DNA aptamer raised against receptor for advanced glycation end products suppresses renal tubular damage and improves insulin resistance in diabetic mice

Ami Sotokawauchi, Takanori Matsui, Yuichiro Higashimoto, Yuri Nishino, Yoshinori Koga, Minoru Yagi and Sho-ichi Yamagishi

Abstract

Objective: Interaction of advanced glycation end products (AGEs) with the receptor RAGE plays a role in diabetic nephropathy. However, effects of RAGE-aptamer on tubular damage remain unknown. We examined whether RAGE-aptamer inhibited tubular damage in KKAy/Ta mice, obese type 2 diabetic mice with insulin resistance.

Materials and Methods: Male 8-week-old KKAy/Ta mice received continuous intraperitoneal infusion of either control-aptamer or RAGE-aptamer for 8 weeks. Blood biochemistry and blood pressure, and urinary N-acetyl-β-D-glucosaminidase (NAG) activity and albumin excretion levels were monitored. Kidney and adipose tissue samples were obtained for immunohistochemical analyses.

Results: Although RAGE-aptamer did not affect blood glucose, blood pressure, body weight, or serum creatinine values, it significantly inhibited the increase in urinary NAG activity and HOMA-IR in diabetic mice at 12 and 16 weeks old, respectively. Furthermore, compared with control-aptamer-treated mice, renal carboxymethyllysine, RAGE, and NADPH oxidase-driven superoxide generation were significantly decreased in RAGE-aptamer-treated mice at 12 weeks old with subsequent amelioration of histological alterations in glomerular and interstitial area, while adipose tissue adiponectin expression was increased.

Conclusion: Our present results suggest that RAGE-aptamer could inhibit tubular injury in obese type 2 diabetic mice partly by suppressing the AGE-RAGE-oxidative stress axis and improving insulin resistance.

Keywords
Aptamer, AGEs, diabetic nephropathy, tubular injury, RAGE

Introduction

Several randomized clinical trials revealed that inhibitors of sodium-glucose cotransporter 2 (SGLT2) reduced the risk of renal events in type 2 diabetic patients, regardless of the presence or absence of chronic renal disease.1–6 We have previously reported that uptake of glucose into proximal tubular cells via SGLT2 increases accumulation of advanced glycation end products (AGEs) and expression of their receptor RAGE in the kidneys of diabetic rats, thereby being involved in tubulointerstitial damage, a key mover in diabetic nephropathy.7–9 Furthermore, we recently found that albuminuria-lowering effects of SGLT inhibitors could be partly ascribed to its protective action against tubular damage in type 2 diabetic patients.10 These observations suggest that activation of AGE-RAGE axis via SGLT2 in the tubulointerstitial areas could contribute to the progression of diabetic nephropathy, and therefore inhibition of the interaction of

1Department of Pathophysiology and Therapeutics of Diabetic Vascular Complications, Kurume University School of Medicine, Kurume, Japan
2Department of Chemistry, Kurume University School of Medicine, Kurume, Japan
3Department of Pediatric Surgery, Kurume University School of Medicine, Kurume, Japan
4Department of Medicine, Division of Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, Tokyo, Japan

Corresponding author:
Sho-ichi Yamagishi, Department of Medicine, Division of Diabetes, Metabolism, and Endocrinology, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan.
Email: shoichi@med.kurume-u.ac.jp
AGEs with RAGE may be a therapeutic target for diabetic nephropathy.

Anti-RAGE neutralizing antibodies have been shown to reduce mesangial area expansion and increase in urinary albumin excretion in both type 1 and type 2 diabetic animals. However, to date, RAGE antibodies have not been approved as a therapeutic tool for diabetic nephropathy due to safety concern and/or limited clinical utility. We have recently shown that DNA aptamer raised against RAGE (RAGE-Apt) significantly inhibits the binding of AGEs to RAGE and subsequently attenuates the AGE-induced reactive oxygen species generation and inflammatory reactions, including RAGE expression in human cultured mesangial cells. Moreover, RAGE-Apt significantly blocked the development and progression of diabetic nephropathy in type 1 diabetic rats although it did not affect glycemic parameters or blood pressure. However, the effects of RAGE-Apt on tubular damage in KKAY/Ta mice, obese type 2 diabetic mice with insulin resistance remain unknown. In this study, we examined whether RAGE-Apt inhibited tubular damage in KKAY/Ta mice.

