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REVIEW ARTICLE

N-acetyl-seryl-aspartyl-lysyl-proline is a valuable endogenous antifibrotic peptide for kidney fibrosis in diabetes: An update and translational aspects

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ABSTRACT

N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous peptide that has been confirmed to show excellent organ-protective effects. Even though originally discovered as a modulator of hemotopoietic stem cells, during the recent two decades, AcSDKP has been recognized as valuable antifibrotic peptide. The antifibrotic mechanism of AcSDKP is not yet clear; we have established that AcSDKP could target endothelial–mesenchymal transition program through the induction of the endothelial fibroblast growth factor receptor signaling pathway. Also, recent reports suggested the clinical significance of AcSDKP. The aim of this review was to update recent advances of the mechanistic action of AcSDKP and discuss translational research aspects.

INTRODUCTION

With the recent type 2 diabetes pandemic, the number of people with diabetic complications could increase in the future. Diabetic kidney disease, such as diabetic nephropathy or other kidney insults, is the major cause of end-stage renal disease and renal replacement therapy. Kidney fibrosis, which is characterized by matrix deposition and glomerulosclerosis, is the final pathway of progressive kidney diseases. Fundamentally, organ fibrosis is an important tissue repair process; progressive kidney fibrosis might be due to disruption of the normal wound healing mechanisms[1,2]. The primary factors in the fibrosis pathway; for example, cellular induction of fibrosis or production of excess extracellular matrix (ECM), are difficult to identify. All cell types, either resident or non-resident, in the kidney, such as resident fibroblasts, tubular epithelial cells, pericytes, endothelial cells, vascular smooth muscle cells, mesangial cells, podocytes and migrated bone marrow derived cells or inflammatory cells, are involved in this process[3,4]. All these cell types could play vital roles in kidney fibrosis. Additionally, the conversion programs of cells from an epithelial or endothelial phenotype to a mesenchymal phenotype through epithelial–mesenchymal transition (EMT) and endothelial–mesenchymal transition (EndMT) could promote the generation of matrix-producing mesenchymal-like cells[4,5]. However, there has been intensive discussion about the contribution or even the presence of EMT or EndMT. Most likely, the complete form of EMT or EndMT would make a minor contribution, if any; the presence of partial EMT or EndMT, the transition phase of epithelial/endothelial cells into mesenchymal cells, would likely have a major impact, because mesenchymal marker expression is common in either epithelial or endothelial cells in fibrotic kidney biopsies[6–8]. Therefore, targeting these mesenchymal programs is essential when considering therapeutic options for kidney fibrosis in diabetes. Diabetes is associated with endothelial damage induced by various insults, such as high glucose, hyperinsulinemia, oxidative stress and transforming growth factor (TGF)-β. The present author and co-authors have investigated endothelial damage and the EndMT program among the many potential mesenchymal programs in diabetic kidneys.

N-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) is an endogenous peptide that has antifibrotic effects. The suppressive effects of AcSDKP on EndMT have been intensively analyzed. In this

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review, AcSDKP as a therapeutic option for diabetic kidney disease is focused on, especially the recent advances in endothelial protection induced by AcSDKP.

ACSDKP SYNTHESIS

AcSDKP is a tetrapeptide isolated from fetal calf bone marrow[9], and studies have shown its strong antifibrotic properties in various organs of multiple disease models. Although the molecular pathway of endogenous AcSDKP synthesis has not been fully elucidated, thymosin β4 (Tβ4), a globular-actin-sequestering peptide, is the strongest candidate for the AcSDKP precursor (Figure 1)[10,11]. Small interfering ribonucleic acid (RNA) knockdown of Tβ4 in HeLa cells resulted in significant suppression of AcSDKP levels[11]. Elegantly, Lenfant *et al.*[10] showed that [^{3}H] AcSDKP was produced when radiolabeled [^{3}H] Tβ4 was incubated with either bone marrow cells or bone marrow lysate. AcSDKP is the N-terminal sequence of Tβ4 (Figure 1), and a subsequent study showed that prolyl oligopeptidase (POP; in some studies described as prolyl endopeptidase) is responsible for Tβ4-mediated AcSDKP production (Figure 1)[12]. Interestingly, POP has shown to be suppressed in patients with relapsing–emitting multiple sclerosis[13].

