Hibiscus sabdariffa Polyphenolic Extract Inhibits Hyperglycemia, Hyperlipidemia, and Glycation-Oxidative Stress while Improving Insulin Resistance

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Hibiscus sabdariffa Polyphenols Alleviate Insulin Resistance and Renal Epithelial to Mesenchymal Transition: A Novel Action Mechanism Mediated by Type 4 Dipeptidyl Peptidase

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Supporting Information

ABSTRACT: The epithelial to mesenchymal transition (EMT) is important in renal fibrosis. Ser307 phosphorylation of insulin receptor substrate-1 (IRS-1 (S307)) is a hallmark of insulin resistance. We report that polyphenol extracts of *Hibiscus sabdariffa* (HPE) ameliorate diabetic nephropathy and EMT. Recently it has been observed that type 4 dipeptidyl peptidase (DPP-4) inhibitor linagliptin is effective for treating type 2 diabetes and albuminuria. We investigated if DPP-4 and insulin resistance are involved in renal EMT and explored the role of HPE. In high glucose-stimulated tubular cells, HPE, like linagliptin, inhibited DPP-4 activation, thereby regulating vimentin (EMT marker) and IRS-1 (S307). IRS-1 knockdown revealed its essential role in mediating downstream EMT. In type 2 diabetic rats, pIRS-1 (S307) abundantly surrounds the tubular region, with increased vimentin in kidney. Both the expressions were reduced by HPE. In conclusion, HPE exerts effects similar to those of linagliptin, which improves insulin resistance and EMT, and could be an adjuvant to prevent diabetic nephropathy.

KEYWORDS: *diabetic nephropathy, epithelial to mesenchymal transition, insulin resistance, polyphenol extracts of Hibiscus sabdariffa, type 4 dipeptidyl peptidase*

INTRODUCTION

Renal fibrosis, characterized by abnormal accumulation of extracellular matrix in glomeruli and tubulointerstitium,[1,2] is a common feature occurring in chronic kidney disease.[3] Although the expanded interstitial fibroblasts result in fibrosis,[4] emerging evidence suggests that epithelial to mesenchymal transition (EMT) of renal tubular epithelial cells, with increasing vimentin, plays a critical role in renal fibrosis. In terms of the risk factors, diabetes mellitus is the major cause of chronic kidney disease, and its incidence and impact are continually increasing worldwide.[5]

Hyperglycemia and hypertension are generally considered as the critical factors in diabetic nephropathy. However, despite careful control of these parameters, renal damage continues to progress in most patients. The key pathogenic mechanism involved in the induction and progression of diabetic nephropathy still remains active and unmodified by the present therapies.[5] Once renal function begins to deteriorate, there is concern about the dosage of most antidiabetic drugs since the metabolic and pharmacological controls become more difficult and complicated. Hence, new treatment paradigms are needed urgently.[6]

It is noteworthy that type 2 diabetes, characterized with insulin resistance as a result of insulin signaling defects, is indeed the most prevalent form of diabetes. Insulin action involves a series of signaling cascades initiated by binding to its receptor, eliciting tyrosine phosphorylation of insulin receptor substrates, and leading to activation of phosphatidylinositol 3-kinase (PI3K), Akt (also known as protein kinase B), and the downstream mediator.[7] However, high glucose was shown to increase the phosphorylation of Ser307 of insulin receptor substrate-1 (IRS-1 (S307)), and thus hinder the response signals, and glucose utilization.[8] Dysregulation of IRS-1 (S307) was also found in TNF- and PMA-stimulated human embryonic kidney cells, while overcome by therapeutics improving insulin sensitivity.[9]

Inhibitors of type 4 dipeptidyl peptidase (DPP-4) have recently emerged as a useful tool for treatment of type 2 diabetes mellitus. Its mode of action is based on inhibiting the degradation of type 1 glucagon-like peptide, an incretin that

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stimulates glucose-dependent insulin secretion from pancreatic beta-cells and suppresses glucagon release from alpha-cells, thereby improving glucose control.[10] Recent observations suggested the direct protective role of DPP-4 inhibitor beyond its glucose-lowering potential. DPP-4 inhibition improved albumin excretion and associated renal parameters in streptozotocin (STZ)-induced diabetic animals, while the effects were independent of blood glucose or systolic blood pressure.[11,12] In the clinical trial, DPP-4 inhibitors lower the albuminuria in type 2 diabetic patients, independent of the level of HbA1C.[13] Among the approved DPP-4 inhibitors, the excretion of linagliptin in the urine is particularly low, which may be a beneficial property in diabetic patients suffering from renal impairment.

