Review article: Expert Opinion

Thymosin-β4: a key modifier of renal disease

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ABSTRACT

Introduction: There is an urgent need for new treatments for chronic kidney disease (CKD). Thymosin-β4 is a peptide that reduces inflammation and fibrosis and has the potential to restore endothelial and epithelial cell injury, biological processes involved in the pathophysiology of CKD. Therefore, thymosin-β4 could be a novel therapeutic direction for CKD.

Areas covered: Here, we review the current evidence on the actions of thymosin-β4 in the kidney in health and disease. Using transgenic mice, two recent studies have demonstrated that endogenous thymosin-β4 is dispensable for healthy kidneys. In contrast, lack of endogenous thymosin-β4 exacerbates mouse models of glomerular disease and angiotensin-II-induced renal injury. Administration of exogenous thymosin-β4, or its metabolite, Ac-SDKP, has shown therapeutic benefits in a range of experimental models of kidney disease.

Expert opinion: The studies conducted so far reveal a protective role for thymosin-β4 in the kidney and have shown promising results for the therapeutic potential of exogenous thymosin-β4 in CKD. Further studies should explore the mechanisms by which thymosin-β4 modulates kidney function in different types of CKD. Ac-SDKP treatment has beneficial effects in many experimental models of kidney disease, thus supporting its potential use as a new treatment strategy.

Keywords: Ac-SDKP, cytoskeleton, fibrosis, glomerulus, inflammation, kidney disease, podocyte, thymosin-β4

1. Introduction

Chronic kidney disease (CKD) is defined as a gradual loss of kidney function over a period of months or years. The worldwide prevalence of CKD is currently rising due to the aging population and increased incidence of obesity, hypertension and diabetes. [1] CKD patients also have increased risk of developing cardiovascular disease compared to age-matched individuals in the general population. [2] Current treatment strategies to slow CKD include lifestyle changes and pharmacological inhibitors that aim to treat underlying risk factors, such as high blood pressure. [3] Despite these interventions, a significant proportion of patients with CKD develop end stage renal failure; [4] a devastating condition that requires lifelong dialysis and transplantation.

The major causes of CKD are diseases that affect the glomerulus, the filtration unit of the kidney, such as hypertension, diabetic nephropathy and glomerulonephritis. [5] Irrespective of the underlying aetiology, the progression to end stage renal failure proceeds through common pathophysiological mechanisms. CKD is accompanied by an inflammatory response characterised by leukocyte infiltration, which is driven by the release of chemokines and expression of adhesion molecules by renal cells. [6] The degree of inflammation can differ depending on the underlying cause and the stage of CKD, [7] but plays a major role in the progression of most kidney disorders including glomerulonephritis, [7] diabetic nephropathy [8] and hypertensive nephropathy [9]. Inflammation subsequently leads to extracellular matrix accumulation, fibrosis and loss of epithelial cell function, correlating with declining renal function. In addition to inflammation and fibrosis, in glomerular disease the endothelial and epithelial (podocytes) cells that compose the glomerular filtration barrier are damaged due to changes in angiogenic growth factors and perturbation of the podocyte cytoskeleton

resulting in disruption to the glomerular filtration barrier, defective filtration and proteinuria. [10-12] Therefore, treatments which modulate inflammation, fibrosis, angiogenesis and the cell cytoskeleton have the potential to reduce the morbidity and mortality associated with CKD.

Thymosin-β4 is a naturally-occurring peptide and in humans is encoded by the *TMSB4X* gene on the X- chromosome. [13] It is the major G-actin–sequestering protein in mammalian cells and prevents actin polymerisation into filaments thus having a critical role in maintaining cytoskeletal dynamics. [14] Thymosin-β4 has been shown to modulate several cellular functions including cell motility, [15] differentiation, [16] survival, [17] angiogenesis, [18] inflammation, [19] and fibrosis. [20] Whilst some of these effects are linked to the G-actin-sequestering function of thymosin-β4, others are mediated via G-actin-independent mechanisms. Thymosin-β4 can form a functional complex with PINCH-1 and integrin-linked kinase (ILK) promoting cell survival [17] and anti-inflammatory actions by preventing tumor necrosis factor-α-mediated NF-κB activation. [21] In lamellipodia, thymosin-β4 binding to ILK has been shown to promote the release of matrix metalloproteinases and enhance cell migration. [15] Thymosin-β4 can also promote phagocytosis by interacting with stabilin-2, a receptor involved in the phagocytosis of apoptotic cells. [22]