Materials and methods

Preparation and selection of RAGE-Apt

Preparation and selection of RAGE-Apt and control-aptamer (Ctrl-Apt) were performed using SELEX as described previously. Sequences of phosphorothioate-modified aptamers are followed: RAGE-Apt, 5′-ccTgATATggtTcAACCcgccCTTAATgTggTcTAc-3′; Ctrl-Apt, 5′-tcggCctggGgcggcCagttcGggtccAgtcgcGg-gag-3′; phosphorothioate nucleotides are indicated as capital letters.

Animal experiments

Male 8-week-old KKAY/Ta mice (CLEA Japan, Inc., Tokyo, Japan) were used in the present experiments. RAGE-Apt (2.0 pmol/day/kg body weight) or Ctrl-Apt was intraperitoneally administered to mice by an ALZET osmotic pump (Durect Corp., Cupertino, CA, USA). At baseline and after 4- or 8-week treatment, blood pressure was measured by a tail-cuff sphygmomanometer BP-98A (Softron Co., Ltd., Tokyo, Japan), and mice were housed in metabolic cages for 8 h to collect urine, sacrificed and then measured by a tail-cuff sphygmomanometer BP-98A (Softron Co., Ltd., Tokyo, Japan), and mice were housed in metabolic cages for 8 h to collect urine, sacrificed and then blood, kidney, and adipose tissue samples were obtained. Total cholesterol, high-density lipoprotein-cholesterol (HDL-cholesterol), triglycerides, creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), and blood urea nitrogen levels were measured using a chemistry analyzer FUJIFILM Corp., Tokyo, Japan). Fasting blood glucose was measured by an LBIS Mouse Insulin enzyme-linked immunosorbent assay kit (U-type; FUJIFILM Corp.). Urinary creatinine was measured by a Serotec CRE-L (Serotec Co., Ltd, Sapporo, Japan). Urinary N-acetyl-β-D-glucosaminidase (NAG) activity was measured by a colorimetric assay kit (BioVision Inc, Milpitas, CA, USA). Serum cystatin C and adiponectin levels were measured with mouse cystatin C (Abcam, Cambridge, UK) and mouse adiponectin enzyme-linked immunosorbent assay kits (R&D Systems Inc, Minneapolis, MN, USA), respectively.

Immunohistochemical analysis

Kidneys and visceral adipose tissues were obtained from KKAY/Ta mice, fixed with 4% paraformaldehyde, embedded in paraffin, cut into 4-µm intervals, and mounted on glass slides. The kidney sections were incubated overnight at 4°C with peroxidase-conjugated monoclonal antibody raised against carboxymethyllysine (CML; TransGenic Inc., Fukuoka, Japan) or anti-RAGE antibody (Santa Cruz Biotechnology Inc., Dallas, TX, USA) followed by Histofine Simple Stain MAX-PO(R) (Nichirei Bioscience Inc., Tokyo, Japan) as described previously. The adipose tissue sections were incubated overnight at 4°C with antibodies raised against adiponectin (Santa Cruz Biotechnology Inc.), and then incubated with goat anti-rabbit IgG (H + L) Alexa Fluor 488 (Invitrogen Corp., Carlsbad, CA, USA). Immunoreactive areas were measured from ten randomly selected fields of kidney and adipose tissue sections per animal using the cellSens version 1.14 software (Olympus Corp., Tokyo, Japan).

Measurement of NADPH oxidase-driven superoxide generation

Renal NADPH oxidase-driven superoxide generation was measured by a luminescence assay in 50 mmol/L phosphate buffer (pH 7.0) containing 1 mmol/L ethylene glycol tetraacetic acid, 150 mmol/L sucrose, 5 µmol/L lucigenin as the electron acceptor, and 100 µmol/L NADPH as the substrate as described previously.