Hibiscus sabdariffa, whose calyces are used as a daily drink in North Africa and Middle and South Asia, was shown to be bioeffective in many ways.[14,15] At least 18 phenolic compounds were found in the polyphenol extracts of *Hibiscus sabdariffa* L. calyx (HPE).[16] Our previous publications have shown the antidiabetic potential of HPE.[16,17] Using the type 2 diabetic rats exhibiting insulin resistance,[18] we demonstrated that HPE has a benefit in diabetic nephropathy by inhibiting albuminuria and hyperfiltration, and reversing the renal fibrosis and associated biomarkers.[19] Recently, we reported that HPE attenuates renal EMT as inhibiting high glucose-induced vimentin and loss of cell junction. HPE recovered the basement membrane surrounding tubular region in diabetic kidneys.[20]

In the present study, using the in vitro and in vivo model, we aim to investigate if insulin resistance signal and DPP-4 could be involved in renal EMT, and further explore the putative role of HPE on this mechanism for preventing diabetic nephropathy.

MATERIALS AND METHODS

Preparation of HPE. *Hibiscus sabdariffa* L. calyx was purchased from Taitung Area Farmer's association. HPE was prepared as previously reported, and its chemical composition was analyzed with liquid chromatography−tandem mass spectrometry. Phenolic compounds including gallic acid, galloyl ester, protocatechuic acid, caffeic acid, caffeoyl quinic acid, chlorogenic acid, and quercetin derivative were found in HPE (shown in the Supporting Information).[16]

Cell Culture. HK-2 cells purchased from Food Industry Research and Development Institute were cultured in keratinocyte serum free medium (Gibco BRL) with 5 ng/mL epidermal growth factor and 50 μg/mL bovine pituitary extract. The cells were grown in a humidified incubator at 37 °C in an atmosphere of 5% CO_2 and 95% air. HK-2 cells were inoculated into a 6 cm dish at a density of 5×10^5 cells for 24 h and then treated with 30 mM glucose with or without various concentrations (0.05−5 mg/mL) of HPE for 48 h.

Cytotoxicity Assay. HK-2 cells were seeded at a density of 1×10^6 cells/mL in a 24-well plate and incubated with linagliptin at various concentrations (1−100 μM) for 24 h. Thereafter, the medium was changed and cells were incubated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/mL) for 4 h. The viable cell was directly proportional to the production of formazan. Following dissolution in isopropanol, the result was read at 563 nm with a spectrophotometer (Hitachi, U-3210).

Western Blot Analysis. Cells were harvested into lysis buffer containing 50 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, and 5% mercaptoethanol and then lysed by sonication. Cell lysate was centrifuged at 9300*g* for 20 min at 4 °C, and the supernatant was collected as the protein sample. After quantifying, equal amounts of protein samples (50 μg) were subjected to 10% SDS−polyacrylamide gel electrophoresis and transferred to nitrocellulosemembranes (Millipore, Bedford, MA). Membranes were blocked with 5% nonfat milk powder with 0.1% Tween-20 in TBS and then incubated with the primary antibody at 4 °C overnight against the following targets. IRS-1: Santa Cruz (1:1000). pIRS-1 (S307): Cell Signaling (1:500). DPP4: Abcam (1:1000). PI3K: Santa Cruz (1:1000). pPI3K (p85, Tyr 458): Cell Singaling (1:500). Antibodies of IRS-1 and PI3K were purchased from Santa Cruz (Santa Cruz, CA), and DPP4 was from Abcam (Cambridge, U.K.). Antibodies of pIRS-1 (S307) and pPI3K were from Cell Singaling, Danvers, MA. Afterward, membranes were washed three times with 0.1% Tween-20 in TBS and incubated with the secondary antibody (1:5000) conjugated to horseradish peroxidase (GE Healthcare, Little Chalfont, Buckinghamshire, U.K.). Band detection was thereafter revealed by enhanced chemiluminescence using ECL Western blotting detection reagents and exposed in FUJFILM Las-3000 (Tokyo, Japan). Protein quantitative was determined by densitometry using FUJFILM-Multi Gauge V2.2 software.

