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ORIGINAL ARTICLE

Glucagon-like peptide-1 receptor regulates receptor of advanced glycation end products in high glucose-treated rat mesangial cells

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Abstract

Background: Hyperglycemia-induced advanced glycation end products (AGEs) and receptor for AGEs (RAGEs) play major roles in diabetic nephropathy progression. In previous study, both glucagon-like peptide-1 (GLP-1) and peroxisome proliferator-activated receptors delta (PPARδ) agonists were shown to have anti-inflammatory effect on AGE-treated rat mesangial cells (RMCs). The interaction among PPARδ agonists, GLP-1, and AGE-RAGE axis is, however, still unclear.

Methods: In this study, the individual and synergic effect of PPARδ agonist (L-165 041) and siRNA of GLP-1 receptor (GLP-1R) on the expression of GLP-1, GLP-1R, RAGE, and cell viability in AGE-treated RMCs were investigated.

Results: L-165 041 enhanced GLP-1R mRNA and protein expression only in the presence of AGE. The expression of RAGE mRNA and protein was enhanced by AGE, attenuated by L-165 041, and siRNA of GLP-1R reversed L-165 041-induced inhibition. Cell viability was also inhibited by AGE. L-165 041 attenuated AGE-induced inhibition and siRNA GLP-1R diminished L-165 041 effect.

Conclusion: PPARδ agonists increase GLP-1R expression on RMC in the presence of AGE. PPARδ agonists also attenuate AGE-induced upregulated RAGE expression and downregulated cell viability. The effect of PPARδ agonists needs the cooperation of GLP-1R activation.

Keywords: Advanced glycation end products; Glucagon-like peptide-1 receptor; Peroxisome proliferator-activated receptors delta; Rat mesangial cell

1. INTRODUCTION

Diabetes mellitus is one of the fastest-growing global health issues in the world.[1] Nearly 537 million adults 20 to 79 years old worldwide have diabetes. The number is about 10.5% of people in that age group.[2] Diabetic nephropathy is the leading cause of end-stage renal disease in the world.[3] Advanced glycation end products (AGEs) and receptor for AGE (RAGE) play a major role in pathogenesis of diabetic nephropathy.[4]

RAGE is a member of the immunoglobulin superfamily. RAGE mediates cellular responses and binds to several kinds of damage-associated molecular pattern molecules, such as AGEs.[5] RAGE and its ligands stimulate and trigger cascades of proinflammatory process. Its reactions are involved in a wide spectrum of diseases, including diabetes mellitus, Parkinson's disease, and cancer. AGE/RAGE axis signaling stimulates second messengers and causes subsequent reactions among proinflammatory enzymes, cytokines, and adhesion molecules.[6] AGE/RAGE axis was also reported to activate nicotinamide adenine dinucleotide phosphate oxidase 1, attenuate expression of superoxide dismutase 1, and then result in oxidative stress promoting diabetes-mediated vascular calcification.[7] In animal study, extracellular matrix components extracted from diabetic mice-altered cardiac fibroblast function through the AGE/RAGE signaling cascade.[8] This signal cascade also induced endothelial dysfunction in a kidney failure animal model.[9]

The transcription factors peroxisome proliferator-activated receptors (PPARs) belong to the subfamily 1 of the nuclear hormone receptor superfamily of transcription factors. Three PPAR subtypes have been identified: PPARα, PPARγ, and PPARβ/δ.[10] They are involved in several physiological processes including lipid metabolism, insulin resistance, cancer development, and anti-inflammatory process.[11–13] PPAR delta (PPARδ) agonists were shown to inhibit hyperglycemia-induced proinflammatory cytokine expression in kidney cells and improve rat mesangial cell (RMC) survival rate.[14] PPARδ also exhibits a renoprotective role by its downstream signaling including RAGE and NF-κB pathway.[15] Some reports revealed PPARδ activation attenuates proteinuria by restoring podocytopathy and rescuing nephrin loss in type 2 diabetic animal model.[16] At present, PPARδ agonist has potential to become a promising treatment for the patient with diabetic nephropathy.

Glucagon-like peptide-1 (GLP-1) is a kind of hormone, also named incretin, released from enteroendocrine L cells.[17] GLP-1 can decrease blood glucose by suppressing glucagon secretion.[18] At present, GLP-1 agonists have been widely used clinically to

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control blood glucose levels in diabetes patients.[19] GLP-1 was also reported to have effect in attenuating oxidative damage via Nrf2 signaling[20] and inhibiting AGE-induced upregulation of inflammatory mediators.[14] Recombinant human GLP-1 exhibits renoprotective effect by alleviation of tubulointerstitial injury via inhibiting phosphorylation of MAPK and nuclear factor-kappa B without influencing fasting blood glucose or body weight.[21] On the other hand, neuroprotection effect of GLP-1 was found to enhance autophagy in a parkinsonian rat model.[22]

In previous studies, both GLP-1 agonists (exendin-4) and PPARδ agonists (L-165 041) showed anti-inflammatory effect in attenuated AGE-induced interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) production, RAGE mRNA and protein expression, and cell death in RMCs.[14] Synergic effect of exendin-4 and L-165 041 in inhibiting cytokines production was also found. The interaction among PPARδ agonists, GLP-1, and AGE-RAGE axis is still unclear. This study tries to investigate the relationship among them.

