Deletion of MK2 signalling in vivo inhibits small Hsp phosphorylation but not diabetic nephropathy

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Abstract
It is supposed that some stress-induced heat shock proteins (Hsps) are regulated through e.g. stimulation of the p38MAPK/MK(MAPKAP)-2 signalling pathway. It has been postulated from in vitro experiments that phosphorylation of Hsp25(rodents)/Hsp27(human), the major phosphorylation substrate of MK2, is responsible for mesangial contractility and glomerular hyperfiltration in the diabetic kidney. To verify this hypothesis in vivo we studied the renal function of nondiabetic and streptozotocin (STZ)-induced, diabetic MK2−/− mice in comparison to wild-type (WT) control mice. Following 8 weeks of hyperglycaemia, light microscopy showed increased glomerulosclerosis and tubulointerstitial renal fibrosis in both diabetic study groups. Protein analysis demonstrated that Hsp25 phosphorylation is stimulated upon high-glucose condition but inhibited in the diabetic MK2−/− mice. However, we found the kidney–body weight ratio significantly increased in diabetic WT and MK2−/− mice. No difference regarding the increased expression of the extracellular matrix proteins and TGF-β1 between both diabetic study groups was observed. Importantly, diabetic MK2−/− mice showed no protection against renal hyperfiltration in the diabetic state and the development of diabetic albuminuria. Although activation of p38MAPK has been previously shown in diabetes mellitus, our results indicate that blockade of the downstream MK2/Hsp25 signalling pathway does not interfere with the development of early diabetic nephropathy.

Keywords: diabetic nephropathy; heat shock protein (Hsp); MK2

Introduction

Diabetic nephropathy is the leading cause of end-stage renal failure in the western world [1]. It involves various renal functional changes including renal hyperperfusion/filtration, mesangial expansion, basement membrane thickening and increased capillary permeability leading to the onset of albuminuria and subsequent overt proteinuria [2]. It is postulated that diabetic nephropathy may result from a local interplay of metabolic and haemodynamic factors mediated through direct effects of high-glucose condition and/or auto- and paracrine actions of various cytokine and growth factors [2]. Multiple studies suggested a potential mechanism for crosstalk between high-glucose condition and angiotensin II by the increased production of reactive oxygen species (ROS) serving as a causal link between the diabetic milieu and the activation of the local renin–angiotensin system for the initiation and progression of early diabetic nephropathy [2,3].

Heat shock proteins (Hsps) are highly conserved molecular chaperones that prevent inappropriate intra- and intermolecular interactions of polypeptide chains [4,5]. These proteins are constitutively expressed, but mostly induced and activated, by diverse stress factors mainly in cardiovascular tissues [4,5]. In the kidney, small heat shock proteins (sHsps) such as Hsp25(rodents)/Hsp27(human) and αB-crystallins are involved in the function of mesangial cells as well as podocytes and presumably contribute to the volume-regulatory remodelling of the cytoskeleton in medullary cells during changes in the extracellular tonicity [4]. Certain sHsps are also induced in various pathological states of the kidney [4–8]. The expression of individual sHsps in specific kidney diseases often displays characteristic time courses and intrarenal distribution patterns suggesting that sHsps are involved in the recovery, but possibly also in the initiation and/or maintenance phases of the disturbances [4].

The major signal transduction pathway leading to phosphorylation/activation of murine Hsp25 at Ser15 and Ser86 residues (respectively Ser15, Ser78 and Ser82 residues of human Hsp27) includes MAPKAP(MK)-2 and its upstream activator p38MAPK [9,10]. Interestingly, it has been demonstrated that the p38MAPK signalling pathway is activated in the vasculature and the kidney in diabetes mellitus [11–13]. The specific and/or overlapping functions of the downstream targets of p38MAPK, however, are currently unknown.
We and others have previously identified Hsp25 as a major phosphorylation substrate of the angiotensin II signalling pathway in vascular smooth muscle cells mediated by p38MAPK activation [14,15]. Furthermore, it has been proposed that increased production of ROS in diabetes mellitus may be linked causally to a loss of glomerular contractile reactivity, and thus hyperfiltration, in early diabetic nephropathy by an altered mesangial cell contractile responsiveness through phosphorylation of Hsp25 (Figure 1) [8]. These observations suggest a regulatory role for Hsp25...
and its phosphorylation in the vasoconstriction/dilatation cycle of intrarenal arterial vessels [16].