Histopathological examinations

The kidneys were fixed in 4% paraformaldehyde and embedded in paraffin, sectioned at 4-µm intervals and mounted on glass slides. The sections were stained with
hematoxylin and eosin for light microscopic analysis. Glomerular area delimited by the internal edge of theBowman’s capsule was measured using image J software as described previously.15 Two-micrometer paraffin sections were stained with periodic acid-Schiff’s reagent. The cortical interstitial area and intra-tubular area were measured from ten randomly selected fields of tissue sample per each animal using the cellSens version 1.14 software (Olympus Corp., Tokyo, Japan).

Statistical analysis
All values were presented as mean ± standard deviation. Statistical comparisons were performed using ANOVA followed by Tukey-Kramer test (except for creatinine and cystatin C in Table 1, Figures 1(a), (c)–(e) and 2), Tukey HSD test (cystatin C at 12 weeks old), or Steel-Dwass test (creatinine, cystatin C at 16 weeks old, and Figure 1(b)); p < 0.05 was considered significant. All statistical analyses were performed with the use of JMP Pro version 14.0 (SAS Institute Inc., Cary, NC, USA).

Results
As shown in Table 1, compared with control mice at 8 weeks old, body weight, mean blood pressure, fasting blood glucose, triglycerides, AST, and blood urea nitrogen levels were significantly elevated in Ctrl-Apt-treated KKAY/Ta diabetic mice at 16 weeks old. In addition, body weight, mean blood pressure, and blood urea nitrogen levels were significantly higher in Ctrl-Apt-treated KKAY/Ta diabetic mice at 16 weeks old than those in control mice at 8 weeks old, whereas fasting blood glucose in Ctrl-Apt-treated mice and AST in RAGE-Apt-treated mice at 16 weeks old were significantly increased. There were no significant differences of these parameters between Ctrl-Apt-treated mice and RAGE-Apt-treated mice at both 12 and 16 weeks old, except for triglycerides; RAGE-Apt treatment significantly increased triglycerides in KKAY/Ta diabetic mice at 12 weeks old, whereas it decreased triglycerides in diabetic mice at 16 weeks old compared with Ctrl-Apt. Compared with control mice at 8 weeks old, serum adiponectin levels were significantly decreased in both Ctrl-Apt-treated and RAGE-Apt-treated KKAY/Ta diabetic mice at 16 weeks old, whereas not at 12 weeks old. Although adiponectin levels were lower in RAGE-Apt-treated KKAY/Ta mice at 12 weeks old compared with Ctrl-Apt-treated mice at the same age, there was no significant difference of circulating adiponectin levels between the two group mice at 16 weeks old (Table 1).

NAG activities in the urine were significantly elevated in Ctrl-Apt-treated KKAY/Ta diabetic mice at 12 and 16 weeks old compared with control mice at 8 weeks old, both of which were reduced by the treatment with RAGE-Apt (Table 1). Although cystatin C levels in KKAY/Ta mice at 12-week and 16-week old were significantly higher than those of control mice at 8-week-old, there was no significant difference of cystatin C levels between Ctrl-Apt- and RAGE-Apt-treated KKAY/Ta mice at 12-week and 16-week old (Table 1).

As shown in Figure 1(a) to (c), compared with Ctrl-Apt-treated mice at 12 weeks old, renal CML, RAGE, and

Table 1. Characteristics of animals.