2. METHODS

2.1. Cell culture and reagents

RMCs were incubated in low glucose (5.56 mM) media Dulbecoo's modified Eagle's medium (GIBCO 10567) with 10% fetal bovine serum (FBS) at 37 °C in humidified atmosphere with 5% CO_2. The experiments were performed after 4 to 6 passages and 80% confluence. When the mesangial cells showed aggregate growth and fuse gradually, we switched the cells to serum-free medium. L-165 041 is a type of PPARδ agonist from Sigma.[14] We used 1-μM L-165 041 in the following experiment.[14]

2.2. Preparation and characterization of AGEs

AGEs were produced by incubating 10 mg/mL of fatty acid-free bovine serum albumin (BSA) with 25-mM glyceraldehyde and 1-mM diethylenetriamine pentad acetic acid in 0.1 M phosphate-buffered saline (pH 7.4) at 37 °C for 7 days. Unbound sugars were removed by dialysis in 10-mM phosphate-buffered saline (pH 7.4) for 24 hours. The protein content was determined by Lowry assay, using BSA as the standard. Estimation of AGE content by spectrofluorometry with excitation wavelength of 390 nm and emission wavelength of 450 nm revealed a 6.5-fold increase in fluorescence for AGE–BSA compared with control BSA. According to the concentration pretest experiments, a concentration of 200 μM of AGE was chosen for the following experiments.[15]

2.3. RNA isolation and reverse transcription

The protocol was described previously.[15] Total cellular RNA was isolated from RMC using the single-step acid guanidinium thiocyanate/phenol/chloroform extraction method. For reverse transcription, 1 μg of RNA was incubated with 200 U of HiScript I reverse transcriptase (Bionovas Biotechnology, Toronto, Canada) in a buffer containing a final concentration of 20 mmol/L Tris/HCl (pH 7.8), 100 mmol/L NaCl, 0.1 mmol/L Ethylenediaminetetraacetic acid, 1 mmol/L Dithiothreitol, 50% glycerol, 2.5 mol/l poly (dT)12–18 oligomer, and 0.5 mmol/L of each dNTP at a final volume of 20 μL. The reaction mixture was incubated at 45 °C for 1 hour and then at 70 °C for 15 minutes to inactivate the enzyme. The produced cDNA was used to generate DNA product by polymerase chain reaction (PCR).

2.4. Real-time PCR

The cDNA had a 10-fold dilution in nuclease-free water and was used for the Smart Quant Green Master Mix (Protech Technology Enterprise Co., Taipei, Taiwan): 2 μL of cDNA solution, 0.5 μmol/L primers, 5 mmol/L magnesium chloride, and 2 μL of Master SYBRGreen in nuclease-free water with a final volume of 20 μL. The initial denaturizing phase was 5 minutes at 95 °C followed by an amplification phase as detailed below: denaturation at 95 °C for 10 seconds, annealing at 55 °C for 10 seconds, elongation at 72 °C for 15 seconds, and detection at 79 °C for 45 cycles. Amplification, fluorescence detection, and postprocessing calculation were performed using the Applied Biosystems Incorporation step 1 apparatus. The primers used for PCR were: RAGE: forward, 5′ AAGCCCCTGGTGCCTAATGAG3′, reverse, 5′CACCAATTGGACCTCCTCCA3′; GLP-1: forward, 5′CATTCACAGGGCACATTCACC3′, reverse, 5′ACCAGCCAAGCAATGAATTCCTT3′; GAPDH: forward, 5′AGACAGCCGCATCTTCTTGT3′, reverse, 5′TTCCCATTCTCAGCCTTGAC3′. Individual PCR product was analyzed for DNA sequence to confirm the purity of the product.[14]

2.5. RNA interference

The protocol was described previously.[14] RMCs were transfected with 800-ng PPARδ annealed siRNA oligonucleotide (sc-36 306) or siRNA of green fluorescent protein (GFP). PPARδ siRNA is a pool of three target-specific 20 to 25 nt siRNA according to a computer program provided by Santa Cruz. GLP-1 receptor (GLP-1R) siRNA was sc-45 760 from Santa Cruz. The negative control, GFP siRNA was used: sense: 5′–GGCUACGUCCAGGAGCGCACC; antisense: 5′–UGCGCUCCUGGACGUAGCCUU (Dharmacon Inc., Lafayette, CO, USA).

2.6. Western blot analysis

Total protein samples were mixed with sample buffer, boiled for 5 minutes, separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis under denaturing conditions, and electroblotted to nitrocellulose membranes (Amersham Pharmacia Biotech, Chicago, IL, USA). The nitrocellulose membranes were blocked in blocking buffer, incubated with human anti-GLP-1R or anti-RAGE (Santa Cruz Biotechnology, Santa Cruz, CA, USA) antibodies, washed, and incubated with horseradish peroxidase-conjugated secondary antibodies. Signals were visualized by enhanced chemiluminescent detection.[15]

2.7. Cell viability test

RMC were seeded onto 96-well plates in medium containing 10% FBS and then incubated with L-165 041, or control medium alone in 5% CO_2 for 18 hours at 37 °C. The cell viability was determined using the Water-Soluble Tetrazolium 8 assay kit (Kishida Chemical Co., Ltd., Japan) following the manufacturer's instructions.[14]

2.8. Statistical analysis

The data were expressed as mean ± SEM. A Tukey test was used for comparing parametric variables between the two groups, while analysis of variance with repeat measurement design was used for time-course changes. Statistical significance was evaluated by Tukey test (GraphPad Software Inc., San Diego, CA, USA). A p-value of less than 0.05 was considered significant.