Assay for DPP-4 Activity. Cells were seeded on 96-well plate (1×10^6/well). After treatment under various conditions, cells of each well were lysed with 100 μL of NP-40 lysis buffer (containing 10 mM HEPES (pH 7.5), 142.5 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA, and 0.2% NP-40) and centrifuged at 9300*g* for 20 min at 4 °C. The supernatants were collected, and the protein concentrations were determined with the Bradford assay. DPP4 activity was measured by DPP4/CD26 Assay Kit for Biological Samples (Enzo Life Sciences). Briefly, H-Gly-Pro-pNA, a chromogenic substrate of DPP-4, is hydrolyzed into the dipeptide Gly-Pro and the product 4-nitroaniline, whose rate of appearance would be measured spectrophotometrically at 405 nm. The activity was normalized with protein concentration, then in proportion to the control. Linagliptin, a gift from Boehringer Ingelheim, was used as DPP4 inhibitor in the experiment.

siRNA Transfection. HK-2 cells were seeded at 1×10^5 cells in each 6 cm Petri dish and incubated for 24 h. Transfection of siRNA (IRS-1 of human) was performed by the Lipofectamine 2000 Reagent Protocol. The final concentration of siRNA (SR302452A: rGrGrArU-rArGrGrArCrArArArUrUrCrArGrUrArArArCrAGG) in transfection medium (keratinocyte serum-free medium without bovine pituitary extract and EGF) was 45 nM; incubation was carried out for 6 h. The transfection medium was subsequently replaced with normal cultured medium and incubated for another 12 h. After that, cells were treated with different conditions. Cell proteins were then extracted and prepared for Western blotting.

Animal Experiment. The animal experimental project was approved by the Animal Model Experimental Ethics Committee of Chung-Shan Medical University based on the European Community guidelines (IACUC approval No: 1007). A type 2 diabetic model was carried out according to our previous publication.[16,18] Briefly, male Sprague−Dawley rats (weight 250 ± 20 g) obtained from LuxBiotech Co., Taiwan, were divided into several groups ($n = 8$ for each group), including the control (normal diet), FAT + STZ (high fat diet and streptozotocin injection), FAT + STZ + low HPE (with 100 mg/kg HPE added), and FAT + STZ + high HPE (with 200 mg/kg HPE added). Seven weeks later, when the average body weight attained 425 ± 15 g, the FAT groups were injected with a dose of STZ 35 mg/kgbw. About 2 weeks later, when the hyperglycemic status was confirmed, rats were tube-fed with or without different doses of HPE. The metabolic features, histological findings, and biomolecule expression of this model have been shown in our previous reports.[16,19] Safety evaluation revealed that HPE did no harm to liver, kidney, or cardiovascular tissue. After rats were sacrificed, the kidney chops were added to radioimmunoprecipitation assay (RIPA) buffer and protein inhibitors and homogenized at 4 °C. The tissue homogenates were centrifuged at 9300*g* for 20 min at 4 °C, and the resulting supernatants (whole-tissue extracts) were used for Western blot analysis.

Immunohistochemistry. The kidneys were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned into 4 μm, and processed for IHC examination. The primary antibody of pIRS-1 (S307) was from Novus, Littleton, CO. Immunostaining was completed using the UltraSensitive S-P kit (Maxim Co., streptavidin peroxidase method).

Statistical Analysis. The statistical software SPSS v.12.0 was used to analyze the data. One-way ANOVA was performed ($p < 0.05$), while Bonferroni's multiple comparison was used for post-test.

RESULTS

HPE Inhibits the High Glucose-Induced Expression of Vimentin and Phosphorylation of IRS-1 (S307). Compared with the control, high glucose increased nearly 3-fold of the vimentin, while HPE inhibited the expression dose-dependently. HPE at 0.5 and 1 mg/mL reduced 45% and 55% of vimentin, respectively (Figure 1A). As well, the phosphorylation of IRS-1 (S307) was increased 2-fold by high glucose. Treatment of HPE inhibited the phosphorylation significantly (Figure 1B). These results implicate that there might be a correlation between EMT and the insulin resistance signals; both are attenuated by HPE. Treatment with HPE has been tested by cytotoxicity assay, and it is nontoxic for HK-2 cells.[20]