|                  | 8-week-old |         | 12-week-old |         | 16-week-old |         |
|------------------|------------|---------|-------------|---------|-------------|---------|
|                  | Ctrl-Apt   | RAGE-Apt| Ctrl-Apt    | RAGE-Apt| Ctrl-Apt    | RAGE-Apt|
| Number           | 5          | 6       | 5           | 6       | 6           | 6       |
| Body weight (g)  | 33.4 ± 2.0 | 41.9 ± 1.3†† | 40.9 ± 1.4†† | 45.7 ± 2.8†† | 42.8 ± 3.3†† |
| Heart rate (beats/minute) | 704 ± 49  | 731 ± 23 | 738 ± 26    | 749 ± 25 | 730 ± 33    |
| Mean blood pressure (mmHg) | 73 ± 8     | 98 ± 6†† | 97 ± 6††    | 89 ± 8†† | 93 ± 3††    |
| Fasting blood glucose (mg/dL) | 119 ± 16   | 200 ± 19†† | 215 ± 23†† | 201 ± 39† | 176 ± 71†   |
| Total cholesterol (mg/dL) | 115 ± 25   | 105 ± 6  | 91 ± 6      | 124 ± 28 | 104 ± 11    |
| HDL-cholesterol (mg/dL) | 136 ± 32   | 98 ± 16  | 115 ± 29    | 134 ± 35 | 103 ± 15    |
| Triglycerides (mg/dL)   | 129 ± 11   | 183 ± 26† | 242 ± 15††  | 234 ± 31†† | 160 ± 23*   |
| Creatinine (Cr) (mg/dL) | 0.15 ± 0.06| 0.23 ± 0.05| 0.25 ± 0.06 | 0.34 ± 0.09| 0.20 ± 0.08 |
| AST (U/L)          | 39 ± 4     | 113 ± 20†† | 140 ± 22†† | 91 ± 14  | 102 ± 40†   |
| ALT (U/L)          | 25 ± 1     | 59 ± 10  | 67 ± 37     | 56 ± 20  | 53 ± 15     |
| Blood urea nitrogen (mg/dL) | 22.8 ± 2.3 | 46.9 ± 1.5†† | 43.1 ± 3.6†† | 48.4 ± 7.3†† | 37.1 ± 9.6†   |
| Urinary NAG activity (U/mg Cr) | 0.47 ± 0.06 | 0.65 ± 0.07† | 0.48 ± 0.09† | 0.98 ± 0.21†† | 0.58 ± 0.11*** |
| Urinary albumin (mg/g Cr) | 632.1 ± 214.7 | 1409.7 ± 1104.1 | 1561.4 ± 862.3 | 1162.9 ± 569.2 | 504.9 ± 101.2 |
| Serum cystatin C (ng/mL) | 571.5 ± 171.6 | 1089.5 ± 182.0†† | 1163.8 ± 222.5†† | 1285.9 ± 325.2† | 1089.2 ± 201.7† |
| Serum adiponectin (µg/mL) | 2.96 ± 0.25 | 3.50 ± 0.18 | 2.78 ± 0.39† | 2.33 ± 0.26† | 2.34 ± 0.29†    |
| Kidney weight/body weight (%) | 0.74 ± 0.03 | 0.78 ± 0.11 | 0.79 ± 0.13 | 0.77 ± 0.12 | 0.67 ± 0.12    |

Data are mean ± SD. †p < 0.05, ††p < 0.01 compared with 8-week-old; *p < 0.05, **p < 0.01 compared with Ctrl-Apt.
NADPH oxidase-driven superoxide generation levels were significantly decreased in RAGE-Apt-treated mice at 12 weeks old. There were no significant differences of CML, RAGE, and NADPH oxidase-driven superoxide generation levels between Ctrl-Apt- and RAGE-Apt-treated KKAy/Ta mice at 16 weeks old.
As shown in Figure 1(d), compared with 8-week-old mice, glomerular area was significantly increased in Ctrl-Apt-treated KKAy/Ta mice at 12 and 16 weeks old. Furthermore, both glomerular and cortical interstitial areas in 16-week-old RAGE-Apt-treated mice were significantly smaller than those of Ctrl-Apt-treated mice at the same age (Figure 1(d) and (e)). In addition, glomerular and cortical interstitial areas in RAGE-Apt-treated 12-week-old diabetic were slightly, but not significantly, decreased compared with those in Ctrl-Apt-treated mice at the same age ($p = 0.41$ for glomerular areas and $p = 0.25$ for interstitial areas). Intra-tubular area did not differ among the three groups. Adiponectin expression in adipose tissues of RAGE-Apt-treated mice at 12 weeks old were significantly higher than that in Ctrl-Apt-treated mice at the same age (Figure 2(a)). Although adipose adiponectin levels in Ctrl-Apt- and RAGE-Apt-treated KKAy/Ta mice at 16 weeks old were higher than those in 8-week-old mice, there was no significant difference of adipose adiponectin between the two groups (Figure 2(a)).