3. RESULTS

3.1. PPARδ agonist (L-165 041) reversed the increment of GLP-1 mRNA induced by AGE, but had no effect on GLP-1 protein

In previous study, L-165 041 attenuated AGE-induced IL-6 production with dose-dependent manner in RMC. GLP-1 also

had the same effect of diminishing AGE-induced IL-6 expression. Furthermore, L-165 041 and GLP-1 showed synergic effect in inhibiting IL-6 and TNF-α production.[14] To clarify the relationship between GLP-1 and PPARδ agonists, we examined the GLP-1 mRNA expression level treated with AGE then L-165 041. As shown in Fig. 1A, we measured GLP-1 mRNA expression in RMC by real-time PCR, we found GLP-1 mRNA levels increased significantly by AGE but attenuated by coadministration of L-165 041 (Fig. 1A). Next, we measured the GLP-1 protein expression to examine the effect of AGE treatment with/without L-165 041. As shown in Fig. 1B, compared with the control group, GLP-1 protein expressions showed insignificant changes under treatment with AGE or coadministration of AGE and L-165 041 (Fig. 1B). It may be due to the different time points of protein expression than mRNA.

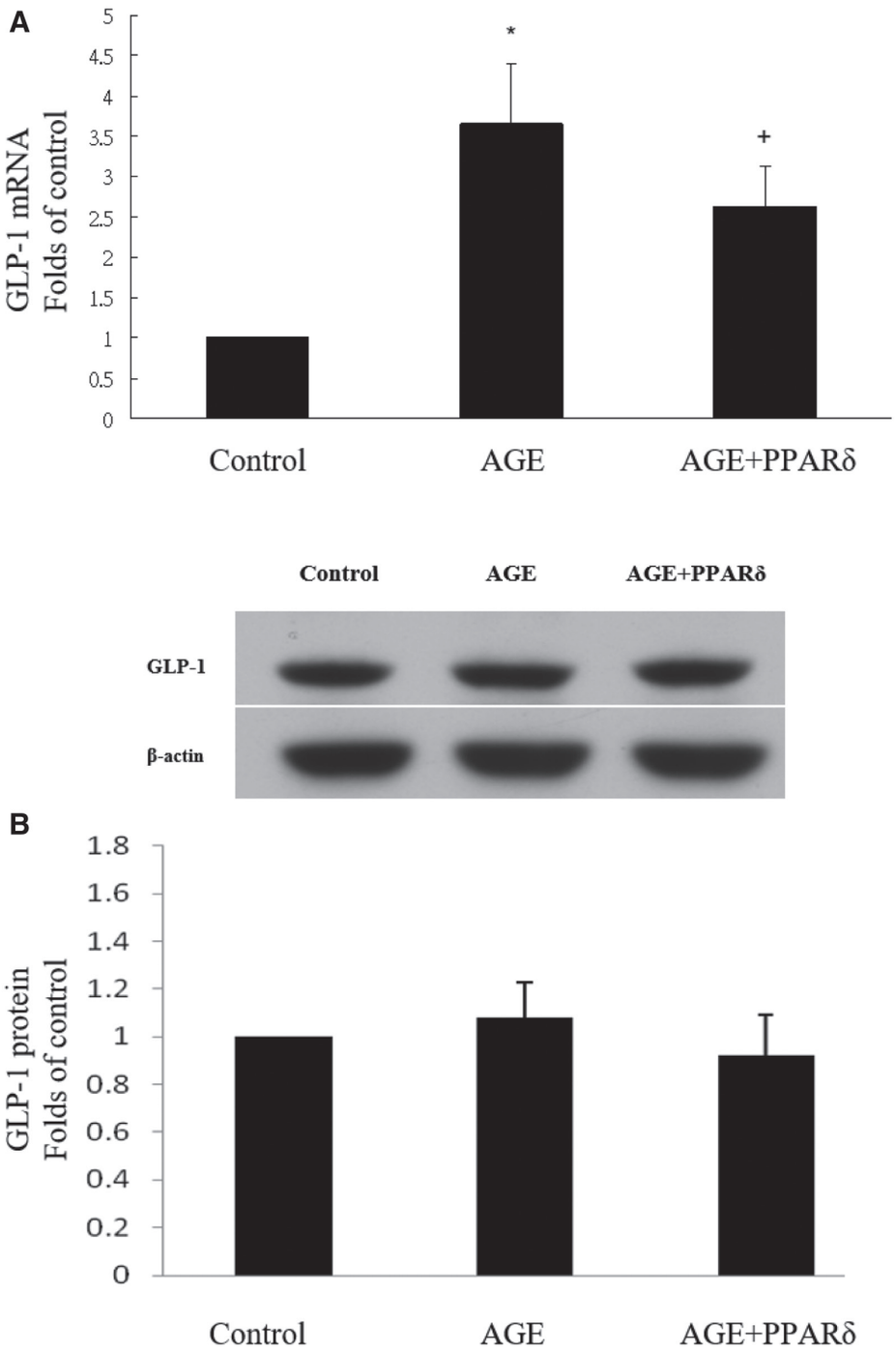

Fig. 1 AGE increased GLP-1 mRNA expression but attenuated by giving PPARδ agonist (L-165 041) (A). Coadministration of AGE and L-165 041 showed insignificant changes in GLP-1 protein (B). *$p < 0.05$ when compared with control. +$p < 0.05$ when compared with AGE group. AGE = advanced glycation end product; GLP-1 = glucagon-like peptide-1; PPARδ = peroxisome proliferator-activated receptor delta.