In animal models, exogenous thymosin-β4 has beneficial effects in diverse pathologies including myocardial infarction, [23] stroke, [24] dry eye, [25] and inflammatory lung disease, [26] and there are clinical trials assessing thymosin-β4 treatment in wound healing and cardioprotection. [27] Thymosin-β4 derivatives also have therapeutic properties. Thymosin-β4 sulfoxide has anti-inflammatory properties and can disperse neutrophils *in vitro* [28] and

reduces macrophage accumulation following injury in zebrafish and mice. [29] This effect was shown to be mediated by thymosin-β4 sulfoxide blocking the production of the pro-inflammatory cytokine interferon-γ from T-cells and subsequently reducing the adhesive properties of activated monocytes and enhancing their cell death by increasing the production of reactive oxygen species. [29] The tetrapeptide N-acetyl-seryl-aspartyl-lysyl-proline (Ac-SDKP), which is generated by successive cleavage of thymosin-β4 by the enzymes meprin-α [30] and prolyl oligopeptidase (POP) [31] and is degraded by angiotensin I-converting enzyme, [32] is able to reduce fibrosis in animal disease models. [33-35]

Given the role of thymosin-β4 in modulating the actin cytoskeleton, angiogenesis, inflammation and fibrosis, processes that are critical in CKD progression, several studies have explored the effects of thymosin-β4 in the kidney. In this review, we will summarise the current understanding of the role of thymosin-β4 in the kidney and its therapeutic potential in renal disease as established using experimental animal models.

2. Experimental evidence on the role of thymosin-β4 in the kidney

2.1. Thymosin-β4 expression in the kidney in health and disease

A number of studies have examined thymosin-β4 expression in the healthy mouse kidney. Guinobert *et. al.* demonstrated that *Tmsb4x* transcripts were present in the embryonic mouse kidney as early as embryonic day 12 and increased throughout development reaching a peak at 7 days after birth. [36] *Tmsb4x* mRNA was present in the mature kidney (8-week old mice) but at reduced levels. [36] The localisation of *Tmsb4x* mRNA was also analysed during active nephrogenesis (embryonic day 18) and in the mature kidney. During nephrogenesis, *Tmsb4x* was expressed in differentiating glomeruli and in the interstitium

surrounding developing tubules. [36] In the mature kidney, *Tmsb4x* transcripts were localised to collecting ducts and glomeruli, with a pattern suggesting thymosin-β4 expression in podocytes, specialised epithelial cells that are critical for glomerular function. [36] Microarray analysis also found *Tmsb4x* levels were enriched in purified podocytes isolated from transgenic mice with podocyte-specific GFP expression (*MafB-GFP*) compared with the rest of the kidney cortex . [37] Our group has also detected *Tmsb4x* transcripts in both developing and mature mouse glomeruli. [38] Furthermore, using immunohistochemistry thymosin-β4 protein was localised in glomeruli from embryonic day 18, 1-week-old postnatal and 8-weeks-old adult mice. In adult glomeruli, thymosin-β4 protein co-localised with the podocyte markers nephrin and nestin, thus demonstrating the expression of thymosin-β4 in podocyte cells, whereas endothelial cells, identified by platelet endothelial cell adhesion molecule (PECAM/CD31) staining, were not positive for thymosin-β4. [38] There is limited data regarding the expression of thymosin-β4 in human kidneys. We found that *TMSB4X* mRNA was expressed in both the glomerular and tubulointerstitial compartments in adult human kidneys. [38] This is in contrast to previous reports that could not detect thymosin-β4 protein in fetal or adult glomeruli by immunohistochemistry, but found thymosin-β4 positivity in fetal and adult collecting ducts and tubules. [39]

The expression of thymosin-β4 in CKD was first explored by a proteomic study using the rat remnant kidney model. [40] The investigators performed unilateral nephrectomy of the right kidney and ligation of the branches of the left renal artery resulting in 5/6 renal ablation, which results in glomerulosclerosis and interstitial fibrosis. Using laser capture microdissection, sclerotic and non-sclerotic glomeruli were isolated from the remnant kidney as well as normal glomeruli from the nephrectomised kidney. [40] Proteomic analysis