In this study we challenged the hypothesis that phosphorylation of Hsp25 mediates the development of diabetic nephropathy by in vivo analysis of MK2−/− mice while using the streptozotocin (STZ)-induced diabetic stress model.

Material and methods

Knock-out mice

Experiments were performed with male MK(MAPKAP)-2−/− knock-out (KO) mice in comparison to appropriate SV129-C57BL/6J-hybrid wild-type (WT) littermate control animals from the strain, which were used to generate the MK2−/− KO mice. The mice were generated at the Max-Delbrück Center (MDC), Berlin, Germany, by the research group of Matthias Gaestel, as published previously [17]. The mice were viable and fertile, grew to normal size and did not exhibit obvious behavioural defects.

Animal studies

Experiments were performed with male MK2−/− mice in comparison to SV129-C57BL/6J-hybrid WT as previously described [18]. The animals received a standard diet with free access to tap water. All procedures were carried out according to guidelines from the American Physiological Society and were approved by local authorities. Eight to twelve-week-old, weight-matched mice received either 125-mg/kg body weight streptozotocin (Sigma-Aldrich, Munich, Germany) in 50 mM sodium citrate (pH 4.5) or sodium citrate buffer intraperitoneally on Days 1 and 4. Glucose levels from tail blood were measured with the glucometer Elite (Bayer, Leverkusen, Germany) every other day. Animals with glucose levels >18 mmol/l on two consecutive measurements were regarded as hyperglycaemic and glucose measurements were extended to once weekly. The mice received no insulin within the complete study period. Ketonuria did not occur (data not shown). After 8 weeks of hyperglycaemia, we obtained the kidney for consecutive morphological and immunohistochemical analyses. For this purpose, animals were killed according to the following protocol: after anaesthesia with Avertin (2.5%), a laparotomy was performed and urine was collected by puncturing the bladder with a 23-gauge needle. The abdominal aorta was canulated with a 23-gauge needle and the organs were perfused with a lactated Ringer solution. After ligation of the left renal artery, the left kidney was removed and stored at −80°C until use.

Immunohistochemistry

Immunohistochemistry was performed as described recently [18]. For indirect immunofluorescence, non-specific binding sites were blocked with 10% normal donkey serum (Jackson Immuno Research Lab, West Grove, USA) for 1 h. Specimens were incubated for 2 h in PBS-1% Tween 20 (PBST) containing 5% powdered skim milk. After three washes with

Western blotting

Mouse tissues were lysed in 50 mM Tris–HCl (pH 7.5), 1 mM EGTA, 1 mM EDTA, 1 mM sodium orthovanadate, 50 mM sodium fluoride, 1 mM sodium pyrophosphate, 0.27 M sucrose, 1% (vol/vol) Triton X-100, 0.1% (vol/vol) 2-mercaptoethanol and complete proteinase inhibitor cocktail (Roche, East Sussex, UK). The lysates were centrifuged at 13 000 rpm for 5 min at 4°C, and the supernatants were removed and stored at −80°C until use. Soluble protein extract was run on 10% polyacrylamide SDS gels and transferred to Hybond ECL membranes (Amersham Biosciences, Freiburg, Germany). Western blotting was performed as described recently [14]. Blots were incubated for 2 h in PBS-1% Tween 20 (PBST) containing 5% powdered skim milk. After three washes with
increased phosphorylation of p38MAPK in diabetic WT mice compared to nondiabetic controls as previously demonstrated [14]. Notably, diabetic MK2\(^{−/−}\) mice also show increased activation of the upstream p38MAPK signalling pathway in the diabetic state. However, no significant group difference between both, WT and MK2\(^{−/−}\), mice is observed. We then studied the major substrate of MK2, Hsp25. Figure 2B also demonstrates increased phosphorylation (activation) of the Hsp25 signalling pathway in the diabetic WT animals compared to nondiabetic WT controls. Interestingly, the increased Hsp25 phosphorylation in the diabetic state was almost completely inhibited, but not abolished, in the MK2\(^{−/−}\) mice (\(P < 0.05\) compared to diabetic WT mice).