3.2. GLP-1R existed in rat renal mesangial cell

Because of AGE/L-165 041 treating GLP-1 protein expression showing insignificant increase, we examined the expression of GLP-1R. Using real-time PCR, we were able to identify the mRNA expression in RMC. Therefore, we confirmed the existence of GLP-1R in RMC (Fig. 2).

3.3. PPARδ agonist (L-165 041) increased GLP-1R mRNA and protein expressions in rat renal mesangial cell pretreated with AGE

We used real-time PCR to observe a series of expressions of GLP-1R mRNA in different conditions. First, compared with the control group, RMC pretreated with AGE or L-165 041 alone showed increased insignificantly expression of GLP-1R mRNA. But GLP-1R mRNA expression enhancement could be seen significantly in RMC pretreated with AGE followed by administration of L-165 041. This increment was attenuated significantly by giving siRNA of PPARδ. Then, we used siRNA of GFP to confirm the role of L-165 041 (Fig. 3A).

Moreover, we also wanted to evaluate GLP-1R protein expression by using Western blot. We observed a significant increase of GLP-1R protein expression in RMC with coadministration of AGE and L-165 041. But this elevation was reversed after giving siRNA of PPARδ. Insignificant increase of GLP-1R protein expressions was observed in both L-165 041 alone or AGE alone group (Fig. 3B).

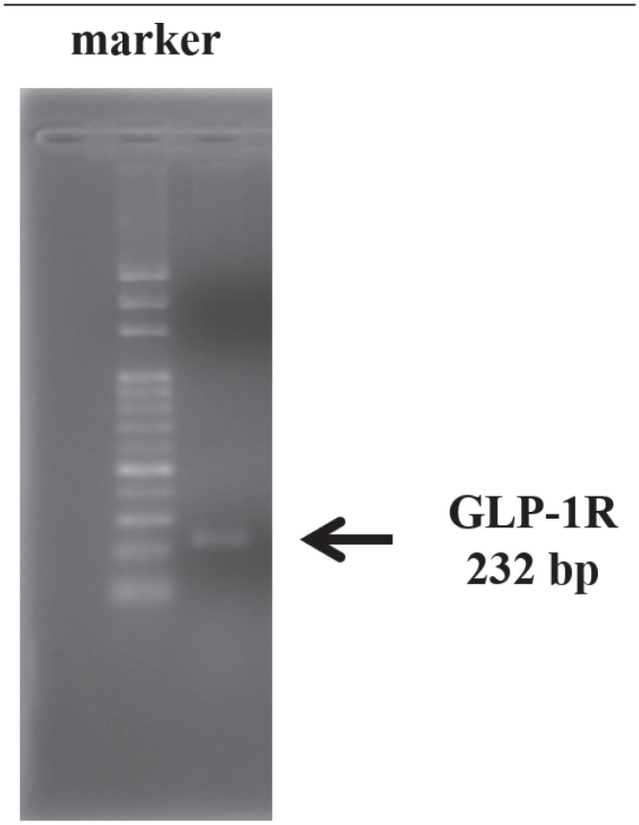

Fig. 2 GLP-1R mRNA was identified in rat renal mesangial cell by real-time PCR. GLP-1R = glucagon-like peptide-1 receptor; PCR = polymerase chain reaction.

3.4. GLP-1R played a role for PPARδ agonist (L-165 041) inhibiting AGE-induced RAGE mRNA and protein expressions

RAGE and AGE are keys for causing diabetic nephropathy. We examined the role of GLP-1R in AGE-induced RAGE upregulation. As shown in Fig. 4, AGE significantly induced RAGE mRNA (Fig. 4A) and protein (Fig. 4B) expressions in RMC. L-165 041 significantly attenuated AGE-induced RAGE mRNA and protein levels. This inhibitory effect of L-165 041 was reversed significantly by treating siRNA of GLP-1R. In addition, RAGE mRNA and protein expressions in group with administration of siRNA of PPARδ without L-165 041 had similar levels compared with the expressions in AGE group with coadministration of both siRNA of PPARδ and L-165 041 (Fig. 4A, B).

3.5. GLP-1R had a role in the regulation of cell viability through PPARδ agonist (L-165 041) and AGE

In previous study, we knew AGE could induce cell apoptosis, including mesangial cell. Here, we monitored the cell viability of RMC by WST-1 under some treatments. As shown in Fig. 5, AGE significantly decreased RMC viability. And L-165 041 significantly attenuated AGE-induced cell death. This inhibitory effect of L-165 041 was significantly reversed by giving siRNA of GLP-1R. Only presentation of siRNA of GLP-1R without L-165 041 showed no changes compared with coadministration of L-165 041 and siRNA of GLP-1R.

4. DISCUSSION

This study shows that PPARδ agonists significantly increase GLP-1R expression on RMC cells in the presence of AGE and inhibit AGE-enhanced RAGE mRNA and protein expression. The inhibition of PPARδ agonists on RAGE expression can be reversed by siRNA of GLP-1R. The AGE-induced cell death is also prevented by PPARδ agonists, and the effect of PPARδ agonists is diminished by siRNA of GLP-1R.