found that thymosin-β4 levels were significantly increased three-fold in sclerotic *versus* normal glomeruli and immunostaining localised thymosin-β4 predominately to endothelial cells with no staining detected in podocytes or infiltrating macrophages. [40] Other studies demonstrated high thymosin-β4 expression in macrophages, myofibroblasts and tubular epithelia in the unilateral ureteral obstruction (UUO) model, where the ureter is ligated leading to a rapid reduction in renal blood flow and glomerular filtration rate in the obstructed kidney and subsequent inflammation and fibrosis. [35] We examined thymosin-β4 expression in the nephrotoxic serum nephritis model, which replicates some of the pathologic features of human crescentic glomerulonephritis. [41] In this model, mice are injected with nephrotoxic serum that contains antibodies against the mouse glomerular basement membrane, thus initiating an immune response resulting in glomerular lesions. [41] Nephrotoxic nephritis involves the injury of intrinsic glomerular cells, including podocytes, as well as leukocyte infiltration, glomerulosclerosis, and tubulointerstitial fibrosis. [42] We found that 21 days after disease induction the mRNA level of *Tmsb4x* in the whole kidney homogenates was not significantly altered compared with healthy controls. Thymosin-β4 protein in diseased kidneys was strongly localised in podocyte cells and in infiltrating macrophages that accumulate around the glomerulus. [38] We also assessed thymosin-β4 expression in kidney biopsy specimens obtained from patients with either rapidly progressive glomerulonephritis or lupus nephritis and found no change in glomerular or tubulointerstitial *TMSB4X* mRNA levels compared with living donor control kidneys. [38] Collectively, these studies show that thymosin-β4 is present in murine and human healthy and diseased kidneys. There are some discrepancies reported in the pattern of thymosin-β4 expression in these studies which may reflect differences between species, type and stage

of CKD or the tools available to detect thymosin-β4, such as the antibodies and fixation methods used. [43]

2.2. The role of endogenous thymosin-β4 in kidney function in health and disease

Despite the evidence for the expression of thymosin-β4 in the kidney, until recently there were no studies exploring the importance of endogenous thymosin-β4 for kidney function. To address this, we used male mice with a global loss of thymosin-β4 (*Tmsb4x*$^{-/y}$) and compared them with male wild-type littermates (*Tmsb4x*$^{+/y}$). [38] Given that thymosin-β4 plays a role in actin binding, [14] we initially hypothesized that the lack of endogenous thymosin-β4 might disrupt the highly branched architecture of glomerular podocytes and subsequently impair renal function. [11] However, we found that lack of thymosin-β4 did not affect podocyte architecture examined by electron microscopy. There were also no differences in renal function as *Tmsb4x*$^{-/y}$ mice had albumin excretion and blood urea nitrogen levels similar to those of wild-type *Tmsb4x*$^{+/y}$ mice at the ages of 1, 3, and 6 months. [38] These results demonstrate that endogenous thymosin-β4 is dispensable in healthy glomeruli.

To investigate the role of endogenous thymosin-β4 in glomerular disease, we utilised the nephrotoxic serum nephritis model. [42] We found that glomerular disease was exacerbated in mice lacking thymosin-β4. [38] *Tmsb4x*$^{-/y}$ mice had significantly worse renal function (evidenced by increased albuminuria, albumin to creatinine ratio, plasma creatinine, blood urea nitrogen and reduced creatinine clearance) and more severe glomerular injury compared with wild-type *Tmsb4x*$^{+/y}$ mice 21 days after the induction of glomerular disease. Lack of thymosin-β4 was shown to worsen glomerular disease progression by two distinct

mechanisms; aberrant podocyte migration and increased accumulation of macrophages around the glomerulus linked to interstitial fibrosis (**Figure 1**). The effects of exogenous thymosin-β4 on the cytoskeleton and cell migration have been studied before, [14, 15, 17, 44-47] however, this was the first study to examine how endogenous thymosin-β4 modulates the cytoskeleton and migration potential of podocyte cells. *In vitro,* lack of endogenous thymosin-β4 increased podocyte migration in a wound-healing assay. This was mirrored *in vivo* by a redistribution of podocytes in *Tmsb4x*$^{-/y}$ mice with glomerular disease from the glomerular tuft, where they contribute to filtration barrier integrity, toward the Bowman capsule. [38] The increased podocyte migration was associated with increased actin stress fibers and activation of RhoA *in vitro*, indicating a potential target of thymosin-β4 action on the podocyte cytoskeleton. Exogenous thymosin-β4 reduces inflammation in several disease settings. [19, 26, 35, 48] In the context of glomerular disease, we found that lack of endogenous thymosin-β4 did not affect macrophage infiltration at the early stage of the disease (day 7), but significantly increased macrophage accumulation in the peri-glomerular region at the late stage (day 21), which was accompanied by increased fibrosis. [38] Macrophage accumulation may result from the absence of the thymosin-β4-derivative, thymosin-β4 sulfoxide, which has been shown to disperse inflammatory macrophages. [29]