The Tβ4 peptide also shows antifibrotic and tissue-protective effects. Tβ4 is a 43 amino acid peptide (4.9 kDa) that sequesters globular-actin and regulates polymerization of F-actin[14,15]. Tβ4 is ubiquitously expressed in various organs[14,15]. Tβ4 administration through either the intracardiac or the intraperitoneal route significantly rescued cardiac function and promoted neovascularization in a mouse model of experimental myocardial infarction[16]. Tβ4 also induced epicardial progenitor cell mobilization[17]. These organ-protective effects of Tβ4 are primarily mediated by the synthesis of AcSDKP[18,19]. In addition to Tβ4, Tβ15, which has the AcSDKP peptide sequence, might also contribute to the genesis of AcSDKP[20,21]. The Tβ15 concentration is believed to be low compared with that of Tβ4 (~1,000-fold lower); Tβ15 levels could be induced in some disease states, such as cancer[20,21]. Interestingly, although the levels of AcSDKP were mostly suppressed, they were not completely abolished in mice with Tβ4 deficiency in the kidney and heart. These results might indicate that peptides homologous to Tβ4 (such as Tβ15) could compensate for the absence of Tβ4[22]; however, caution is required in interpreting these data, because the AcSDKP level in the organs was determined with enzyme immunoassays for plasma/serum or urine, and such assays often show false positive values.

In the generation process of AcSDKP from Tβ4, recent evidence suggested the requirement of additional enzymatic cleavage processing. Kumar *et al.*[23] focused on the biology of POP. POP can cleave the peptide that is shorter than the sequence of 30 amino acids. However, Tβ4 is indeed 43 amino acids; therefore, theoretically, an additional enzymatic cleavage process should be required. Kumar *et al.*[23] clearly showed that meprin-α is a potential responsible enzyme to cleave Tβ4 and subsequently enable POP to cleave Tβ4 fragments to synthesize AcSDKP. Indeed, meprin-α knockout mice showed significantly lower levels of AcSDKP. In regard to this, exogenous-derived

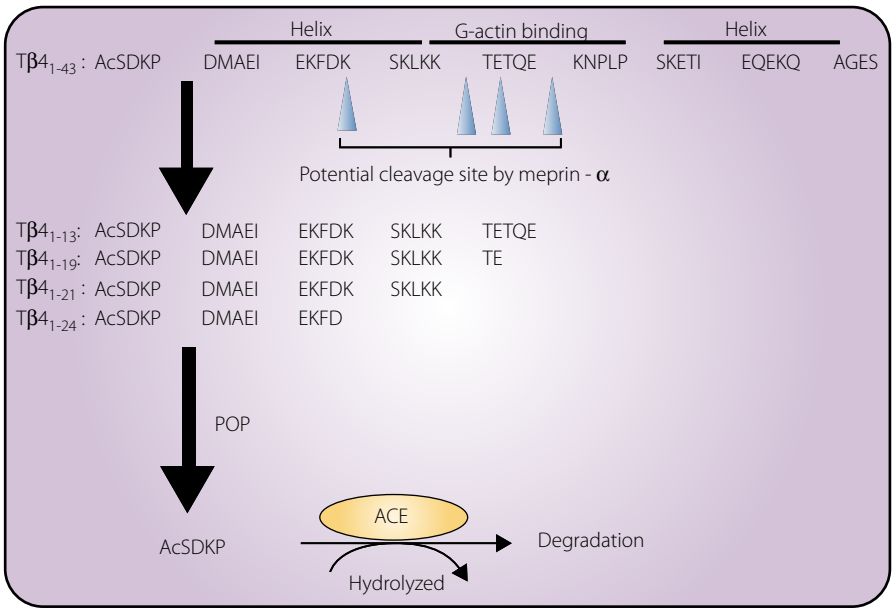

Figure 1 | Generation of *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP) from precursor polypeptide thymosin β4 (Tβ4). Tβ4 involves the amino acid sequence AcSDKP in the N-terminal. Maprin-α digested Tβ4 peptide into shorter sequence which can be cleaved by prolyl oligopeptidase (POP). In the second step, POP cleaves the AcSDKP sequence containing a shorter fragment of Tβ4 (1–13~24), and AcSDKP is excised out. Generated AcSDKP is degraded by angiotensin-converting enzyme (ACE).

Tβ4-increased AcSDKP levels *in vivo* were diminished by actinonin, the potential meprin-α inhibitor[23]. These results suggested two-step generation of AcSDKP from Tβ4 through meprin-α and then POP (Figure 1).

ACSDKP DEGRADATION AND THE ROLE OF ANGIOTENSIN-CONVERTING ENZYME

Angiotensin-converting enzyme (ACE) was confirmed to hydrolyze AcSDKP (Figures 1,2). AcSDKP is maintained at a low level in the plasma, and the AcSDKP concentration increased fivefold after captopril administration[24]. ACE has N-terminus (ACE^N) and C-terminus (ACE^C) zinc-binding catalytic domains that are responsible for the cleavage of target substrates[25,26]. The amino acid homology between the ACE^N and ACE^C domain is approximately 60%; approximately 89% homology can be observed in the catalytic regions[26]. The *ACE* gene in higher organisms is believed to be the result of the ancient gene duplication[27], and the resultant ACE, which has two catalytic sites, is found in many organs and cells (somatic ACE). In contrast, an ACE composed of only the ACE^C without the ACE^N is only found in the testis, and this testis ACE is vital for fertility[28–30]. The testis ACE is likely the primitive type of ACE[26]. This duplication of the *ACE* gene occurred in early evolution, and separate promoter regions regulate the different enzyme expression levels (Figure 2)[27,31,32]. These similar, but distinct, catalytic domains were described in detail in a previous report[33].

