypertrophy in diabetes does not develop in all glomeruli concurrently [23, 24, 25]. Moreover, we previously demonstrated that nephrin messenger RNA and protein expression were different between relatively small and large glomeruli isolated from early diabetic rats [26]. Based on these findings, we hypothesized that there would be a wide variation of glomerular size in diabetes and that the expression of multiple genes in addition to nephrin would also differ according to the size of the glomeruli. The results of this study show that the expression of phospho-Akt is decreased in DM-LG, while its expression is increased in DM-SG. This provides another explanation for the inconsistent results regarding the changes in Akt activity under diabetic conditions [15, 19, 20, 21, 22] and suggests differential ongoing events according to glomerular size in diabetic nephropathy.

In addition to hypertrophy, apoptosis has also been implicated in the pathogenesis of diabetic nephropathy [4, 5, 6]. Cell culture experiments using mesangial cells under high-glucose conditions and in vivo studies using various models of diabetes have suggested that self-limited low-grade proliferation occurs initially, leading to an increase in mesangial cell number [7, 36]. In the late stage of diabetic nephropathy, however, mesangial cell loss is observed and apoptosis is surmised to be involved [4]. These findings suggest that apoptosis may be a homeostatic mechanism regulating the mesangial cell population. On the other hand, the number of podocytes is decreased in the glomeruli of patients with type 1 and type 2 diabetes [37, 38], and a good deal of evidence has shown that apoptosis is also implicated in the process of podocyte loss under diabetic conditions [39]. In the present study, the activity of Akt was significantly...
decreased in DM-LG along with increased expression of active fragments of Caspase-3, Bax and phospho-p53 protein and decreased Bcl-2 protein expression. Since the phosphoinositide-3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway is considered a typical pro-survival/antiapoptotic pathway, these findings suggest that apoptosis is more operative in more hypertrophied diabetic glomeruli. The results of this study also showed that the numbers of total glomerular cells and podocytes were significantly decreased in DM-LG. When these data were compared to the results of our previous study with 6-week C and DM rats [26], total glomerular cells as well as podocyte numbers were significantly lower in 3-month DM-LG relative to 6-week DM-LG. Furthermore, the changes in the expression of apoptosis-related molecules were insignificant in 6-week DM-LG (data not shown). Double immunofluorescence staining for WT-1 and active fragments of Caspase-3 revealed that apoptosis within 3-month DM-LG was significantly increased mainly in podocytes but somewhat in other glomerular cells, maybe mesangial cells. Taken together, we assume that apoptosis occurs principally after 6 weeks of DM induction especially in more hypertrophied diabetic glomeruli, resulting in fewer glomerular cells and podocytes in DM-LG. A recent study by Menini et al. [40] also suggested that glomerular cell apoptosis was not an early feature in the course of experimental diabetic glomerulopathy, since it is preceded by glomerular hypertrophy, supporting our assumption.

CKIs are members of cell cycle regulatory proteins and are known to play an important role in the development of cellular hypertrophy under diabetic conditions. CKIs expression was found to be increased under diabetic conditions, both in vitro and in vivo, and was closely associated with cellular and glomerular hypertrophy [29, 30, 31, 41, 42]. In addition, recent studies have suggested that CKIs are involved in protecting nonmalignant cells from apoptosis [32, 43, 44]. Moreover, several recent studies have suggested that there is an interaction between the PI3K/Akt/mTOR pathway and the expression of CKIs [16, 45]. Taken together, the evidence suggests that CKIs...
as important molecules not only in the development of cellular hypertrophy but also in cell survival pathways by protecting against apoptosis under diabetic conditions via coordination of the cell cycle and cell death programs. Considering these facts, we also determined the expression of CKIs in relatively small and large diabetic glomeruli and found that p27Kip1 and p21Cip1 expression was increased only in DM-SG. Even though they are difficult to confirm, the results of the present study suggest that the still-ongoing hypertrophic process or the cellular response to apoptotic stress may be linked to the increase in CKIs expression in DM-SG. Contrary to most previous studies, on the other hand, p27Kip1 and p21Cip1 expression was not increased in DM-LG. As aforementioned, since CKI expression is dependent on the PI3K/Akt/mTOR pathway, the differential expression of Akt activity may partly contribute to the differential expression of CKIs. In addition, the CKI expression in a relative small proportion of LG to total glomeruli (11.3 ± 1.2%) in 3-month DM rats may be masked by their expression in DM-SG in most previous studies, which used whole glomeruli for experiments [31, 41, 42]. In this study, cyclin D1 expression, which is central to the regulation of the G1 to S phase transition and has been known to be involved in apoptosis in neuron cells [46, 47], was increased in DM-SG but not in DM-LG. An increase in cyclin D1 expression was also demonstrated in renal cortex of diabetic mice and high-glucose-stimulated mesangial cells and was associated with glomerular and mesangial cell hypertrophy [48]. In addition, Jiang et al. [49] suggested that increased expression of cyclin D1 with a concomitant increase in p21Cip1 expression induced G0/G1 progression but a cell cycle arrest at G1 to S transition, leading to cell hypertrophy. Collectively, increased CKIs and cyclin D1 expression only in DM-SG may imply that the hypertrophic process is underway in less hypertrophied diabetic glomeruli, while it is completed and replaced by apoptosis in more hypertrophied diabetic glomeruli.

TGF-β1, an important mediator in the pathogenesis of diabetic nephropathy, is also involved in cell growth, differentiation, ECM production and apoptosis [11]. TGF-β1 exerts these diverse effects by binding its receptor complex and subsequently activating the same members of the Smad family of transcription factors. Recently, however, in addition to the Smad pathway (canonical pathway), the TGF-β1 receptor complex has been demonstrated to induce non-Smad signals in various cell types, including mitogen-activated protein kinases, PI3K, Akt and mTOR (noncanonical pathway) [50]. Ultimately, integration of Smad and non-Smad signaling pathways determines the nature of the cellular response. Consistent with these previous findings, the results of the present study showed that in DM-SG, Akt might be activated in part by TGF-β1 via noncanonical pathway, while in DM-LG, in which Akt activity was not increased and apoptosis was in progress, TGF-β1 might exert its effect via the classical Smad pathway.

In conclusion, Akt phosphorylation was significantly decreased in relatively large diabetic glomeruli along with concomitantly increased protein expression of Bax, active fragments of Caspase-3 and phospho-p53 and decreased Bcl-2 protein expression. In addition, the numbers of total glomerular cells and podocytes were significantly lower in relatively large diabetic glomeruli. These findings suggest that apoptosis may be more operative in more hypertrophic glomeruli, resulting in fewer glomerular cells and podocytes in diabetic kidney disease.

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Conflict of interest statement. None declared.

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