PPARδ regulates important cellular metabolic functions that contribute to maintaining energy balance.[11] The anti-inflammatory and antiapoptosis effects of PPARδ and GLP-1R agonists have been shown in many previous studies. Activation of PPARδ by PPARδ agonists in human umbilical cord vein cells attenuated endoplasmic reticulum (ER) stress induced by the plasma from patients with lupus nephritis.[23] PPARδ agonists also attenuated C-reactive protein-induced proinflammation in cardiomyocytes[24] and AGE-induced apoptosis in human embryonic kidney cells (HEK293).[25] GLP-1 was shown to attenuate AGE-induced RAGE mRNA expression and oxidative stress in human proximal tubular epithelial[26] and mesangial cells.[27] A GLP-1R agonist,exendin-4, also inhibited high glucose (30 mmol/L)-induced transforming growth factor-β1 and connective tissue growth factor expression in human mesangial cells.[28] In clinical studies with obese children and adolescents, increased serum proinflammatory cytokines showed negative correlation with GLP-1R or PPARα levels in leukocytes.[29] A GLP-1R agonist, semaglutide, was shown to reduce vascular inflammation in a rabbit model of advanced atherosclerosis in a study using positron emission tomography as an investigating tool.[30]

Few papers talked about the interaction between GLP-1 and PPARδ. A PPARδ agonist, GW501516, enhances glucose- and bile acid-induced GLP-1 release by intestinal L cells *in vitro*. GW501516 also enhanced the increase in plasma GLP-1 level after an oral glucose load in wild-type and diabetic mice.[31]