Only the ACE^N is contributing AcSDKP hydrolysis (Figure 2). In fact, the testis (where the germinal-type ACE without the ACE^N is expressed) is known to have higher levels of AcSDKP than other organs[34,35]. A seminal report from Li *et al.*[36] showed that ACE^N-knockout mice, which showed high levels of AcSDKP, had significantly less bleomycin-induced lung fibrosis, as shown by lung histology and hydroxyproline levels, than ACE^C knockout mice, suggesting the vital role of the ACE^N in AcSDKP degradation. Furthermore, they investigated whether S-17092, a POP inhibitor, could decrease the lung-protective effects in ACE^N knockout mice, and as expected, S-17092 abolished lung protection in these mice. AcSDKP suppressed bleomycin-induced lung fibrosis in wild-type mice. Overall, that study showed that physiological elevation of AcSDKP through inhibition of the ACE^N leads to endogenous antifibrosis programs in the lungs[36].

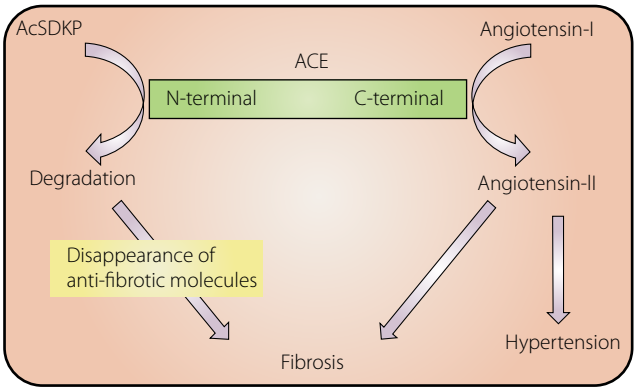

Figure 2 | The role of the distinct two catalytic sites of angiotensin-converting enzyme (ACE) in tissue fibrosis. The C-terminal catalytic site of ACE shows a higher affinity to angiotensin I. In contrast, the N-terminal catalytic site of ACE shows a high affinity to *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP), and AcSDKP could be only degraded by the N-terminal catalytic site.

KIDNEY FIBROSIS

After kidney insults, profibrotic cytokines accumulate in the microenvironment and subsequently stimulate target cell activation, producing ECM, which is fundamental for renal fibrogenesis. The majority of matrix-producing cells responsible for the production of interstitial matrix components (including fibronectin and type I and type III collagens) are fibroblasts[37]. Activated fibroblasts or myofibroblasts, which are characterized by increased expression of alpha smooth muscle actin (αSMA), are believed to be a significant source of ECM-producing renal cells. However, almost all cell types in the kidney (either resident or non-resident kidney cells) play a vital role in ECM production[38]. As described earlier, these cell types develop a profibrotic phenotype, and TGF-β, a profibrotic cytokine that plays a central role in fibrogenesis, has an essential role in this process. Thus, inhibition of either TGF-β or the TGF-β-stimulated Smad transcription factor signaling pathway shows antifibrotic effects[39]. Activated fibroblasts in the kidney express αSMA and are often called myofibroblasts, which show unique contractile properties[37]. However, αSMA expression was observed not only in fibroblasts, but also in tubular cells[6] and endothelial cells[8], suggesting that these cells could contribute to kidney fibrosis through EMT or EndMT program. Although the presence of these programs is controversial, at least in diabetic models, these processes are most likely important in kidney fibrosis. Interestingly, most studies did not detect EMT or EndMT in unilateral ureteral obstruction or ischemic reperfusion injury models. Unilateral ureteral obstruction is often utilized in kidney fibrosis studies, but the relationship of this model to human kidney disease is unclear. Differences are observed in the progression time and the background physiological condition. In diabetic models, EMT or EndMT has been detected (>150 papers on EMT and 20 papers on EndMT). Therefore, these programs should still be credible to be investigated in kidney fibrosis in diabetes.

ANTIFIBROTIC EFFECTS OF ACSDKP

AcSDKP has been confirmed to show antifibrotic organ-protective effects in diverse preclinical models[18,40–45]. In the first study of *db*/*db* mice[42], the present author and co-authors showed the preclinical utility of AcSDKP in protection against diabetic kidney disease, with a focus on kidney fibrosis.