In parallel, dependence of Hsp25 phosphorylation upon the p38MAPK/MK2 signalling pathway under high-glucose condition was analysed by immunoblot analysis and quantitated by densitometry (Figure 3). Several signalling cascade proteins (p38MAPK, MK2, Hsp25) and their phosphorylation status were investigated. Again, it is demonstrated that the p38MAPK signalling pathway is significantly stimulated in both WT and MK2\(^{−/−}\) diabetic mice (Figure 3). The activation of p38MAPK pathway subsequently leads to phosphorylation of the final substrate Hsp25 in diabetic WT mice. As described previously, the deletion of the MK2 gene results in complete loss of MK2 expression and reduction of the p38MAPK protein level [21]. Although the remaining p38MAPK is hyperphosphorylated and Hsp25 protein expression is increased in response to diabetic conditions in WT controls, no increase in Hsp25 phosphorylation at serine 86 is detectable in diabetic MK2\(^{−/−}\) mice. These results confirm the initial hypothesis that phosphorylation/activation of Hsp25 does occur in the diabetic kidney in a MK2-dependent manner.

Previously, it has been suggested that activation of the p38MAPK/MK2 signalling pathway is involved in the regulation of hyperperfusion and -filtration in the diabetic kidney [8]. Thus, we next studied renal hypertrophy and fibrosis in our MK2\(^{−/−}\) mice to further reveal the role of the phosphorylation of the Hsp25 signalling pathway in the development of early diabetic nephropathy. During the study period, a significant decrease in body weight was observed in both diabetic WT (\(P < 0.01\) versus controls) and MK2\(^{−/−}\) mice (\(P < 0.001\) versus controls) probably due to persistent hyperglycaemia in the two diabetic study groups (Table 1). When calculating the kidney/body weight ratio, we found a significant increase in the diabetic WT animals compared to the nondiabetic control animals (\(P < 0.01\) versus controls) (Table 1). Notably, the MK2\(^{−/−}\)

### Table 1. Baseline and final blood glucose levels and body and kidney weight in WT and MK2\(^{−/−}\) mice after 8 weeks of diabetes.

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>Glucose (mmol/l) Day 0</th>
<th>Glucose (mmol/l) Week 8</th>
<th>Body weight (g) Day 0</th>
<th>Body weight (g) Week 8</th>
<th>Kidney weight (mg) Week 8</th>
<th>Kidney/body weight ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT control</td>
<td>13</td>
<td>7.4 ± 0.9</td>
<td>5.7 ± 0.5</td>
<td>23.5 ± 4.5</td>
<td>26.5 ± 3.5</td>
<td>178 ± 28</td>
<td>6.7 ± 1.0</td>
</tr>
<tr>
<td>WT diabetic</td>
<td>15</td>
<td>6.5 ± 0.7</td>
<td>24.2 ± 6.3(***)</td>
<td>24.3 ± 3.1</td>
<td>21.5 ± 3.7(**)</td>
<td>189 ± 40</td>
<td>9.4 ± 2.6(***)</td>
</tr>
<tr>
<td>MK2(^{−/−}) control</td>
<td>14</td>
<td>7.1 ± 0.6</td>
<td>5.4 ± 0.6</td>
<td>23.1 ± 3.4</td>
<td>27.6 ± 3.3</td>
<td>152 ± 33</td>
<td>5.4 ± 0.4</td>
</tr>
<tr>
<td>MK2(^{−/−}) diabetic</td>
<td>16</td>
<td>6.8 ± 0.5</td>
<td>26.4 ± 5.4(***)</td>
<td>25.7 ± 5.4</td>
<td>21.8 ± 4.2(***)</td>
<td>179 ± 36</td>
<td>9.4 ± 1.8(***)</td>
</tr>
</tbody>
</table>