HOMA-IR was significantly increased in 16-week-old Ctrl-Apt-treated mice compared with control mice at 8 weeks old, which was reduced by the treatment with RAGE-Apt (Figure 2(b)). Although HOMA-IR was slightly higher in RAGE-Apt-treated mice at 12 weeks old compared with Ctrl-Apt-treated mice at the same age, these values did not differ from the HOMA-IR of 8-week-old mice (Figure 2(b)).

**Conclusion**

AGEs have been shown to induce oxidative stress generation, apoptotic cell death, inflammatory and fibrotic reactions in human cultured proximal tubular cells via the interaction with RAGE. Indeed, RAGE-Apt or antibodies raised against RAGE have been reported to block these harmful effects of AGEs on proximal tubular cells. However, the pathological role of AGE-RAGE axis in tubulointerstitial damage in type 2 diabetic nephropathy has not been fully elucidated. In order to investigate the time-course effects of RAGE-Apt on tubular damage and insulin resistance in type 2 diabetic animals, we used here 8-week-old prediabetic KKAy/Ta mice as a control group. Furthermore, to examine the effects of RAGE-Apt on AGE-RAGE axis and adiponectin and subsequent tubular damage in type 2 diabetic animals, we investigated renal CML, RAGE, NADPH oxidase activity, and adipose adiponectin expression in KKAy/Ta mice at both 12-week-old and 16-week-old. In this study, we found for the first time that RAGE-Apt treatment significantly inhibited the increase in urinary NAG activities, a marker of tubulointerstitial injury and dysfunction in obese type 2 diabetic mice with insulin resistance, which was associated with reduction of CML, RAGE, and NADPH oxidase-driven superoxide generation in the kidneys of KKAy/Ta diabetic mice at 12 weeks old. Injury and apoptosis of proximal tubular cells could contribute to tubular atrophy, tubulointerstitial fibrosis and atubular glomeruli, which are prognostic histological markers to predict the progression of renal dysfunction in diabetic patients. Given that urinary NAG activity could also predict renal prognosis in patients at early stage of diabetic nephropathy, our present study suggests that inhibition of the AGE-RAGE axis in tubulointerstitial areas by RAGE-Apt may be a novel therapeutic strategy for treatment of diabetic nephropathy.
Anti-RAGE neutralizing antibodies have already been shown to play a protective role against experimental diabetic nephropathy. However, aptamers have several advantages over antibodies for blocking the deleterious function of target protein. Compared with antibodies, aptamers can be easily selected with relatively low production costs. When used in an in vivo therapeutic application, aptamers can more efficiently penetrate into various tissues with less immunogenicity over antibodies. These findings may suggest the clinical feasibility of RAGE-Apt for treatment of diabetic nephropathy.

RAGE-Apt reduced urinary albumin excretion levels by half in KKAy/Ta diabetic mice at 16 weeks old, but its effects were not statistically significant. That meant that the present data did not provide evidence against null-hypothesis. However, in the present study, all the KKAY/Ta mice at 12-week-old were sacrificed for immunohistochemical analyses. Therefore, we could not evaluate the serial change of urinary albumin over time in each animal. In addition to this, small sample size may limit the statistical power of the findings. On the other hand, histological examinations revealed that compared with 8-week-old mice, glomerular areas were significantly increased in Ctrl-Apt-treated KKAY/Ta mice at 12 and 16 weeks old, the latter of which was significantly attenuated by the treatment with RAGE-Apt. Moreover, cortical interstitial area in RAGE-Apt-treated KKAY/Ta diabetic mice at 16 weeks old was significantly smaller than those in Ctrl-Apt-treated mice at the same age. Given these beneficial effects of RAGE-Apt on histological alterations in the kidneys of KKAY/Ta mice in association with the reduction of CML, RAGE, and NADPH oxidase-derived superoxide generation in both glomeruli as well as tubulointerstitial areas, it is conceivable that inhibition of glomerular AGE-RAGE axis by RAGE-Apt may also play a protective role in our animal models. It would be interesting to examine whether long-term treatment with RAGE-Apt could prevent the increase in urinary albumin excretion, glomerular sclerosis, and tubulointerstitial fibrosis in KKAY/Ta mice.