Although various studies have shown the apparent antifibrotic effects *in vivo* and the direct effects of AcSDKP on culture fibroblasts *in vitro*, the effects of AcSDKP on fibroblast activation/myofibroblast differentiation programs are still unclear. Peng *et al.*[46] reported that AcSDKP inhibited TGF-β1-induced differentiation of human cardiac fibroblasts into myofibroblasts, as shown by increased expression of αSMA and the embryonic isoform of smooth muscle myosin. Xu *et al.*[47] reported that AcSDKP inhibits myofibroblast accumulation in silicotic nodules in the lung and the TGF-β1-induced myofibroblast differentiation of pulmonary fibroblasts. In a study of the AcSDKP-inhibited myofibroblast differentiation program, the present author and co-authors focused on the anti-EndMT effects of AcSDKP associated with TGF-β-Smad signal transduction[33,48–51].

More than 15 years ago, the present author and co-authors found that AcSDKP inhibited TGF-β-induced Smad2 phosphorylation, and the anti-TGF-β/Smad pathway is the key to understanding the antifibrotic effects of AcSDKP in rat cardiac fibroblasts and human mesangial cells[52,53]. Indeed, AcSDKP is the first endogenous circulatory polypeptide shown to inhibit TGF-β-induced Smad2 phosphorylation (only a Smad2 phosphorylation antibody was available at this time). Smads are TGF-β superfamily-specific transcription factors and are essential players in signal transduction[39,54–56]. Smad transcription factors are categorized as follows: (i) receptor-regulated (R)-Smads (Smad2 and 3); (ii) common (co)-Smads (Smad4); and (iii) inhibitory (I)-Smads (Smad6 and 7). After TGF-β binding to the type II receptor, this receptor physically interacts with and subsequently induces serine residue phosphorylation on the type I receptor (TGF-βRI)[57]. The phosphorylated type I receptor interacts with and phosphorylates R-Smads, and phosphorylated R-Smads subsequently interact with co-Smads and translocate into the nucleus with the help of importin-β[58]. The R-co-Smad heterodimer binds to the Smad-binding elements of the target promoter deoxyribonucleic acid regions, whereas I-Smads localize to the nucleus[53] and translocate to the cytoplasm from the nucleus after stimulation with TGF-β to inhibit R-Smad phosphorylation by type I receptors.

The precise molecular mechanisms by which AcSDKP suppresses TGF-β-induced R-Smad phosphorylation are still unclear; I-Smads could have a role in this process. The present author and co-authors first reported that the I-Smad Smad7 was translocated after AcSDKP incubation in human mesangial cells,[53] and several groups have shown that the Smad7 levels were indeed elevated by AcSDKP administration *in vivo*[43]. However, alternative molecular mechanisms by which AcSDKP-inhibited R-Smad phosphorylation could be linked to fibroblast growth factor (FGF) receptor (FGFR)[49] and FGFR-associated induction of the microRNA (miRNA) let-7-mediated suppression of TGF-β1[59].

FGFR1

Fibroblast growth factor signaling, mediated by FGFRs, has an important role in maintaining endothelial homeostasis and cardiovascular integrity[60–63]. The FGFR family, a subfamily of receptor tyrosine kinases, consists of four members (FGFR1, FGFR2, FGFR3 and FGFR4)[64]. Chen *et al.*[59] reported that FGF/FGFR1 signaling suppressed TGF-β-induced EndMT through inducing the miRNA let-7. The present author and co-authors showed that a fibrotic strain of diabetic mice displayed suppression of FGFR1 on endothelial cells, and that AcSDKP restored the levels of endothelial FGFR1 in these mice[49]. FGFR1 is a fundamental molecule that inhibits the TGF-β/Smad signaling pathway in endothelial cells[65]. Additionally, a key adaptor of the FGFR signaling pathway, FGF receptor substrate 2, has been shown to suppress EndMT induction, and endothelial-specific FGF receptor substrate 2 knockout mice showed increased levels of EndMT- and EndMT-derived smooth muscle cells[59]. A deficiency in mesodermal FGF receptor substrate 2 resulted in misalignment and hypoplasia of the cardiac outflow tract[66]. FGFR1 also plays an essential role in cardiomyocyte development and cardiac chamber identity in the developing ventricle[67,68].

EFFECT OF ACSDKP ON THE FGFR1-MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE KINASE 4 PATHWAY

Mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4), a member of the Sterile 20 family of kinases, has various biological roles[69]. In endothelial cells, MAP4K4 has been shown to suppress integrin β1 activity by inducing moesin[70]. Integrin β1 stimulates EMT and organ fibrosis through TGF-β/Smad signaling activation[71–73]. The present author and co-authors showed that the interaction between dipeptidyl peptidase (DPP)-4 and integrin β1 induces EndMT, and that this interaction disrupts endothelial homeostasis[74]. Misshapen (msn) kinases (the mammalian orthologs of MAP4K4) have been shown to suppress TGF-β/Smad signaling[75].