Subsequently, Kumar *et. al.* also reported that the absence of endogenous thymosin-β4 does not affect kidney weight, albuminuria, systolic blood pressure, inflammation or fibrosis in otherwise healthy mice. [49] The authors then infused angiotensin-II for six weeks (980ng/kg/minute) to induce hypertension in *Tmsb4x*$^{+/y}$ and *Tmsb4x*$^{-/y}$ adult mice. Angiotensin-II raised systolic blood pressure in both *Tmsb4x*$^{+/y}$ and *Tmsb4x*$^{-/y}$ mice compared with base-line measurements prior to infusion, but there was no significant

difference between the two groups. Angiotensin-II infusion also led to renal injury, demonstrated by low levels of albuminuria, macrophage infiltration, increased expression of intracellular molecule-1 (ICAM-1) and fibrosis assessed by increased collagen content and α smooth muscle actin (αSMA); these parameters were significantly more pronounced in *Tmsb4x*$^{-/y}$ compared with *Tmsb4x*$^{+/y}$ mice.[49]

These findings demonstrate that endogenous thymosin-β4 has a protective effect in two models of renal disease and thus indicate that administration of exogenous thymosin-β4 may have a therapeutic effect in this pathology. Further studies are necessary to determine if endogenous thymosin-β4 has a similar role in other types of CKD and to fully dissect the mechanisms by which thymosin-β4 mediates its effects on epithelial cells, inflammation and fibrosis.

2.3. The therapeutic potential of thymosin-β4 and its derivatives in experimental models of kidney disease

Recent studies have provided evidence that administration of thymosin-β4 is beneficial in animal models of kidney disease. In the UUO mouse model of interstitial fibrosis, administration of thymosin-β4 at a dose of 150 μg/day intraperitoneally reduced fibrosis at the late (day 14) but not the early (day 5) stage of the disease. [35] In this model, thymosin-β4 treatment did not affect inflammation as macrophage number was not altered at either disease stage. Instead, the authors provided evidence that thymosin-β4 administration reduced plasminogen activator inhibitor-1 (PAI-1) expression and transforming growth factor-β (TGF-β) 1 signalling, [35] both of which are important pro-fibrotic pathways in CKD. The importance of the PA-1 pathway was further indicated by the absence of any

improvement in fibrosis when PAI-1 knock-out mice that had also undergone UUO were treated with thymosin-β4. [35] Another study demonstrated a beneficial effect of exogenous thymosin-β4 in the rat UUO model. [50] Adult Sprague Dawley male rats underwent UUO or sham surgery and were subsequently treated daily with 1 or 5 mg/kg thymosin-β4 or saline administered by intragastric lavage. Treatment with thymosin-β4, was shown to alleviate fibrosis assessed by light microscopy, reduce TGF-β and αSMA upregulation, restore E-Cadherin protein levels and reduce apoptosis of renal tubular cells. These effects were particularly pronounced in the high dose thymosin-β4 group compared with the UUO group administered saline. [50] Overall, these studies provide evidence for an anti-fibrotic effect of thymosin-β4 in renal interstitial fibrosis through inhibition of TGF-β signalling.

The therapeutic potential of thymosin-β4 was also assessed in a mouse model of diabetic nephropathy. Twelve-week-old *KK Cg-Ay/J* mice, which exhibit type 2 diabetes mellitus, were injected with either saline or 100 ng/10 g/day thymosin-β4 intraperitoneally for three months. [51] Thymosin-β4-treated mice had reduced albuminuria and albumin to creatinine ratio compared with saline-treated *KK Cg-Ay/J* mice indicating improved renal function. In addition, thymosin-β4 treatment attenuated the renal pathological changes shown in saline-injected *KK Cg-Ay/J* mice. [51] Thymosin-β4 treatment also improved hyperglycaemia in this model [51] and this effect may have contributed to the improvements in renal function and structure. However, this study did not investigate the mechanisms that mediated the effects of thymosin-β4.