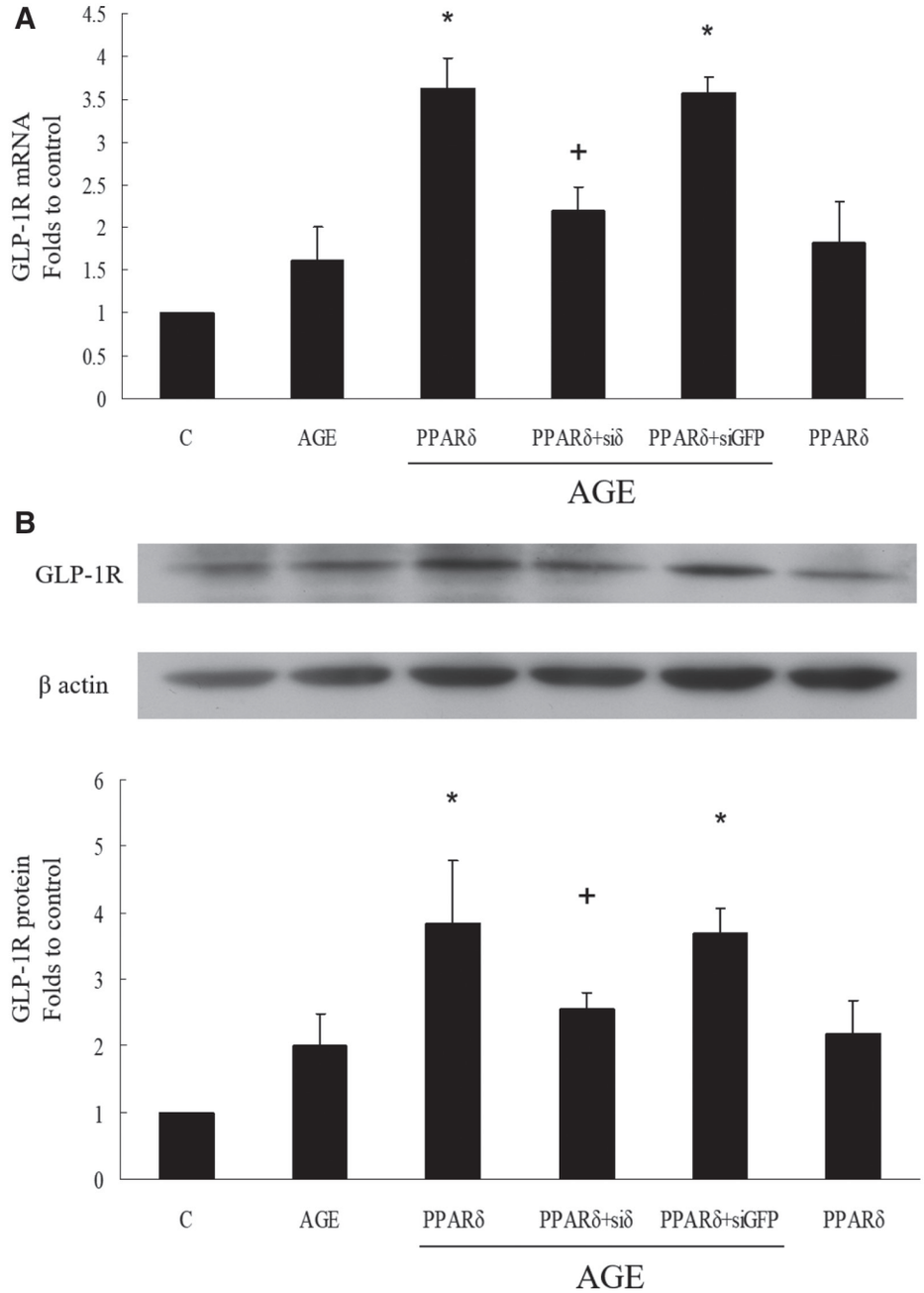

Fig. 3 GLP-1R mRNA (A) and protein (B) expressions in RMC treated with AGE and PPARδ agonist (L-165 041). L-165 041 increased GLP-1R mRNA and protein expressions with existence of AGE. It could be attenuated with siRNA of PPARδ administration. *$p < 0.05$ when compared with AGE group. +$p < 0.05$ when compared with AGE with PPARδ group. AGE = advanced glycation end product; GLP-1R = glucagon-like peptide-1 receptor; PPARδ = peroxisome proliferator-activated receptor delta; RMC = rat mesangial cell.

Activation of PPARδ or GLP-1R is all reported to exhibit a protective effect against lipotoxic apoptosis in pancreatic β cells.[32] GW501516 decreased apoptosis in isolated rat β cells and robustly stimulated GLP-1R expression under palmitate-treated lipotoxic conditions. In this study, PPARδ agonist L-165 041 increased GLP-1R receptor expression and inhibited AGE-induced RAGE upregulation and cell death through the help of GLP-1R. The results are compatible with previous studies.

The synergistic effect of GLP-1 and PPARδ agonists was reported in previous studies. Both exendin-4 (a GLP-1R agonist) and L-165 041 (a PPARδ agonist) significantly attenuated AGE-induced IL-6 and TNF-α production, RAGE expression, and cell death in RMC. Similar anti-inflammatory potency was seen between 0.3-nM exendin-4 and 1-μM L-165 041. Synergic effect of exendin-4 and L-165 041 was shown in inhibiting cytokines production.[14] In a clinical study, combined GLP-1R agonist exenatide and PPARγ agonist pioglitazone significantly greater

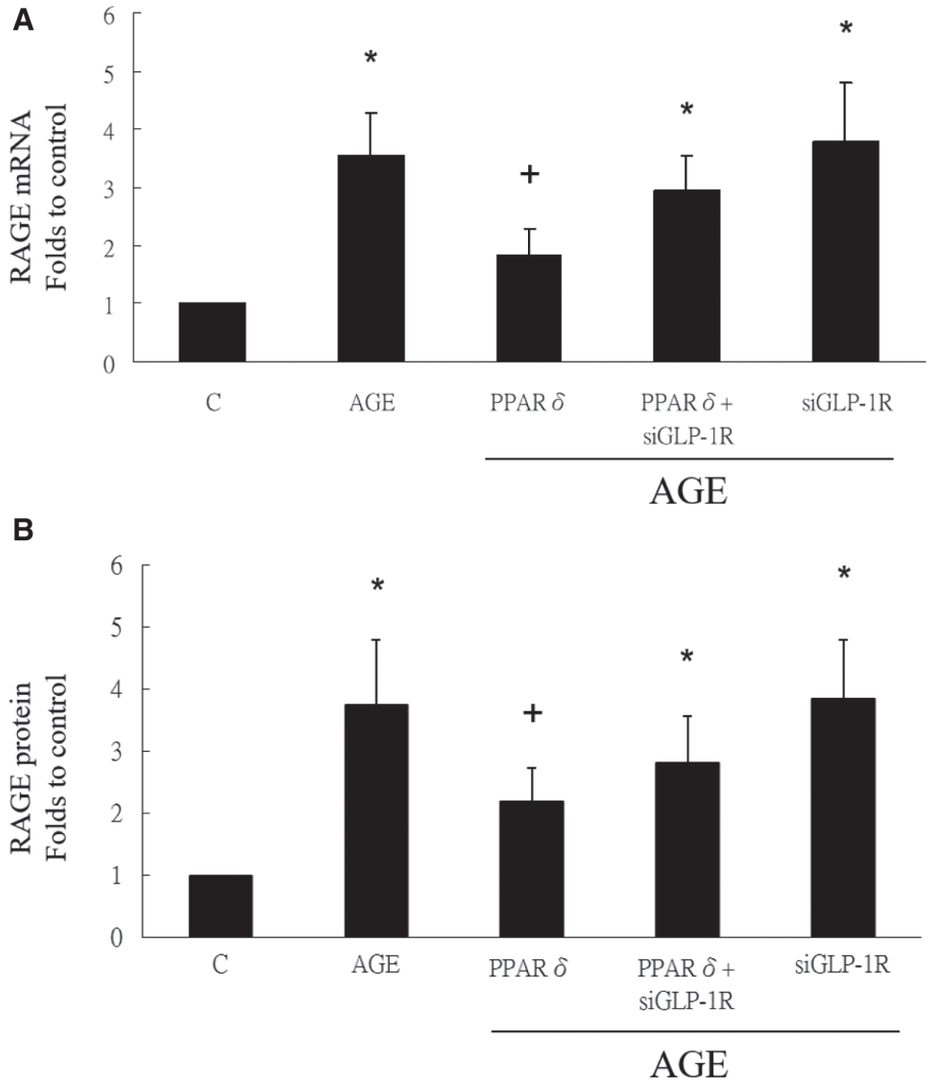

Fig. 4 GLP-1R played a role for PPARδ agonist (L-165 041) inhibiting AGE-induced RAGE mRNA (A) and protein (B) expressions. AGE increased RAGE mRNA and protein expressions. But L-165 041 significantly attenuated the AGE-induced RAGE expression. The inhibitory of L-165 041 was revered by siRNA of GLP-1R. *$p < 0.05$ when compared with control. +$p < 0.05$ when compared with AGE group. AGE = advanced glycation end product; GLP-1R = glucagon-like peptide-1 receptor; GLP-1R = glucagon-like peptide-1 receptor; PPARδ = peroxisome proliferator-activated receptor delta; RAGE = receptor for AGE.