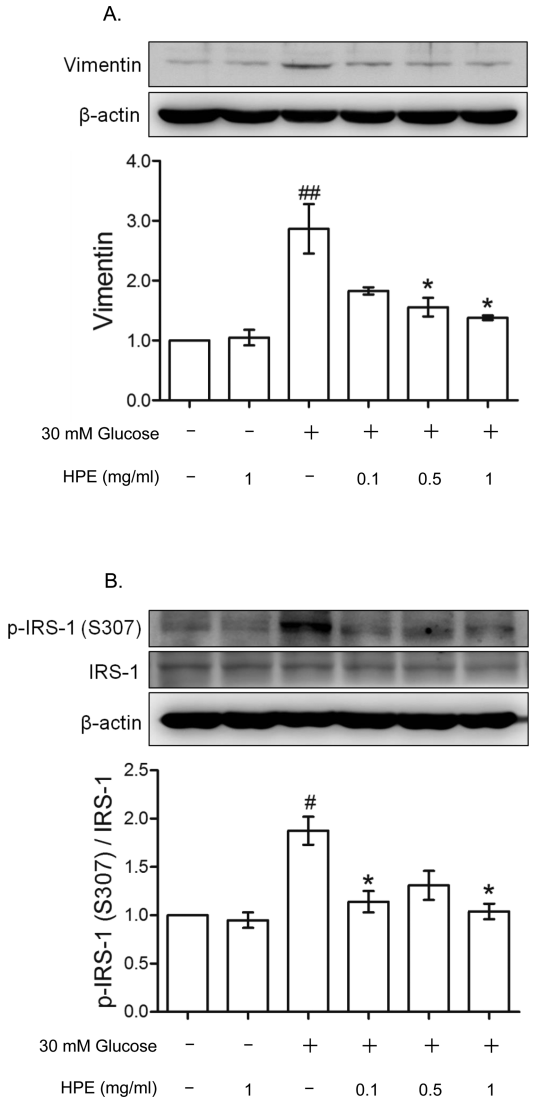

Figure 1. Effect of HPE on vimentin and IRS-1 (S307). HK-2 cells were incubated for 48 h under normal or high glucose (30 mM) conditions with or without different concentrations of HPE. Cell proteins were extracted and analyzed with Western blot: (A) vimentin; (B) IRS-1 (S307). Protein levels of vimentin and the ratio of IRS-1 (S307)/IRS-1 were calculated as fold compared with the control. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the control. $^{*}p < 0.05$, compared with the high glucose-treated.

HPE Inhibits DPP-4 Activity Induced by High Glucose. Figure 2A showed that neither high glucose nor HPE affect the DPP-4 protein level. However, by measuring DPP-4 activity, the alteration was discovered significantly. High glucose stimulated the activation of DPP-4 about 2.6-fold. Although the dose 0.1 mg/mL could not suppress the activation, 0.5 and 1 mg/mL HPE showed a good inhibitory effect to decrease DPP-4 activity even below that in the control (Figure 2B). Hence DPP-4 could be involved in the high glucose and HPE-regulated signals.

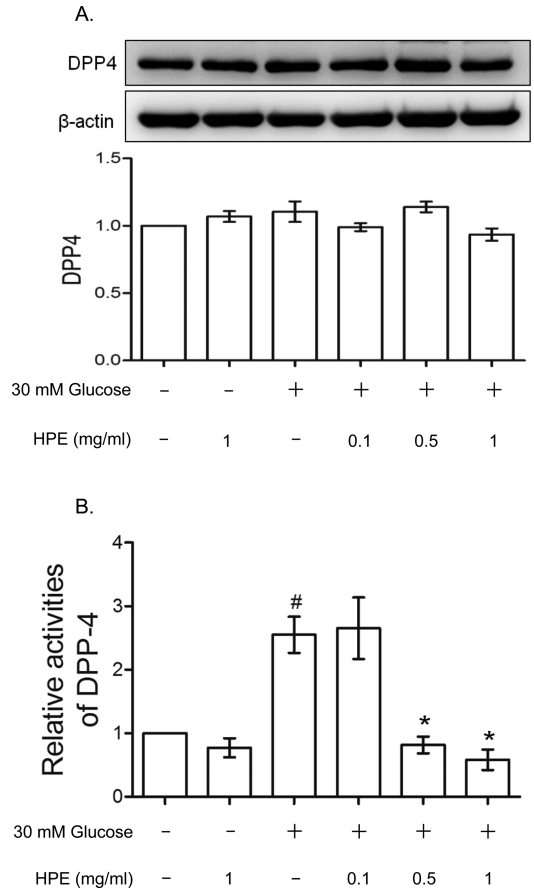

Figure 2. Effect of HPE on DPP-4. HK-2 cells were incubated for 48 h under normal or high glucose (30 mM) conditions with or without different concentrations of HPE. (A) DPP-4 proteins were extracted and analyzed with Western blot. (B) DPP-4 activity was analyzed. Protein levels and activity were calculated as fold compared with the control. Data are presented as means ± SD ($n = 3$) and analyzed with ANOVA. $^{\#}p < 0.05$, compared with the control. $^{*}p < 0.05$, compared with the high glucose-treated.

Linagliptin Was Revealed To Be Nontoxic and Effectively Inhibited DPP-4. Linagliptin was used as DPP-4 inhibitor to investigate the putative role of DPP-4. The test of cytotoxicity assay revealed that linagliptin is nontoxic under 2 μM (Figure 3A). In comparison with the control, 1 μM linagliptin reduced 80% of DPP-4 activity (Figure 3B). Hence it was the dose chosen in the following experiment.