A number of studies have investigated the role of the thymosin-β4 derivative, Ac-SDKP, in kidney disease. Plasma Ac-SDKP is elevated in patients with chronic renal failure compared with healthy individuals and levels are further enhanced in patients treated with ACE inhibitors. [52, 53] This increase is attributed to both inhibition of ACE, which degrades Ac-SDKP, and to declining glomerular function with CKD resulting in impaired renal clearance of excess Ac-SDKP. It is unclear whether the increase of circulating Ac-SDKP mediates some of the beneficial effects of ACE inhibitor treatment in CKD. [54]

A study investigating the role of endogenous Ac-SDKP in fibrosis demonstrated that downregulation of Ac-SDKP in rats by administrating a POP inhibitor (S17092, 40 mg/kg/day) increased collagen deposition and promoted cardiac and renal perivascular fibrosis and glomerulosclerosis. [55] These findings suggested that exogenous Ac-SDKP could have an anti-fibrotic effect. Indeed, many subsequent studies have shown Ac-SDKP treatment has beneficial effects in a wide range of CKD animal models.

Administration of Ac-SDKP (400 μg/kg/day) in a rat UUO model, reduced kidney fibrosis, decreased the number of macrophages in the kidney and lowered the renal expression of αSMA, monocyte chemoattractant protein-1 (MCP-1) and TGF-β1 two weeks after UUO. [56] In another study, Ac-SDKP treatment (1.6 mg/kg/day) via osmotic minipump also decreased fibrosis at both early (day 5) and late (day 14) time-points [35] in the UUO murine model as evidenced by significant decrease in fibronectin, collagen I, PAI-1, TGF-β1 signalling and reduced number of renal macrophages and myofibroblasts. Finally, another group reported that although, administration of Ac-SDKP (1 mg/kg/day) by osmotic minipump decreased collagen I and III deposition in renal cortical tissue 7 days post-UUO indicating reduced

fibrosis, this had no effect on interstitial injury assessed by light microscopy or inflammation. [57] This discrepancy may be due to the lower dose used by these investigators.

Ac-SDKP treatment has also shown therapeutic potential in models of hypertensive nephropathy induced by nephron reduction. Exogenous Ac-SDKP (800 μg/kg/day) administration by osmotic minipump was initiated either 1 week before or 3 weeks after nephrectomy to compare effectiveness as a preventative or interventional therapy in rats with 5/6 nephrectomy (5/6Nx)-induced hypertension. [58] In both protocols, Ac-SDKP improved albuminuria, glomerular filtration rate, macrophage infiltration, glomerulosclerosis and renal collagen content. [58] In addition, Ac-SDKP reversed the loss of nephrin, a molecule critical for the integrity of the glomerular filtration barrier, providing a potential mechanism by which Ac-SDKP may elicit its therapeutic benefit in this animal model. [58] Subsequently, this group demonstrated that Ac-SDKP is also renoprotective in deoxycorticosterone acetate-salt hypertensive mice [59] and Dahl salt-sensitive hypertensive rats. [60] In both cases, treatment with Ac-SDKP (800 or 1600 μg/kg/day) improved glomerular damage, albuminuria, inflammation and interstitial fibrosis. These effects were not due to an effect of Ac-SDKP in blood pressure which was unaltered.

A mouse model of systemic lupus erythematosus (SLE) utilising *MRL/MpJ-Faslpr/2J* mice that develop an aggressive form of lupus nephritis with no hypertension was investigated to assess the effect of Ac-SDKP on lupus nephritis. *MRL/MpJ-Faslpr/2J* mice and age-matched control *MRL-MpJ* mice were administered Ac-SDKP (800 μg/kg/day) or vehicle by osmotic minipump from 12 weeks of age for 20 weeks and were assessed at 32 weeks of age. Ac-SDKP treatment had no effect on control *MRL-MpJ* mice. *MRL/MpJ-Faslpr/2J* mice had

impaired renal function and glomerular damage which was reduced by Ac-SDKP treatment when compared with vehicle-treated mice. [61] This was accompanied by a reduction in macrophage and T-cell infiltration in the kidney and lower renal levels of chemokines and cytokines (complement C5-9, RANTES, MCP-5, and ICAM-1). [61] The therapeutic potential of Ac-SDKP was also assessed in another mouse model of lupus (female *NZBWF1* mice) that develop glomerulonephritis at 20-24 weeks of age and non salt-sensitive hypertension and endothelial dysfunction at 34-36 weeks of age. [62] Ac-SDKP (800 µg/kg/day) or vehicle was administered by osmotic minipump to *NZBWF1* SLE female mice and NZW control mice from 24 to 38 weeks of age. Ac-SDKP delayed the onset of albuminuria and prevented glomerulosclerosis in *NZBWF1* mice without affecting the hypertension observed in these animals. [62]