\(\text{**} P < 0.01; \text{***} P < 0.001\) versus control (unpaired \(t\)-test).

PBST, membranes were incubated for 16 h with the primary antibody at 4°C and for 1 h with horseradish peroxidase-conjugated secondary (2000-fold diluted) antibodies at room temperature. Blots were developed with an ECL detection kit (Santa Cruz Biotechnology, Santa Cruz, USA) and the digital chemiluminescence images were taken by a luminescent image analyser LAS-3000 (Fujifilm, Duesseldorf, Germany).

The following antibodies were used: anti-p38MAPK, anti-phospho-p38MAPK and anti-MK2 were used from New England Biolabs ( Ipswich, USA), anti-Hsp25 was used from Stressgene (Ann Arbor, USA), anti-phospho-Hsp25 (Ser86) from Biosource (Solingen, Germany) and anti-GAPDH antibody from Millipore (Schwalbach, Germany).

**Statistics**

Data are shown as mean ± SEM. The data were compared by analysis of variance (ANOVA) with appropriate post-test. Data analysis was performed using InStat software (GraphPad Software, Inc., San Diego, USA).

**Results**

Hyperglycaemia was induced in 7-week-old mice by intraperitoneal injection of STZ on Days 1 and 4. After 8 weeks of hyperglycaemia, the non-fasting blood glucose levels were 24.2 ± 6.3 mmol/l in the diabetic WT and 26.4 ± 5.4 mmol/l in the diabetic MK2\(^{−/−}\) mice (Table 1; \(P < 0.001\) versus control). In the sham-injected mice, the levels remained normoglycaemic with blood glucose levels of 5.7 ± 0.5 mmol/l in WT and 5.4 ± 0.6 mmol/l in the MK2\(^{−/−}\) mice (Table 1).

To study the possible early structural changes in the kidney from (diabetic) MK2\(^{−/−}\) mice we first performed periodic acid Schiff (PAS) staining and semi-quantitative histomorphological analysis of animals after 8 weeks of diabetes mellitus (Figure 1). Figure 1A demonstrates increased glomerular hypercellularity and mesangial expansion in diabetic WT and MK2\(^{−/−}\) animals compared to healthy controls and the nondiabetic MK2\(^{−/−}\) mice, respectively. However, we did not reveal any significant group difference between the diabetic WT and the MK2\(^{−/−}\) mice on the glomerular or tubulointerstitial level (\(P > 0.05\)) (Figure 1B).

We next started investigation of the p38MAPK/MK2/Hsp25 signalling pathway by means of immunohistochemical analysis of kidney tissues. Figure 2A demonstrates
Fig. 2. Immunohistochemistry of phospho-p38MAPK (A) and phospho-Hsp25 (B). Activation of the p38MAPK signalling pathway in the diabetic state is demonstrated in both, WT and MK2−/− mice, whereas deletion of the downstream kinase MK2 leads to reduced activation of its substrate Hsp25 (*, P > 0.05).

mice also showed a significant increase of the kidney/body weight ratio in the diabetic state (P < 0.001 versus controls) compared to nondiabetic control animals, which was equal to the increase in diabetic WT mice.