DPP-4 Mediates the Regulation of Vimentin, IRS-1 (S307), and PI3K. Figure 4 shows that high glucose-induced vimentin and IRS-1 (S307) phosphorylation were significantly

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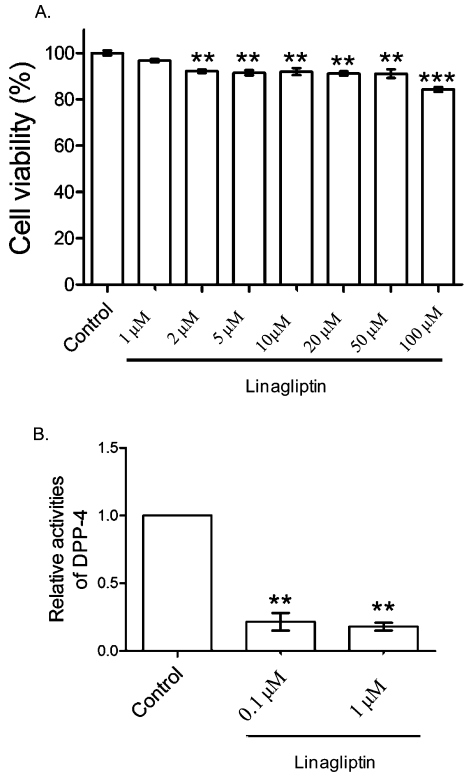

Figure 3. Cytotoxicity and DPP-4 inhibitory effect of linagliptin. (A) HK-2 cells were incubated for 48 h with or without different concentrations of linagliptin. Cell viability was calculated as percentage compared with the control group. (B) DPP-4 activity was analyzed. Data are presented as means ± SD (n = 3) and analyzed with ANOVA. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with the control.

reduced by 1 μM linagliptin or 1 mg/mL HPE. Noticeably, especially IRS-1 (S307) phosphorylation was inhibited by linagliptin or HPE even below that in the control. In contrast, PI3K, a normal response signal transmitting insulin sensitivity, was decreased about 20% by high glucose while recovered by either linagliptin or HPE. These results indicate that DPP-4 activation mediates the downstream resistance and EMT signals, while HPE has potential to improve the pathogenic markers (Figure 4).

IRS-1 Plays the Pivotal Role To Mediate Downstream Expression of Vimentin. For illustrating if vimentin elevation could be downstream of IRS-1, si-RNA of IRS-1 was transfected into the high glucose-incubated tubular cells. Figure 5A shows that the knockdown blocked most of the IRS-1 expression and reduced about 75% of the IRS-1 protein. High glucose-induced vimentin was significantly attenuated by the si-RNA (Figure 5B), indicating that IRS-1 and its phosphorylation could be an essential mediator of downstream EMT.

In addition to the resistance marker, we have further tested if glucose uptake could be involved (shown in the Supporting Information). The finding revealed that no matter whether glucose uptake is significantly altered or not, DPP-4/insulin resistance signal is the critical mediator of renal EMT.

pIRS-1 (S307) and Vimentin Are Coordinately Increased in the Diabetic Kidneys. Immunochemical staining demonstrated that pIRS-1 (S307) appeared rich in type 2 diabetic rats, especially surrounding the tubular region (Figure 6A–C). As previously indicated,[20] vacuole fusions and lipid accumulation were also found in the diabetic tubular