A number of studies have demonstrated the therapeutic potential of Ac-SDKP in animal models of type 1 (streptozotocin (STZ)-induced) and type 2 (*db/db* mice) diabetic nephropathy. Ac-SDKP treatment (1 mg/kg/day for two months delivered by osmotic minipump) was initiated in male Sprague-Dawley rats eight weeks after STZ injection. [33] Ac-SDKP administration improved fibrosis assessed by Sirius red staining and reversed diabetes-induced loss of nephrin in diabetic rats. Despite these molecular and structural changes, Ac-SDKP did not alter renal function with no improvement in albuminuria observed, [33] possibly due to the late initiation of treatment. The effect of Ac-SDKP treatment was also tested in *db/db* mice . [34] Ac-SDKP (1mg/kg/day) was administered from 10 weeks of age for 8 weeks by osmotic minipump. Ac-SDKP did not alter hyperglycaemia, blood pressure or peripheral erythrocyte number but prevented the pathological increase in mesangial matrix expansion observed in vehicle-treated *db/db* mice as assessed by light microscopy. [34] Ac-SDKP

treatment also ameliorated the diabetes-induced overproduction of fibronectin and collagen IV in the glomerulus and reduced plasma creatinine levels compared with vehicle-treated *db/db* mice. [34] These improvements in *db/db* mice following Ac-SDKP treatment were also accompanied by diminished TGF-β signalling within glomeruli. [34] However, albuminuria was not affected by Ac-SDKP treatment, suggesting that the function of the glomerular filtration barrier was not improved by Ac-SDKP treatment.

Subsequent studies explored the therapeutic effect of Ac-SDKP in combination with ACE inhibitors in diabetic nephropathy. Combination treatment with Ac-SDKP (500 μg/kg/day) and the ACE inhibitor, Imidapril, in male CD1 mice with STZ-induced type I diabetes was initiated 16 weeks post-STZ injection and was maintained for 8 weeks. Combination treatment suppressed glomerular mesangial area expansion, fibrosis and reduced the ratio of αSMA-positive to CD31+ cells compared with Imidapril alone. [63] The authors explored the possibility that Ac-SDKP blocks endothelial to mesenchymal transition (EndMT), which has been proposed as a mechanism that contributes to glomerulosclerosis in early diabetic nephropathy, [64] and found evidence that Ac-SDKP inhibits EndMT of human dermal microvascular endothelial cells *in vitro*. [63] In addition, the effect of Ac-SDKP on the expression of microRNAs downregulated in mouse models of CKD and proposed to reduce fibrosis by targeting TGF-β signalling [65] [66] was explored. Treatment with Ac-SDKP alone (500 μg/kg/day for 8 weeks initiated 16 weeks post-STZ administration) [67] or Imidapril and Ac-SDKP [68] [63] restored the expression of anti-fibrotic microRNAs, miR-29 and miR-let-7, in the diabetic kidney.

The therapeutic potential of Ac-SDKP has also been explored in a rat model of nephrotoxic serum nephritis. [69] Ac-SDKP (1mg/kg/day) was administered by osmotic minipump two weeks after disease induction for one month. Ac-SDKP treatment ameliorated proteinuria, blood urea nitrogen, plasma creatinine, glomerulosclerosis and interstitial fibrosis in Ac-SDKP-treated rats compared with rats administered saline. [69] This effect was mediated by diminished TGF-β signalling in the kidney shown by reduced Smad2 phosphorylation and increased Smad7 expression. The renal expression of pro-inflammatory genes (ICAM-1, interleukin-1β, MCP-1 and tumor necrosis factor-α) was also reduced by Ac-SDKP treatment and associated with reduced macrophage accumulation in both the glomerulus and the tubulointerstitium. [69] Ac-SDKP did not alter the total number of peripheral leukocytes suggesting that the main effect of Ac-SDKP was on monocyte infiltration into the kidney.