We next analysed the expression of the profibrotic cytokine transforming growth factor beta 1 (TGF-β1) and extracellular matrix molecules that are involved in the chronic renal fibrosis observed in diabetic nephropathy (Figure 4) [1,2]. First, renal TGF-β1 expression was evaluated by immunohistochemistry (Figure 4A). In both WT and MK2−/− hyperglycaemic mice, a significant increase of glomerular TGF-β1 expression was demonstrated.
Fig. 3. Western blotting of protein extracts from kidney lysates. The p38MAPK signalling pathway is significantly upregulated in both diabetic groups compared to appropriate controls (*, \( P > 0.05; **, \ P > 0.01 \)). The gene KO is confirmed in the MK2\(^{-/-}\) mice. In WT mice, activation of p38MAPK signalling pathway in the diabetic state leads to phosphorylation of the substrate Hsp25 through MK2 signalling. Notably, Hsp25 phosphorylation is almost completely inhibited in MK2\(^{-/-}\) mice.

However, no group difference was observed between the WT and MK2\(^{-/-}\) animals. Secondly, we performed the immunohistological evaluation of fibronectin (Figure 4B) and type III collagen (Figure 4C). Both extracellular matrix molecules were significantly increased under diabetic conditions, but to a similar extent in both WT and MK2\(^{-/-}\) diabetic groups. These results further demonstrate that the p38MAPK/MK2 signalling pathway does not contribute to the high-glucose-induced renal fibrosis in early diabetic nephropathy.

To directly study renal hyperperfusion and glomerular hyperfiltration in early diabetic nephropathy, we next performed measurement of the glomerular filtration rate by means of inulin clearance in MK2\(^{-/-}\) mice. Data are displayed in Table 2. Diabetic WT mice demonstrate a 59% increase (± 11%; \( P > 0.01 \)) in the glomerular filtration rate compared to nondiabetic controls indicating renal hyperperfusion in the diabetic state (312 ± 79 versus 186 ± 34 \( \mu l/min; \ P < 0.01 \)). Similar results were observed between diabetic and control MK2\(^{-/-}\) mice (320 ± 75 versus 190 ± 66 \( \mu l/min; \ P < 0.05 \)). Notably, no significant difference regarding the glomerular filtration rate was observed between the diabetic WT and the diabetic MK2\(^{-/-}\) mice. These results directly prove that hyperfiltration in early diabetic nephropathy is not prevented when phosphorylation/activation of the small heat shock protein Hsp25 is inhibited.

Lastly, we also analysed albuminuria in the MK2\(^{-/-}\) mice as it has also been suggested that phosphorylation of Hsp25 in the diabetic glomeruli may be implicated in podocyte functional and structural abnormalities [33,34].

Table 2. Glomerular filtration rate determined as inulin clearance in WT and MK2\(^{-/-}\) mice after 8 weeks of diabetes.

<table>
<thead>
<tr>
<th>Study group</th>
<th>n</th>
<th>Inulin clearance (( \mu l/min ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT control</td>
<td>8</td>
<td>186 ± 34</td>
</tr>
<tr>
<td>WT diabetic</td>
<td>5</td>
<td>312 ± 79**</td>
</tr>
<tr>
<td>MK2(^{-/-}) control</td>
<td>4</td>
<td>190 ± 66</td>
</tr>
<tr>
<td>MK2(^{-/-}) diabetic</td>
<td>6</td>
<td>320 ± 75*</td>
</tr>
</tbody>
</table>

\( P < 0.05; ** P < 0.01 \) versus control (unpaired \( r \)-test).
Fig. 4. Immunohistochemistry of the profibrotic cytokine TGF-β1 (A) and the extracellular matrix proteins fibronectin (B) and type III collagen (C). All key proteins of chronic renal fibrosis show a significant increase, to a similar extent, in the expression level in both WT and MK2−/− diabetic groups.
After 2 weeks of hyperglycaemia, albuminuria was not yet present in either nondiabetic or diabetic animals (Figure 5). After 8 weeks of diabetes mellitus a significant increase of albuminuria was observed in the WT and the MK2<sup>−/−</sup> rodents. The albumin/creatinine ratio was significantly increased in the diabetic WT mice with a median of 32.5 ± 24.2 g/mol compared to 6.6 ± 5.2 g/mol in the sham-injected control WT animals (P < 0.01). Notably, the albumin/creatinine ratio also remained increased with a median of 19.4 ± 7.3 g/mol in hyperglycaemic MK2<sup>−/−</sup> mice compared to nondiabetic MK2<sup>−/−</sup> control animals (3.0 ± 0.7 g/mol; P < 0.05). The individual data are displayed in Figure 5. In conclusion, these data further underline that also the disturbed integrity of the glomerular filtration barrier in diabetes mellitus is not prevented in the absence of the MK2 gene.