We found that AcSDKP restored the TGF-β2-suppressed levels of FGFR1 on endothelial cells, and that the FGFR1 levels were associated with MAP4K4 phosphorylation (Figure 3). AcSDKP was detected in close proximity to FGFR1. TGF-β2 suppressed FGFR1, and AcSDKP restored the FGFR1 levels in association with the AcSDKP–FGFR1 interaction (Figure 3); however, mutant peptides, such as AcDSPK, AcSDKA and AcADKP, did not alter the levels of FGFR1[50]. FGFR1 physically interacted with phosphorylated MAP4K4, and neutralizing FGFR1 abolished the AcSDKP-induced MAP4K4 phosphorylation (Figure 3). Additionally, knockdown of MAP4K4 in endothelial cells abolished the anti-EndMT and anti-TGF-β1/Smad effects of AcSDKP. Furthermore, the hearts of diabetic mice showed suppressed levels of both FGFR1 and MAP4K4 associated with Smad3 phosphorylation on endothelial cells; AcSDKP intervention normalized all of these parameters[50]. All

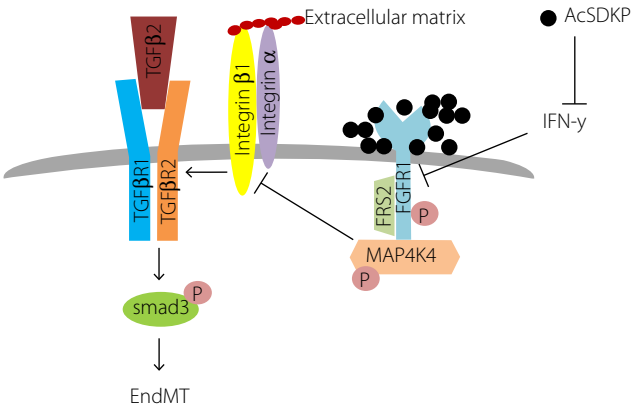

Figure 3 | *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP)–fibroblast growth factor receptor 1 (FGFR1)–mitogen-activated protein kinase kinase kinase kinase 4 (MAP4K4) axis-induced suppression of integrin–transforming growth factor-β receptors (TGFβRs) interaction and endothelial mesenchymal transition (EndMT). AcSDKP shows close proximity and would stabilize FGFR1. Alternatively, AcSDKP suppresses interferon-γ (IFN-γ), which inhibits FGFR1 levels in endothelial cells. FGFR1 physically interacts with MAP4K4 by which integrin β1 signaling is suppressed. Integrin β1 is essential for the heterodimer formations of TGFβRs; integrin β1 suppression directly results in the suppression of TGF-β1-smad signaling and EndMT.

these data showed that the FGFR1–MAP4K4 pathway is involved in the anti-EndMT effects of AcSDKP (Figure 3).

β-KLOTHO

Klotho proteins are type I single-pass transmembrane proteins that share homology with family 1 β-glycosidases, and are composed of α-klotho (KLA)[76], β-klotho (KLB)[77] and γ-klotho[78]. Klotho proteins consist of short intracellular and relatively large extracellular domains with two internal repeats, termed KL1 and KL2[79]. Klotho proteins act as coreceptors of FGFRs, facilitating the binding of the FGF19 subfamily on FGFRs (e.g., KLA for FGF23 and KLB for FGF19/FGF21)[80,81], and have emerged as essential metabolism-regulating factors through their interactions during the past decades[82–88].

KLA was shown to have anti-aging effects[76], and the functions of KLA in endothelial cells and the cardiovascular system have been reported[89–92]. However, the biological significance of KLB is still unclear. KLB is expressed in human umbilical vein endothelial cells[93] and human brain microvascular endothelial cells[94], where KLB contributes to the formation of the blood–brain barrier.