decrease in hepatic fat and plasma triglyceride than pioglitazone alone in type 2 diabetic patients.[33]

This study showed PPARδ activation diminished AGE-induced RAGE upregulation and cell death, and the effect of PPARδ agonists should be supported by the presence of GLP-1R. Adenosine monophosphate-activated protein kinase (AMPK) may play a major role in the interaction between PPARδ and GLP-1R. AMPK is a serine/threonine protein kinase and known as a cellular energy sensor to restore energy homeostasis at cell levels in conditions of metabolic stress.[34] Activation of AMPK inhibits mammalian Target Of Rapamicyn (mTOR) activity and prevents protein synthesis as well as cell growth.[35] mTOR is a serine/threonine protein kinase and plays a key role in regulating the growth and division of cells.[36] Inhibition of mTOR is going to inhibit cell proliferation and decrease inflammation. Activation of PPARδ was shown to increase AMPK phosphorylation and decrease ER stress-induced oxidative stress.[37] AMPK was reported to mediate PPARδ effect on bone regeneration,[38] insulin sensitivity,[39] and inflammation.[40] Activation of GLP-1R was also shown to have anti-inflammatory[41] and cardioprotective[42] effect through AMPK. The effects of PPARδ activation in inhibiting RAGE expression and increasing cell viability may be mediated through AMPK with cooperation of GLP-1R activation.

As far as we know, this is the first paper to investigate the influence of PPARδ agonists on the expression of GLP-1R and RAGE in the presence of AGE and the role of GLP-1R in cytoprotective effect of PPARδ. Further studies are needed to evaluate the underlying signaling pathways responsible for the interaction between PPARδ and GLP-1R.

In conclusion, both PPARδ and GLP-1R agonists play important roles in attenuating AGE-RAGE axis reaction and RMC survival. Through siRNA technique, we find the relationship between PPARδ and GLP-1R in anti-inflammatory response. PPARδ has anti-inflammatory effect to ameliorate RAGE expression by upregulation of downstream GLP-1R expression, and AMPK may play a major role in the interaction between PPARδ and GLP-1R. This is the first time to demonstrate

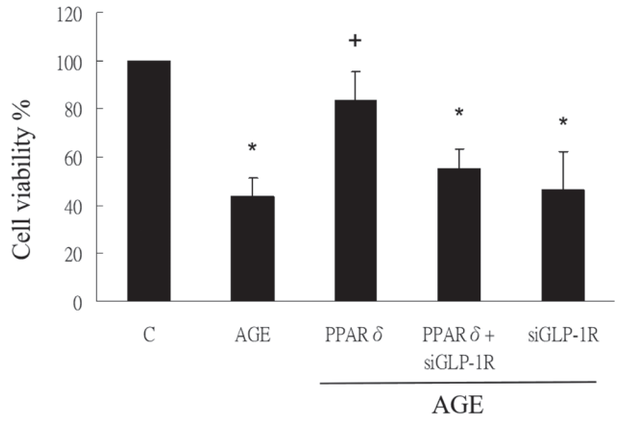

Fig. 5 GLP-1R had a role in the regulation of cell viability through PPARδ agonist (L-165 041) and AGE. AGE decreased cell survival rate, but it could be reversed by treating L-165 041. However, L-165 041's effect improving cell viability would be attenuated by giving siRNA of GLP-1R. *$p < 0.05$ when compared with control. +$p < 0.05$ when compared with AGE group. AGE = advanced glycation end product; GLP-1R = glucagon-like peptide-1 receptor; PPARδ = peroxisome proliferator-activated receptor delta.

PPAR-GLP-1R-RAGE axis in anti-inflammatory process. This finding may be a pharmaceutical development target in treating diabetes and its inflammatory complications in the future. Further studies are needed to further identify the correlation among PPARδ, GLP-1R, and other signaling pathways.