**Discussion**

In the present study, we demonstrate that p38MAPK/MK2-induced sHsp phosphorylation does not contribute to the high-glucose-induced renal hypertrophy and glomerular hyperfiltration or renal bibrosis and albuminuria in the diabetic state. Our findings contradict the postulate that phosphorylation of Hsp25 is responsible for mesangial contractility and glomerular hyperfiltration in the diabetic kidney as previously suggested from *in vitro* experiments [8].

Small Hsps are highly conserved, abundantly expressed proteins, and increased expression of sHsps is believed to enhance the survival of cells exposed to oxidant stress [22]. Protection against oxidative stress thereby may be conferred by two distinct properties of the sHsps. Firstly, nonphosphorylated Hsp25 act as molecular chaperones by maintaining a large multimeric aggregate structure while providing direct protection against intracellular ROS [4,5,22]. Secondly, phosphorylated, but not phosphorylated, Hsp25 is supposed to act as actin-capping protein, which inhibits actin polymerization when bound (Figure 1). Hsp25 phosphorylation thereby stabilizes microfilaments and leads to remodelling of the F-actin network allowing contractility of diverse vascular cell types [5,22]. This assembly–disassembly process is, for example, proposed to mediate proliferation and migration of vascular cells [23–25]. In human endothelial cells, inhibition of vascular endothelial growth factor-induced p38MAPK activation abolishes Hsp25 phosphorylation, actin polymerization and cell migration suggesting also a possible link between Hsp25 and angiogenesis [26]. Also, MK2-deficient murine cells display decreased cell migration [20,27]. Furthermore, Hsp25 is thought to participate in the ischaemia-induced disruption and restructuring of the cytoskeleton in the cortex and outer medulla of the rat kidney [28]. The finding that ischaemia induces Hsp25 in cortical and outer medullary blood vessels, presumably in endothelial cells, suggests similar functions in nontubular cells [29].

In the adult kidney, sHsps display a distribution pattern paralleling the corticopapillary gradient, with high amounts in the papilla and low amounts in the cortex [30,31]. Immunohistochemical investigations have revealed that, in the renal cortex, Hsp25 is present predominantly in nonepithelial cells (glomerular mesangial cells, podocytes and vascular cells) whereas its expression in the medulla is mainly found in proximal and distal tubular cells [6,30,31]. Glomerular mesangial cells and podocytes obtain contractile systems and respond to vasoactive substances by contraction [6]. For example, inhibition of the sHsp phosphorylation prevents angiotensin II contraction of mesangial cells [16]. Previously, we and others have also identified Hsp25 as a major phosphorylation substrate of the
angiotsensin II signalling pathway in vascular smooth muscle cells that is mediated through activation of the p38MAPK signalling cascade [14,15].