3. Conclusion

In conclusion, the studies conducted so far indicate a protective role of endogenous thymosin-β4 in the context of experimental glomerular disease and angiotensin-II-induced renal injury. Administration of exogenous thymosin-β4 has shown promising therapeutic effects in animal models of interstitial fibrosis and diabetic kidney disease. The effects of thymosin-β4 in the kidney are at least partly mediated by its ability to modulate macrophage accumulation leading to inflammation and its anti-fibrotic effect. There is also evidence that endogenous thymosin-β4 limits the migration of podocyte cells and thus prevents their loss from the glomerular tuft, where they are critical for the integrity and function of the glomerulus. More studies are required to explore the therapeutic potential of exogenous thymosin-β4 in different types of CKD, which will strengthen the evidence for using thymosin-β4 as a novel treatment to reduce the morbidity and mortality associated

with CKD. The therapeutic potential of the thymosin-β4 derivative Ac-SDKP has been extensively assessed in a wide range of experimental models of CKD demonstrating anti-inflammatory and anti-fibrotic effects. The findings reviewed here are based on animal models and their relevance to CKD progression in human patients needs to be established in future studies.

4. Expert Opinion

There is substantial evidence supporting the therapeutic potential of exogenous Ac-SDKP to alleviate inflammation and fibrosis in experimental CKD, whilst endogenous Ac-SDKP has a protective role and acts to prevent excess collagen deposition. The anti-inflammatory and anti-fibrotic potential of Ac-SDKP has been demonstrated in animal models of interstitial renal fibrosis and immune-mediated, hypertensive and diabetes-induced glomerular disease thus indicating the effectiveness of the peptide in a wide range of experimental models and warranting further investigation towards testing as a novel treatment in CKD.

In contrast, there are still only a few studies assessing the role and therapeutic potential of thymosin-β4 in CKD and more work is needed to better define its role and therapeutic potential. The studies conducted to date report conflicting results regarding the effectiveness of thymosin-β4 in reducing macrophage accumulation and inflammation. Two studies have demonstrated that endogenous thymosin-β4 prevents macrophage accumulation at the late stage of immune-mediated glomerular disease and following angiotensin-II-induced hypertensive renal disease in mice. In contrast, administration of exogenous thymosin-β4 does not have an effect on macrophage number at either early or late stage interstitial injury in the UUO model. Further studies are required to determine if

this discrepancy is due to differences in the bioavailability of endogenous *versus* exogenous thymosin-β4. Alternatively, the differences may relate to the experimental models being used and therefore it is also necessary to investigate the effectiveness of thymosin-β4 in different types of experimental kidney disease as it has been done for Ac-SDKP. Despite the differences in the anti-inflammatory potential of thymosin-β4 in kidney disease, both endogenous and exogenous thymosin-β4 appear to have anti-fibrotic effects, as demonstrated in glomerular disease and interstitial fibrosis. An exciting recent finding has been the discovery that endogenous thymosin-β4 is important in preventing the aberrant migration of podocytes in glomerular disease. These specialised epithelial cells are central to glomerular function and are a major target of injury in glomerular disease. [11] Maintaining their function is thus an important goal in the road to discovering new treatments for glomerular disease. Future studies are needed to investigate if treatment with exogenous thymosin-β4 has the potential to maintain podocyte structure and function following injury.

The studies on thymosin-β4 have also investigated the mechanisms that mediate its effects on the kidney. Inhibition of TGF-β signalling has been shown as a central pathway that facilitates the anti-fibrotic effect of thymosin-β4 in kidney disease. However, the mechanism by which endogenous thymosin-β4 dampens inflammation in glomerular disease is not clear. Loss of endogenous thymosin-β4 did not alter macrophage infiltration in the kidney at the early stage of glomerular disease, but resulted in a sustained and amplified presence of macrophages at the late stage, which implies a role for thymosin-β4 in the resolution of inflammation. It is currently unclear whether this effect is mediated by thymosin-β4 sulfoxide, which has been shown to disperse macrophages in injury models *in vivo*. [29] Alternatively, thymosin-β4 may act to reduce inflammation by suppressing the NFκB

pathway, as has been demonstrated in the case of inflammatory eye disease. [19] The ability of thymosin-β4 to modulate podocyte motility is probably linked to its actin-binding function. Increased podocyte migration by cells lacking thymosin-β4 was associated with increased actin stress fibers and activation of the Rho-GTPase, RhoA. Modulation of the activity of Rho-GTPases results in podocyte injury and proteinuria, [70, 71] it will therefore be interesting to further investigate the interaction between thymosin-β4 and this novel downstream target. The role of thymosin-β4 in specific cell types and the mechanisms that mediate its effects should be studied further by generating mice allowing conditional deletion or overexpression of *Tmsb4x* in specific cell types.