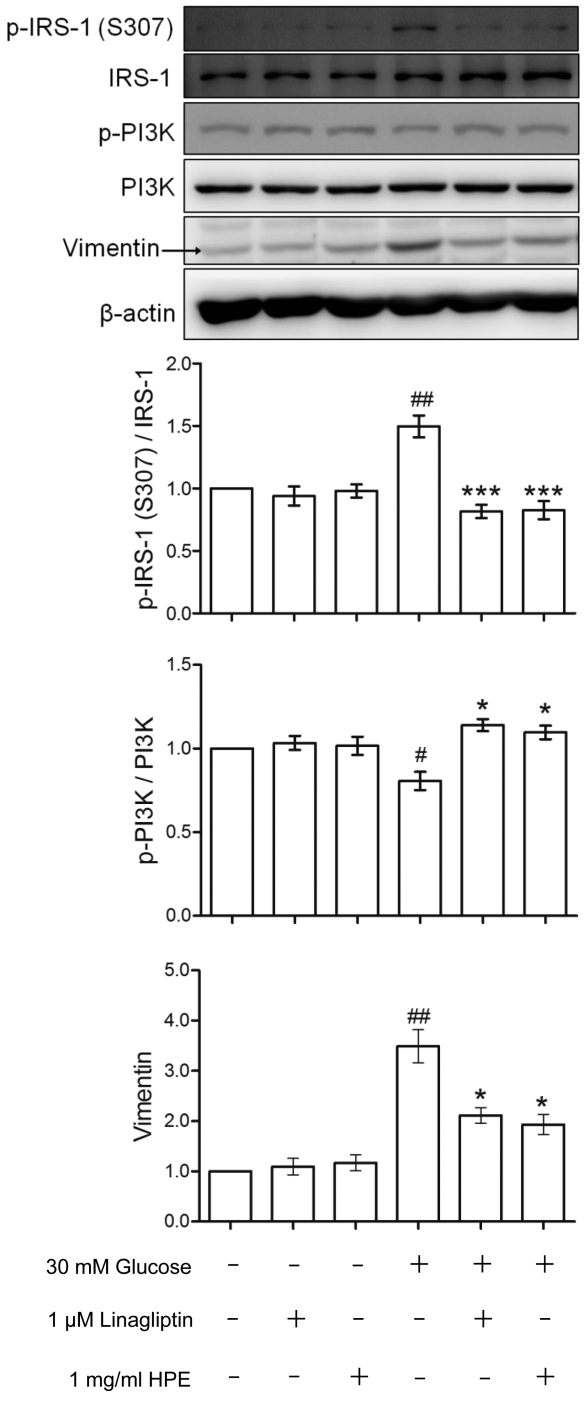

Figure 4. Effect of HPE on IRS-1 (S307), p-PI3K, and vimentin. HK-2 cells were incubated for 48 h under normal or high glucose (30 mM) conditions with or without different concentrations of HPE. Cell proteins were extracted and analyzed with Western blot. The ratios of IRS-1 (S307)/IRS-1 and p-PI3K/PI3K and the level of vimentin were calculated as fold compared with the control. Data are presented as means ± SD (n = 3) and analyzed with ANOVA. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ compared with the control. $^{*}p < 0.05$, $^{***}p < 0.001$, compared with the high glucose-treated.

region, accompanied by the destroyed basement membrane. As well, the protein level of vimentin significantly increased 1.5-fold in diabetic kidneys (Figure 6D). Treatment of HPE, especially at the dose of 200 mg/kg, ameliorated the resistance

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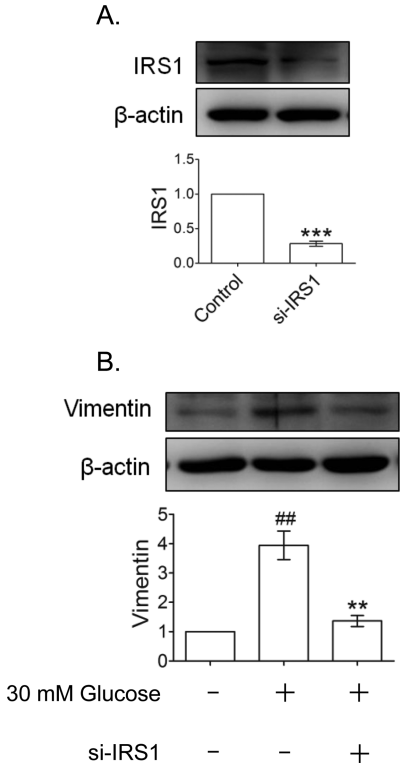

Figure 5. Signal cascades of IRS-1 and vimentin. HK-2 cells were transfected with siRNA and then incubated under different conditions. Cell proteins were extracted and analyzed with Western blot: (A) IRS-1; (B) vimentin. Protein levels were calculated as fold compared with the control. Data are presented as means ± SD (n = 3) and analyzed with ANOVA. $^{\#\#}p < 0.01$, compared with the control. $^{**}p < 0.01$, $^{***}p < 0.001$, compared with the high glucose-treated.

signal, and it reduced the EMT marker even below that in the control, thereby improving the histological changes.

DISCUSSION

We demonstrated the concomitant expression of the pivotal markers of EMT and insulin resistance in renal tubular cells. Treatment of HPE or linagliptin inhibited the high glucose-induced activation of DPP-4, thereby regulating vimentin and phosphorylation of IRS-1(S307), while recovering the signals of PI-3K. Blocking IRS-1 inhibited the elevation of vimentin, suggesting that an essential role of IRS-1 mediates between DPP-4 and the downstream EMT. In the diabetic rat, pIRS-1 (Ser 307) was abundantly expressed especially surround the tubular region, accompanied by increased vimentin in the kidney. Treatment of HPE ameliorated the EMT and resistance and improved the histological change.