It is well described that early renal changes in the diabetic state lead to glomerular and mesangial cell hypocontractility, a condition that contributes to a potential damaging of unregulated glomerular hyperfiltration in the diabetic kidney [32]. Notably, it has previously been proposed that altered mesangial cell contractile responsiveness through phosphorylation of Hsp25 may be a mechanism underlying abnormalities in glomerular haemodynamics in early diabetic nephropathy [8]. Dunlop et al. thereby suggested that the generation of oxygen-derived free radicals in diabetes mellitus may be linked causally to a loss of glomerular contractile reactivity and thus hyperfiltration in the early stages of diabetes mellitus [8]. Our in vivo results from MK2−/− mice, however, clearly demonstrate that prevention of MK2-induced Hsp25 phosphorylation does not inhibit renal hypertrophy and hyperfiltration in the diabetic state. The discrepancy of these findings is obvious, but explained by several facts. First, Dunlop et al. studied glomeruli from rats with experimental diabetes induced by STZ administration, and isolated glomeruli were exposed to free radical stress [8]. Secondly, contractile responsiveness of mesangial cells was determined by the serum-induced contraction of cell-embedded type I collagen gels, meaning an in vitro model of contractility measurement [8]. Lastly, in contrast to this experimental set-up, we chose to directly measure the glomerular filtration rate in MK2−/− mice, which were exposed to STZ or sham injection, by means of inulin clearance. Thereby we have studied the functional role of Hsp25 phosphorylation in early diabetic nephropathy in vivo.

Notably, our results also could not demonstrate a functional role for sHsp phosphorylation in the development of albuminuria in the STZ-induced diabetic stress model. However, we cannot exclude that sHsp phosphorylation is involved in the development of other glucose-independent renal diseases. In the nephrotic puromycin aminonucleoside of focal segmental glomerulosclerosis model, retraction and effacement of glomerular epithelial cell foot processes were associated with enhanced protein expression and phosphorylation of glomerular Hsp25 [33,34]. These increases were postulated to modulate actin polymerization–depolymerization indicating an important role in the development of the observed changes in foot process architecture [6]. Our current results are in contrast to these previous studies and clearly demonstrate in vivo that diabetic MK2−/− mice were not protected against the development of albuminuria, at least in early diabetic nephropathy.

Further approaches would be desirable in the future to study the role of sHsps in diabetic nephropathy. A direct analysis of the MK2/Hsp25 signalling pathway would require an Hsp25-gene-KO model which is so far not available. Instead, we therefore studied a phosphorylation/activation KO mouse model by deletion of the upstream kinase MK2 (MAPKAP-2) with regard to early experimental diabetic nephropathy. Interestingly, the increased Hsp25 phosphorylation in the diabetic state was almost completely inhibited, but not abolished, in the MK2−/− mice suggesting that also other kinases might interfere with the activation of (other) sHsps. In the literature, there is another MK2-related Hsp25 kinase activated by p38MAPK, termed MK3 (MAPKAP-3) [35–37]. However, the MK3 isoform is only very weakly expressed in the kidney and is not induced in the diabetic state or compensatorily upregulated in our MK2 KO model (data not shown). Future analysis of a double KO mutant including the MK2 and the MK3 gene may offer new perspectives on this subject in the future [38].

Furthermore, it is noteworthy to mention that phosphorylation of Hsp25 can also be induced in vitro by the protein kinase C (PKC), specifically the PKC-δ isoform, indicating a functional role of another intracellular serine–threonine kinase signalling cascade in the phosphorylation/activation of Hsp25 [39]. Interestingly, high-glucose-induced PKC isoform activation has been shown to be a major signalling pathway to be activated in diabetic microvascular complications [40]. For example, it has been previously shown that glucose-induced gene expression of endothelin-1 (ET-1) in retinal-endothelial cells, as well as in pericytes, is mediated through PKC isoform activation, mainly the PKC-β- and -δ isoforms [41]. Furthermore, we had demonstrated that high-glucose-induced p38MAPK signalling in vascular smooth muscle cells is mainly activated through PKC-δ isoform signalling [42]. We therefore cannot exclude that other signalling pathways somehow may contribute to the activation of (other) sHsp in diabetic nephropathy.

In summary, we demonstrate that in vivo deletion of high-glucose-induced activation of the p38MAPK/MK2 signalling pathway and its major phosphorylation substrate Hsp25 does not prevent the development of experimental diabetic nephropathy.

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Conflict of interest statement. None declared.

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