In this study, we found that CML, one of the well-characterized AGEs, RAGE, and NADPH oxidase-mediated superoxide generation levels were significantly reduced by RAGE-Apt in 12-week-old KKAY/Ta mice. We have previously shown that neutralizing antibody raised against RAGE or an anti-oxidant N-acetylcysteine inhibits the AGE-induced oxidative stress generation and RAGE expression in human cultured proximal tubular cells. Furthermore, renal levels of CML are decreased in RAGE-deficient diabetic mice. These observations suggest that the AGE-RAGE-NADPH oxidase-evoked superoxide generation could enhance the CML formation and RAGE expression in the diabetic kidneys, whose positive feedback loop was blocked by the treatment with RAGE-Apt. Prognostic value of urinary excretion levels of AGEs for progression of diabetic nephropathy was lost after adjustment for baseline urinary activity of NAG, further supporting the clinical relevance of AGE-RAGE axis activation in tubular damage and subsequent deterioration of renal function in diabetic nephropathy.

In the present study, we found that RAGE-Apt increased adipose tissue adiponectin expression at 12-week-old KKAY/Ta mice and ameliorated insulin resistance evaluated by HOMA-IR at 16-week-old KKAY/Ta mice. Although calculation of HOMA-IR is human-based and that basal concentrations of glucose and insulin are not the same in rodents, it is widely used as a proxy of insulin resistance in rodents as well and an accepted surrogate for estimating insulin resistance. Since we, along with others, have found that (1) blockade of the AGE-RAGE axis improves glycemic control in fructose-fed rats, an animal model of insulin resistance and that (2) insulin sensitivity and serum adiponectin levels are increased in RAGE-deficient mice compared with wild-type mice, inhibition of the AGE-RAGE axis in the adipose tissues may also be a therapeutic target for insulin resistance in obese type 2 diabetes. A ligand of peroxisome proliferator-activated receptor-gamma has been shown to reduce triglycerides and counteract insulin resistance, thereby resulting in amelioration of tubular damage in obese type 2 diabetic animals. Moreover, insulin resistance is associated with tubular damage in elderly individuals. These observations suggest that beneficial effects of RAGE-Apt on triglycerides and HOMA-IR may partly contribute to the protection against tubular injury in KKAY/Ta mice.

A major limitation to this study is the lack of age-matched non-diabetic control group. Indeed, we used a spontaneous model of type 2 diabetes, and comparisons of data were performed between RAGE-Apt- and Ctrl-Apt-treated mice at 12 and 16 weeks old as well as between both groups at each time point and 8-week-old prediabetic mice. From this experimental design, it is impossible to exclude that effects of RAGE-Apt observed here may not be specific to diabetes. In other words, effects of RAGE-Apt on age-related changes may affect the present findings. Second, RAGE-Apt significantly reduced CML, RAGE, and NADPH-oxidase-derived superoxide generation in the kidneys of diabetic mice at 12 weeks old, but not at 16 weeks old. We did not know the reason for the time-varying effects of RAGE-Apt on AGE-RAGE axis. In any case, suppression of renal AGE-RAGE axis by RAGE-Apt in diabetic mice at 12 weeks old may contribute to the beneficial effects of RAGE-Apt on histological alterations in the kidneys of diabetic mice at 16 weeks old. In addition, we did not measure the effects of RAGE-Apt on tubular apoptosis in this model. It would be helpful to examine whether RAGE-Apt could attenuate tubular apoptosis in KKAY/Ta diabetic mice. Further biochemical data that could support the histological findings would strengthen the present results.
Author contributions
S. Yamagishi conceptualized and designed the study; acquired, analyzed, and interpreted data; and drafted the manuscript; and he takes responsibility for the integrity of the data and the accuracy of the data analysis. A. Sotokawauchi, T. Matsui, Y. Higashimoto, Y. Nishino, and Y. Koga acquired, analyzed, and interpreted data. M. Yagi critically revised the manuscript.