To achieve the ultimate goal of establishing the potential benefit of thymosin-β4 as a novel therapy in CKD, further studies are needed to characterise the circulating and kidney levels of thymosin-β4 in patients with CKD, which would be essential for the design of therapeutic interventions.

Article highlights box

- Thymosin-β4 is expressed in the mouse kidney in both health and disease.
- Endogenous thymosin-β4 is dispensable in healthy mouse kidneys.
- In mouse models of immune-mediated and hypertensive renal disease, lack of endogenous thymosin-β4 exacerbates disease progression.
- The protective role of thymosin-β4 is associated with the modulation of podocyte migration, inflammation and fibrosis.
- Treatment with exogenous thymosin-β4 has demonstrated therapeutic benefits in mouse models of interstitial fibrosis and diabetic nephropathy.

- Ac-SDKP treatment is beneficial in a number of experimental models of renal disease including interstitial fibrosis and glomerular disease induced by hypertension, diabetes or inflammation.

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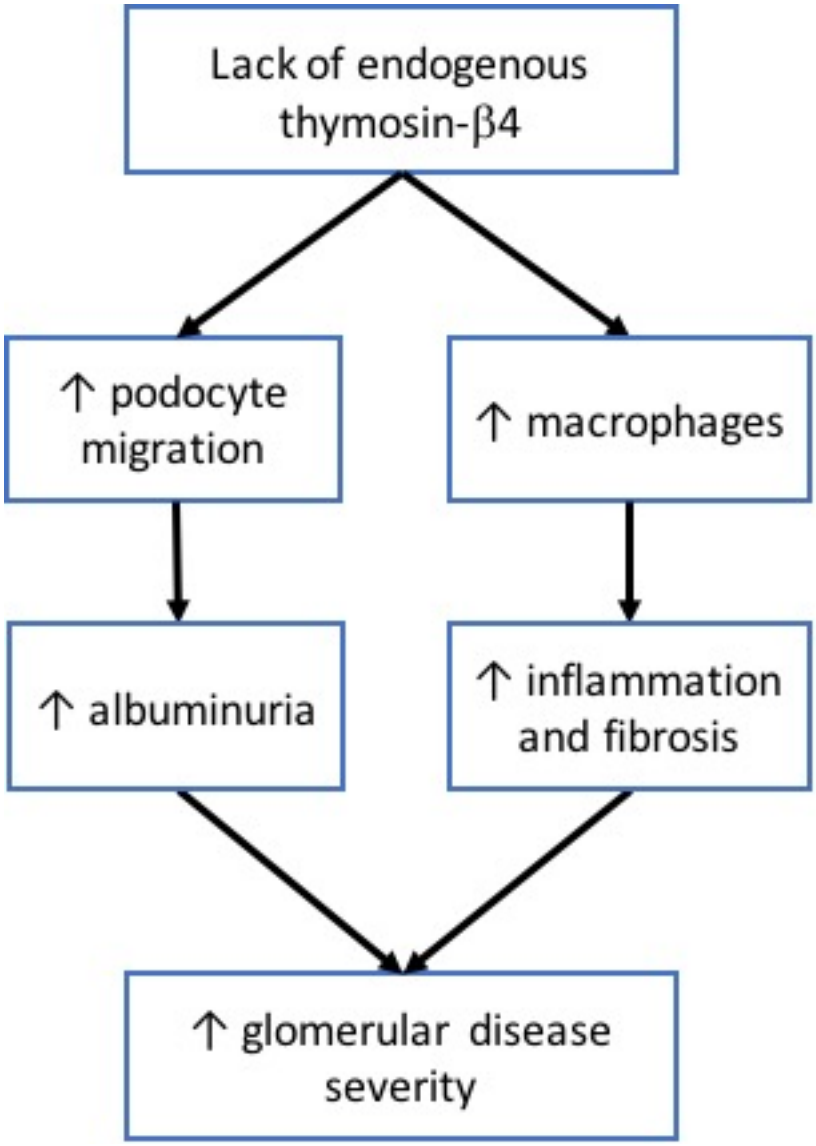
Figure Legends

Figure 1. The role of thymosin-β4 in glomerular disease progression. Lack of endogenous thymosin-β4 in mice with glomerular disease results in (a) increased podocyte migration and increased albuminuria (b) increased accumulation of macrophages surrounding the glomerulus leading to increased inflammation and fibrosis.


Lack of endogenous thymosin-β4
↑ podocyte migration
↑ macrophages
↑ albuminuria
↑ inflammation and fibrosis
↑ glomerular disease severity