EMT is an important process for embryonic development. Moreover, it occurs under pathological conditions, including cancer cell invasion and renal fibrosis.[21−23] EMT involves loss of epithelial cell adhesion, disrupted basement membrane, enhanced cell migration, and invasion.[24] With increasing vimentin, tubular epithelial cells undergo a phenotypic transformation into matrix-producing fibroblasts.[25] Besides measuring the protein markers, we have performed the morphologic observation to identify EMT. Scanning electromicroscopy and immunohistochemical stain proved that high glucose induced typical features of EMT, while HPE attenuated the alteration.[20] In diabetes, transitioned tubular epithelial cells are associated with interstitial fibrosis and renal dysfunction.[26] Our previous report demonstrated the effect of HPE on inhibiting renal fibrosis of type 2 diabetic rats,[19] which should partly be attributed to the improvement of EMT.

IRS-1 (S307), which is phosphorylated via several mechanisms, including insulin-stimulated kinases or stress-activated kinases like JNK1, is a serine residue located near the phosphotyrosine-binding domain of IRS-1. The phosphorylation of Ser307 leading to a conformational change disrupts the interaction between the catalytic domain of the insulin receptor and the phosphotyrosine-binding domain of IRS-1, and thus inhibits insulin signaling and stimulating the phosphatidylinositol 3-kinase and MAPK cascades.[27] IRS-1 (S307) phosphorylation is viewed as a hallmark of insulin resistance in biologically insulin-responsive cells or tissues.[28]

Cellular insulin resistance predominantly affects adipose and muscle cells. However, a spectrum of renal disease is associated with increased markers of insulin resistance, although direct causal mechanisms are not known.[29] Among a variety of kidney cells, podocyte, with kinetics similar to that of muscle cells, was shown to be insulin sensitive with respect to glucose uptake only if nephrin is expressed.[30] Palmitate blocks insulin-stimulated glucose uptake in human podocytes by dysregulating the phosphorylation of the insulin receptor, IRS-1 and PKB, and leads to impaired translocation of GLUT4 to the cell surface.[29] The resistance signals were also found in human embryonic kidney cells.[9] In the present study, high glucose-induced EMT and resistance marker were concomitantly rescued by HPE or linagliptin in renal tubular cells, implying that insulin resistance could get involved in the interstitial fibrosis and diabetic nephropathy. The mediating role was confirmed with IRS knockdown. Moreover, this phenomenon was proved in kidneys of type 2 diabetic rats. Our findings suggest that insulin resistance, attenuated by HPE, should pivotally associate with renal tubular EMT.

Many previous studies showed that DPP-4 inhibitor improved renal function parameters including albuminuria and creatinine clearance, or improved the histological changes of interstitial volume and glomerular basement membrane thickness, while the effects appeared to be independent of blood glucose lowering.[12,13] DPP-4 inhibition on top of angiotensin receptor blocker treatment significantly reduced urinary albumin excretion and oxidative stress in diabetic eNOS knockout mice, and the results were independent of blood glucose or systolic blood pressure.[11] Treatment with DPP-4 inhibitor significantly improved hyperglycemia and insulin resistance in SHR/NDmcr-cp rats, as evidenced by oral glucose tolerance test and homeostasis model assessment for insulin resistance, respectively.[31] In adults with metabolic symdrome, DPP-4 decrease was associated with insulin sensitivity following exercise training and weight loss.[32] There is evidence that DPP-4 inhibitors directly act on collagen metabolism and modulate the fibrosis marker such as transforming growth factor β.[33,34] We recently showed that HPE inhibit the angiotensin receptor-1/transforming growth factor β1 mediated EMT.[20] The cascades could be downstream of DPP-4 and even the subsequent resistance signal. Furthermore, the usual dose of linagliptin (MW = 472.54) for type 2 DM is 2.5 mg daily per os, which corresponds to 5.3 μmol. Taking the serum volume to be approximately 4 L per capita for human, the transient blood concentration could yield 1.325 μM. In the present study, 1.0 μM of linagliptin was used as DPP-4 inhibitor of the experiment, which is indeed correct and feasible.