Declaration of conflicting interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported in part by Grants-in-Aid for Scientific Research (Grant Number 17K08968 (SY) and 19K16387(AS)) from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

ORCID iDs
Ami Sotokawauchi https://orcid.org/0000-0002-4608-9341
Takanori Matsui https://orcid.org/0000-0001-9506-7571
Sho-ichi Yamagishi https://orcid.org/0000-0003-0102-0823

References
1. Wanner C, Inuzuchi SE, Lachin JM, et al. Empagliflozin and Progression of Kidney Disease in Type 2 Diabetes. N Engl J Med 2016; 375: 323–334.
2. Neal B, Perkovic V, Mahaffey KW, et al. Canagliflozin and Cardiovascular and Renal Events in Type 2 Diabetes. N Engl J Med 2017; 377: 644–657.
3. Wiviott SD, Raz I, Bonaca MP, et al. Dapagliflozin and Cardiovascular Outcomes in Type 2 Diabetes. N Engl J Med 2019; 380: 347–357.
4. Mosenzon O, Wiviott SD, Cahn A, et al. Effects of dapagliflozin on development and progression of kidney disease in patients with type 2 diabetes: an analysis from DECLARE-TIMI 58 randomised trial. Lancet Diabetes Endocrinol 2019; 7: 606–617.
5. Perkovic V, Jardine MJ, Neal B, et al. Canagliflozin and Renal Outcomes in Type 2 Diabetes and Nephropathy. N Engl J Med 2019; 380: 2295–2306.
6. Yamagishi S and Matsui T. Protective role of sodium-glucose co-transporter 2 Inhibition against vascular complications in diabetes. Rejuvenation Res 2016; 19: 107–114.
7. Maeda S, Matsui T, Takeuchi M, et al. Sodium-glucose cotransporter 2-mediated oxidative stress augments advanced glycation end products-induced tubular cell apoptosis. Diabetes Metab Res Rev 2013; 29: 406–412.
8. Ojima A, Matsui T, Nishino Y, et al. Empagliflozin, an Inhibitor of Sodium-Glucose Cotransporter 2 Exerts Anti-Inflammatory and Anti-fibrotic Effects on Experimental Diabetic Nephropathy Partly by Suppressing AGEs-Receptor Axis. Horm Metab Res 2015; 47: 686–692.
9. Gilbert RE. Proximal tubulopathy: Prime mover and key therapeutic target in diabetic kidney disease. Diabetes 2017; 66: 791–800.
10. Yanagisawa K, Sotokawauchi A, Nishino Y, et al. Albuminuria-lowering effect of sodium-glucose cotransporter 2 inhibitors could be partly attributable to the attenuation of tubular damage in type 2 diabetic patients. Diabetes Metab Res Rev 2020; e3327.
11. Flyvbjerg A, Denner L, Schrijvers BF, et al. Long-term renal effects of a neutralising RAGE antibody in obese type 2 diabetic mice. Diabetes 2004; 53: 166–172.
12. Jensen LJ, Denner L, Schrijvers BF, et al. Renal effects of a neutralising RAGE-antibody in long-term streptozotocin-diabetic mice. J Endocrinol 2006; 188: 493–501.
13. Matsui T, Higashimoto Y, Nishino Y, et al. RAGE-aptamer blocks the development and progression of experimental diabetic nephropathy. Diabetes 2017; 66: 1683–1695.
14. Yamagishi S, Inagaki Y, Nakamura K, et al. Pigment epithelium-derived factor inhibits TNF-alpha-induced inter-leukin-6 expression in endothelial cells by suppressing NADPH oxidase-mediated reactive oxygen species generation. J Mol Cell Cardiol 2004; 37: 497–506.
15. Matsui T, Nakashima S, Nishino Y, et al. Dipeptidyl peptidase-4 deficiency protects against experimental diabetic nephropathy partly by blocking the advanced glycation end products-receptor axis. Lab Invest 2015; 95: 525–533.
16. Ishibashi Y, Matsui T, Takeuchi M, et al. Beneficial effects of metformin and irbesartan on advanced glycation end products (AGEs)-RAGE-induced proximal tubular cell injury. Pharmacol Res 2012; 65: 297–302.
17. Ishibashi Y, Matsui T, Takeuchi M, et al. Metformin inhibits advanced glycation end products (AGEs)-induced renal tubular cell injury by suppressing reactive oxygen species generation via reducing receptor for AGEs (RAGE) expression. Horm Metab Res 2012; 44: 891–895.
18. Maeda S, Matsui T, Takeuchi M, et al. Pigment epithelium-derived factor (PEDF) inhibits proximal tubular cell injury in early diabetic nephropathy by suppressing advanced glycation end products (AGEs)-receptor (RAGE) axis. Pharmacol Res 2011; 63: 241–248.
19. Kaifu K, Ueda S, Nakamura N, et al. Advanced glycation end products evoke inflammatory reactions in proximal tubular cells via autocrine production of dipeptidyl peptidase-4. Microvasc Res 2018; 120: 90–93.
20. Fu WJ, Xiong SL, Fang YG, et al. Urinary tubular biomarkers in short-term type 2 diabetes mellitus patients: a cross-sectional study. Endocrine 2012; 41: 82–88.
21. Ziyadeh FN and Goldfarb S. The renal tubulointerstitium in diabetes mellitus. Kidney Int 1991; 39: 464–475.
22. Taft JL, Nolan CJ, Yeung SP, et al. Clinical and histological correlations of decline in renal function in diabetic patients with proteinuria. Diabetes 1994; 43: 1046–1051.
23. Mise K, Hoshino J, Ueno T, et al. Prognostic value of tubulo-interstitial lesions, urinary N-Acetyl-β-d-glucosaminidase, and urinary β2-microglobulin in patients with type 2 diabetes and biopsy-proven diabetic nephropathy. Clin J Am Soc Nephrol 2016; 11: 593–601.
24. Sun H and Zu Y. A highlight of recent advances in aptamer technology and its application. Molecules 2015; 20: 11959–11980.
25. Yamagishi SI and Matsui T. Therapeutic potential of DNA-aptamers raised against AGE-RAGE axis in diabetes-related complications. *Curr Pharm Des* 2018; 24: 2802–2809.