We have reported that FGFR1 deficiency induced activation of TGF-β/Smad3 signaling[50]; however, KLB knockdown was not associated with an obvious alteration of Smad3 phosphorylation[51]. As KLB knockdown did not directly alter the FGFR1 levels, FGFR1 could be essential for the regulation of Smad3 phosphorylation. In contrast, the mitogen-activated protein kinase signaling pathways involving mitogen-activated protein kinase kinase (MEK)1/2 and extracellular signal-regulated kinases 1/2 were activated[51]. Consistent with this finding, a MEK inhibitor decreased KLB deficiency-induced EndMT. Liu *et al.*[95] reported that KLB overexpression inhibited EMT, the epithelial program sharing similar molecular mechanisms to EndMT, through the suppression of mitogen-activated protein kinase. Unexpectedly, the KLB-deficient activated mitogen-activated protein kinase pathway without Smad3 phosphorylation is sufficient for the induction of EndMT. Either FGF19 or FGF21, a ligand of the FGFR1–KLB complex, synergistically diminished EndMT and MEK–extracellular signal-regulated kinase pathway activation in AcSDKP-treated cultured endothelial cells (Figure 4)[51]. Inhibition of FGFR1 and KLB protein levels was found in streptozotocin-induced diabetic mouse hearts; AcSDKP restored these levels, suggesting the important *in vivo* effects of AcSDKP on FGFR1 and KLB complex formation[51].These reports showed the essential role of the FGFR1-KLB complex in the suppression of EndMT by AcSDKP, but the qualitative difference between Smad-dependent EndMT and Smad-independent, mitogen-activated protein kinase-dependent EndMT should be further investigated.

MIRNA CROSS-TALK

As described earlier, AcSDKP-induced FGFR1 is associated with the anti-EndMT miRNA let-7 that targets TGF-βRI; miRNA let-7 induction through the AcSDKP–FGFR1 axis played further roles in endothelial protection through inducing miRNA-29[8,96,97] and vice versa (Figure 5).

In a follow-up study, the present authors and co-authors found that AcSDKP-induced let-7 was associated with miRNA 29, and miRNA 29 expression was abolished by an antagomir for miRNA let-7, suggesting antifibrotic cross-talk between miRNA let-7 and miRNA 29[48]. This conclusion is reasonable, because activation of TGF-β signaling significantly suppresses miRNA 29, and the induction of miRNA let-7 by miRNA 29 in endothelial cells is a reasonable sequence (Figure 5). Interestingly, miRNA 29 induction also suppressed EndMT, and was associated with the induction of miRNA let-7[48]. In this complex process, the present author and co-authors identified the precise molecular mechanisms underlying the miRNA cross-talk. MiRNA 29 targets several profibrotic molecules, such as integrin β1 and DPP-4[48]. Integrin β1 and DPP-4 form a complex on the cell surface of endothelial cells, and induce TGF-β receptor heterodimer formation and subsequent activation of the Smad signaling pathway[74]. TGF-β-induced EndMT was also inhibited by the DPP-4 inhibitor linagliptin, which further restored miRNA 29. The induction of miRNA 29 is likely important for the antifibrotic effects of AcSDKP. MiRNA 29 also targets interferon-γ, a cytokine related to inflammation and organ fibrosis. Interferon-γ can inhibit endothelial FGFR1, the key mediator of miRNA let-7 induction and the suppression of EndMT by AcSDKP[59]. The present author and co-authors showed that the AcSDKP–FGFR1 axis was critical for maintaining endothelial