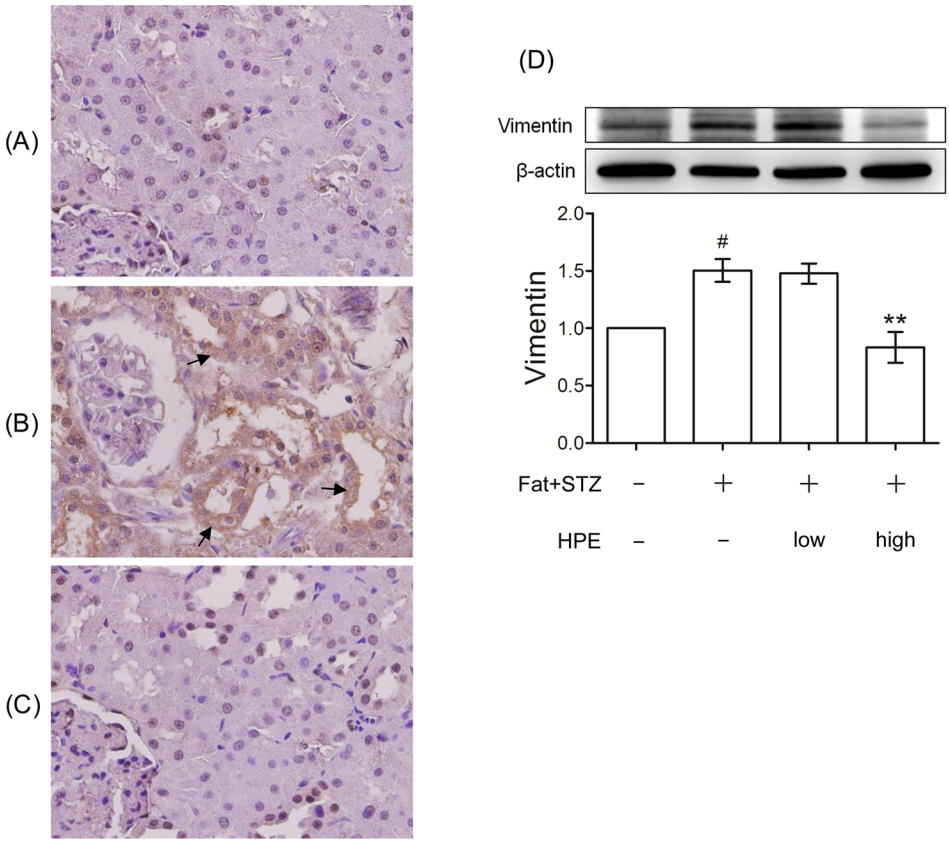

Figure 6. Effect of HPE on the diabetic kidneys. Kidneys of normal and type 2 diabetic rats with or without HPE treatment were examined by IHC (200×) and Western blot. (A–C) Histological expressions of IRS-1 Ser (307) (brown staining as arrow-indicated) in normal, diabetic, and 200 mg/kg HPE treated diabetic groups, respectively. (D) Protein levels of vimentin in the diabetic kidneys. $^{\#}p < 0.05$, compared with the control. $^{**}p < 0.01$, compared with the diabetic group.

In the present study, we have tested some of the components of HPE (shown in the Supporting Information). Although caffeic acid, protocatechuic acid, chlorogenic acid, and gallic acid all have the potential to inhibit DPP-4, a low dose of caffeic acid especially inhibits EMT of renal tubular cells. Gallic acid and cholorogenic acid could also exert some effect in triggering DPP-4 and the downstream EMT. The synergistic effects should be considered. In fact, all the tested compounds were reported to be antidiabetic. Caffeic acid promoted glycolysis and glycogen synthesis, but inhibited gluconeogenesis in insulin-resistant hepatocytes.[35] Caffeic acid protected the kidney from diabetic injuries, reducing blood glucose, HbA1c, blood urine nitrogen, urine output, renal advanced glycation end product (AGE), and inflammatory cytokines, while improving clearance of creatinine.[36] Treatment of protocatechuic acid also reduced plasma HbA1c, urinary glycative albumin, renal AGEs, and receptor of AGE.[37] Chlorogenic acid lowered fasting plasma glucose, TBARS, and lipid hydroperoxides in diabetic rats.[38] Gallic acid stimulated insulin secretagogue by regenerating pancreas β-islets, thus increasing plasma insulin and glucose tolerance.[39] Galloyl ester decreased oxidative stress and AGEs, and improved hepatic and renal function in type 2 diabetic db/db mice.[40]

In conclusion, HPE improves insulin resistance signals and EMT, which is implicated in the pathogenesis of diabetic nephropathy. HPE deserves further investigation and could be an adjuvant to prevent diabetic renal damage.

■ ASSOCIATED CONTENT

Supporting Information

Table of retention time, UV–vis, and mass spectral characteristics for the phenolics identified in HPE (S1); graphic depicting glucose uptake assay (S2); and graphics depicting DPP-4 inhibited by the phenolics of HPE and vimentin reduced by the phenolics of HPE (S3). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

DPP-4, type 4 dipeptidyl peptidase; EMT, epithelial to mesenchymal transition; HPE, polyphenol extracts of *H. sabdariffa*; IRS-1 (S307), phosphorylation of Ser307 of insulin receptor substrate-1; PI3K, phosphatidylinositol 3-kinase; STZ, streptozotocin

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