26. Tan AL, Sourris KC, Harcourt BE, et al. Disparate effects on renal and oxidative parameters following RAGE deletion, AGE accumulation inhibition, or dietary AGE control in experimental diabetic nephropathy. *Am J Physiol Renal Physiol* 2010; 298: F763–F770.

27. Kern EF, Erhard P, Sun W, et al. Early urinary markers of diabetic kidney disease: a nested case-control study from the Diabetes Control and Complications Trial (DCCT). *Am J Kidney Dis* 2010; 55: 824–834.

28. van Dijk TH, Laskewitz AJ, Grefhorst A, et al. A novel approach to monitor glucose metabolism using stable isotopically labelled glucose in longitudinal studies in mice. *Lab Anim* 2013; 47: 79–88.

29. Ojima A, Matsui T, Nakamura N, et al. DNA aptamer raised against advanced glycation end products (AGEs) improves glycemic control and decreases adipocyte size in fructose-fed rats by suppressing AGE-RAGE axis. *Horm Metab Res* 2015; 47: 253–258.

30. Monden M, Koyama H, Otsuka Y, et al. Receptor for advanced glycation end products regulates adipocyte hypertrophy and insulin sensitivity in mice: involvement of Toll-like receptor 2. *Diabetes* 2013; 62: 478–489.

31. Nakano R, Kurosaki E, Shimaya A, et al. YM440, a novel hypoglycemic agent, protects against nephropathy in Zucker fatty rats via plasma triglyceride reduction. *Eur J Pharmacol* 2006; 549: 185–191.

32. Carlsson AC, Calamia M, Risérus U, et al. Kidney injury molecule (KIM)-1 is associated with insulin resistance: results from two community-based studies of elderly individuals. *Diabetes Res Clin Pract* 2014; 103: 516–521.