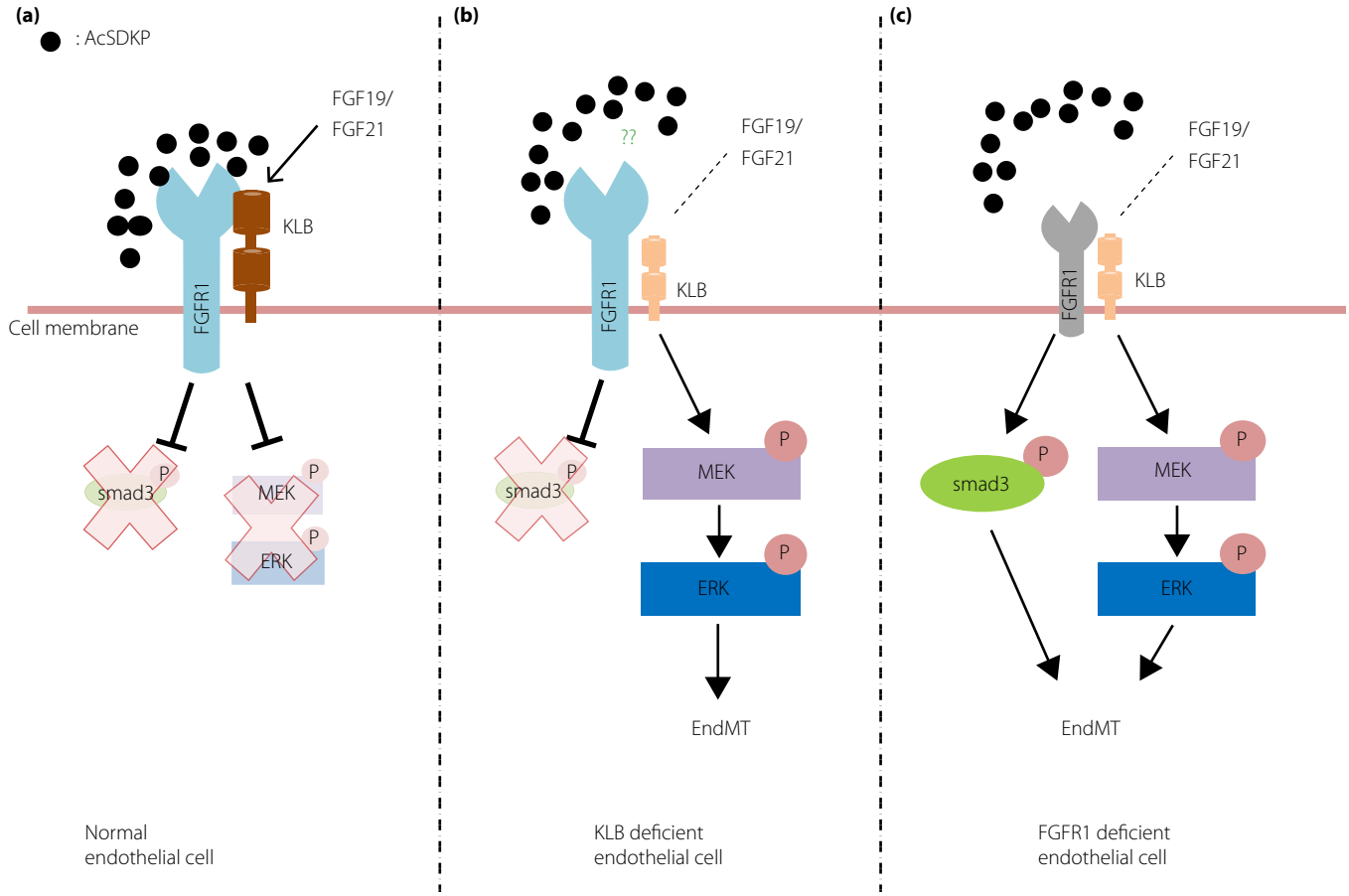

Figure 4 | Fibroblast growth factor receptor 1 (FGFR1)–β klotho complex plays roles in endothelial homeostasis. (a) In normal endothelial cells, *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP)-induced FGFR1 levels and FGFR1–KLB complex. Such FGFR1–KLB complex is essential for the endothelial homeostasis by inhibiting both smad3 and mitogen-activated protein kinase kinase (MEK)–extracellular signal-regulated kinases (ERK) signaling pathway. (b) In the endothelial cells with KLB deficiency, MEK–ERK pathway dependent EndMT could be induced. KLB deficiency is not associated with alteration in FGFR1 levels in endothelial cells. AcSDKP cannot inhibit MEK–ERK pathway-dependent EndMT in KLB-deficient endothelial cells. (c) In the endothelial cells with FGFR1 deficiency, KLB levels are also significantly diminished, and both smad3 and the MEK–ERK pathway are activated. FGFR1-deficient endothelial cells also show AcSDKP-resistant endothelial mesenchymal transition (EndMT).

mitochondrial biogenesis through induction of miRNA let-7b-5p[98]. These data showed that the AcSDKP–FGFR1 axis on endothelial cells is essential for endothelial homeostasis through the cross-talk between miRNA let-7 and miRNA 29 (Figure 5).

PERSPECTIVE: TRANSLATIONAL RESEARCH OF ACSDKP

As described earlier, AcSDKP has the potential to combat fibroproliferative diseases, such as kidney complications in diabetes. Although AcSDKP has not yet been translated into the clinic, recent data have shown that AcSDKP could be a useful clinical biomarker in some disease conditions.

Sodium intake is a known detrimental factor in the clinical outcome of renal diseases[99]. Kwakernaak *et al.*[100] examined the potential relevance of AcSDKP in renal protection. The researchers enrolled 46 non-diabetic chronic kidney disease patients with overt proteinuria who showed mild-to-moderate renal insufficiency. In a cross-over design/double-blind analysis for a 6-week study period with a regular or a low-sodium diet and either lisinopril or lisinopril plus valsartan, sodium restriction was confirmed to increase the plasma level of AcSDKP during either single or dual renin–angiotensin system blockade[100]. Interestingly, a recent report utilizing mice with knockout of the N-terminal sequence of ACE, which resulted in AcSDKP degradation, found that they showed increased levels of AcSDKP associated with enhanced urine excretion of sodium without alterations in renal angiotensin II levels[101]. The molecular regulation and physiological relevance of the interaction of AcSDKP levels and sodium intake/excretion have not yet been elucidated, and further research is required.

Finally, the present author recently reported that the urine levels of AcSDKP could be a potential biomarker of alteration of renal function in normoalbuminuric diabetes patients with an estimated glomerular filtration rate (eGFR) ≥30 mL/min/1.73 m^2[102]. When compared with that observed two decades

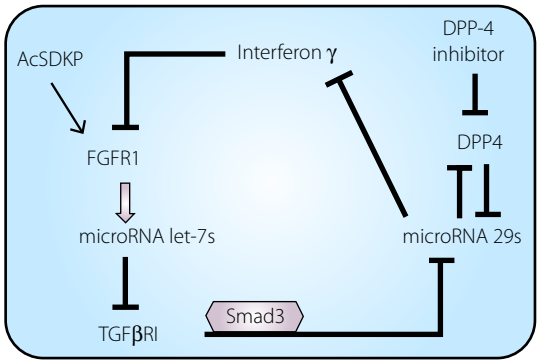

Figure 5 | Micro ribonucleic acid (miRNA) cross-talk between miR 29 and miR let-7 in the anti-endothelial mesenchymal transition action of *N*-acetyl-seryl-aspartyl-lysyl-proline (AcSDKP). AcSDKP increases the levels of fibroblast growth factor receptor 1 (FGFR1) and, subsequently, miR let-7s are induced. MiR let-7 suppressed transforming growth factor β receptor (TGFβR1) and downstream Smad3 signaling, the major miR 29 inhibitory pathway. Therefore, the cells treated with AcSDKP also show induction of miR 29. miR 29 suppressed profibrotic and inflammatory cytokines and proteins, such as interferon-γ, dipeptidyl peptidase-4(DPP-4) and so on. Interferon-γ is known to suppress FGFR1. Interestingly, DPP-4 inhibitor-mediated suppression of DPP-4 subsequently induced miR 29, and such DPP-4 inhibitor-activated miR 29 induced FGFR1-dependent miR let-7, as vice versa.
[Correction added on 1 April 2020, after first online publication: The figure has been amended to match the original.]

ago, the diabetes population with renal deficiency without urine albuminuria has increased[103,104]. The explanation for these changes is unknown, but evidence-based treatment of diabetes patients (such as appropriate blood glucose control and renin–angiotensin system blockade) could result in the reduction of urine albumin with a decrease in eGFR[103,104]. Urine albumin-negative diabetes patients rarely show a progressive decline in renal function, few, but some, can progress to end-stage renal disease[104]. Most importantly, there is no established biomarker for this urine albumin-negative diabetes population. The present author and co-authors have already shown that CD-1 mice, a fibrotic strain with diabetes, displayed a diabetes-associated decline in urine AcSDKP levels[48].

Based on this background, the present author and co-authors hypothesized that the urine AcSDKP-to-urine creatine ratio can predict kidney injury and future renal function. A total of 21 diabetes patients with normoalbuminuria and an eGFR ≥ 30 mL/min/1.73 m^2 were divided into two groups based on the median values: low or high urinary AcSDKP groups (uAcSDKP/Cr^{low} or uAcSDKP/Cr^{high}) in the first morning urine and follow up at ~4 years to monitor eGFR. In the uAcSDKP/Cr^{high} group, the alteration in eGFR ($\Delta eGFR^{op}$ [ΔeGFR observational periods]) showed significant stability compared with that of the uAcSDKP/Cr^{low} group over time ($P = 0.003$, $\chi^2 = 8.58$). Levels of the urine kidney injury molecule-1 (uKim-1), which is known to increase in the injured kidney, were also measured, and in the low uKim-1 group, $\Delta eGFR^{op}$ showed stable kidney function over time compared with that of the high uKim-1 group ($P = 0.004$, $\chi^2 = 8.38$). There was no significant interaction between the levels of AcSDKP and Kim-1. Patients who had both uAcSDKP/Cr^{high} and uKim-1^{low} were much more likely to show stable $\Delta eGFR^{op}$ ($P < 0.001$, $\chi^2 = 30.4$) than other patients. The plasma AcSDKP level ($P = 0.015$, $\chi^2 = 5.94$) can also weakly, but significantly, predict $\Delta eGFR^{op}$. As kidney tubulointerstitial fibrosis is a major determinant of kidney dysfunction, the antifibrotic peptide, AcSDKP, could be a functional biomarker for kidney injury and a future decline in renal function[102].

CONCLUSION

A therapeutic strategy with appropriate molecular biomarkers is essential to prevent kidney disease progression in diabetes. Fibrosis is a pathological process that destroys normal parenchyma; therefore, an antifibrotic strategy based on the mechanisms underlying kidney fibrogenesis could help to inhibit diabetic kidney disease progression. From this point of view, further elucidation of the potential of AcSDKP should be carried out. AcSDKP could be valuable, as both a therapeutic treatment and molecular biomarker in kidney disease, especially for diabetes patients.

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DISCLOSURE

KK is an advisory board member of Boehringer Ingelheim and collaborates with a study on a similar topic contained in this manuscript.

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