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REFERENCES

1. Brown WV. Microvascular complications of diabetes mellitus: renal protection accompanies cardiovascular protection. *Am J Cardiol* 2008;**102**:10L–3L.
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF diabetes atlas: global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. *Diabetes Res Clin Pract* 2022;**183**:109119.
3. Ghaderian SB, Hayati F, Shayanpour S, Beladi Mousavi SS. Diabetes and end-stage renal disease; a review article on new concepts. *J Renal Inj Prev* 2015;**4**:28–33.
4. Sanajou D, Ghorbani Haghjo A, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: current status and future directions. *Eur J Pharmacol* 2018;**833**:158–64.
5. Kim HJ, Jeong MS, Jang SB. Molecular characteristics of RAGE and advances in small-molecule inhibitors. *Int J Mol Sci* 2021;**22**:6904.
6. Hajam YA, Rani R, Ganie SY, Sheikh TA, Javaid D, Qadri SS, et al. Oxidative stress in human pathology and aging: molecular mechanisms and perspectives. *Cells* 2022;**11**:552.
7. Kay AM, Simpson CL, Stewart JA Jr. The role of AGE/RAGE signaling in diabetes-mediated vascular calcification. *J Diabetes Res* 2016;**2016**:6809703.
8. Burr SD, Stewart JA Jr. Extracellular matrix components isolated from diabetic mice alter cardiac fibroblast function through the AGE/RAGE signaling cascade. *Life Sci* 2020;**250**:117569.
9. Wang CC, Lee AS, Liu SH, Chang KC, Shen MY, Chang CT. Spironolactone ameliorates endothelial dysfunction through inhibition of the AGE/RAGE axis in a chronic renal failure rat model. *BMC Nephrol* 2019;**20**:351.
10. Christofides A, Konstantinidou E, Jani C, Boussiotis VA. The role of peroxisome proliferator-activated receptors (PPAR) in immune responses. *Metabolism* 2021;**114**:154338.
11. Liu Y, Colby JK, Zuo X, Jaoude J, Wei D, Shureiqi I. The role of PPAR-delta in metabolism, inflammation, and cancer: many characters of a critical transcription factor. *Int J Mol Sci* 2018;**19**:3339.
12. Hall JA, Ramachandran D, Roh HC, DiSpirito JR, Belchior T, Zushin PH, et al. Obesity-linked ppargamma s273 phosphorylation promotes insulin resistance through growth differentiation factor 3. *Cell Metab* 2020;**32**:665–75.e6.
13. Bougarne N, Weyers B, Desmet SJ, Deckers J, Ray DW, Staels B, et al. Molecular actions of PPARalpha in lipid metabolism and inflammation. *Endocr Rev* 2018;**39**:760–802.
14. Chang JT, Liang YJ, Hsu CY, Chen CY, Chen PJ, Yang YF, et al. Glucagon-like peptide receptor agonists attenuate advanced glycation end products-induced inflammation in rat mesangial cells. *BMC Pharmacol Toxicol* 2017;**18**:67.
15. Liang YJ, Chen SA, Jian JH. Peroxisome proliferator-activated receptor delta downregulates the expression of the receptor for advanced glycation end products and pro-inflammatory cytokines in the kidney of streptozotocin-induced diabetic mice. *Eur J Pharm Sci* 2011;**43**:65–70.
16. Lee EY, Kim GT, Hyun M, Kim S, Seok S, Choi R, et al. Peroxisome proliferator-activated receptor-delta activation ameliorates albuminuria by preventing nephrin loss and restoring podocyte integrity in type 2 diabetes. *Nephrol Dial Transplant* 2012;**27**:4069–79.
17. Lebrun LJ, Lenaerts K, Kiers D, Pais de Barros JP, Le Guern N, Plesnik J, et al. Enteroendocrine L cells sense LPS after gut barrier injury to enhance GLP-1 secretion. *Cell Rep* 2017;**21**:1160–8.
18. Muller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-like peptide 1 (GLP-1). *Mol Metab* 2019;**30**:72–130.
19. Xie Y, Bowe B, Gibson AK, McGill JB, Maddukuri G, Yan Y, et al. Comparative effectiveness of SGLT2 inhibitors, GLP-1 receptor agonists, DPP-4 inhibitors, and sulfonylureas on risk of kidney outcomes: emulation of a target trial using health care databases. *Diabetes Care* 2020;**43**:2859–69.
20. Oh YS, Jun HS. Effects of glucagon-like peptide-1 on oxidative stress and Nrf2 signaling. *Int J Mol Sci* 2017;**19**:26.
21. Yin W, Xu S, Wang Z, Liu H, Peng L, Fang Q, et al. Recombinant human GLP-1(rhGLP-1) alleviating renal tubulointestitial injury in diabetic STZ-induced rats. *Biochem Biophys Res Commun* 2018;**495**:793–800.
22. Bu LL, Liu YQ, Shen Y, Fan Y, Yu WB, Jiang DL, et al. Neuroprotection of exendin-4 by enhanced autophagy in a parkinsonian rat model of alpha-synucleinopathy. *Neurotherapeutics* 2021;**18**:962–78.
23. Toral M, Jimenez R, Romero M, Robles-Vera I, Sanchez M, Salaices M, et al. Role of endoplasmic reticulum stress in the protective effects of PPARbeta/delta activation on endothelial dysfunction induced by plasma from patients with lupus. *Arthritis Res Ther* 2017;**19**:268.
24. Liang YJ, Chen CY, Juang SJ, Lai LP, Shyu KG, Wang BW, et al. Peroxisome proliferator-activated receptor delta agonists attenuated the c-reactive protein-induced pro-inflammation in cardiomyocytes and H9c2 cardiomyoblasts. *Eur J Pharmacol* 2010;**643**:84–92.
25. Liang YJ, Jian JH, Liu YC, Juang SJ, Shyu KG, Lai LP, et al. Advanced glycation end products-induced apoptosis attenuated by PPARdelta activation and epigallocatechin gallate through NF-kappaB pathway in human embryonic kidney cells and human mesangial cells. *Diabetes Metab Res Rev* 2010;**26**:406–16.
26. Park CW, Kim HW, Ko SH, Lim JH, Ryu GR, Chung HW, et al. Long-term treatment of glucagon-like peptide-1 analog exendin-4 ameliorates diabetic nephropathy through improving metabolic anomalies in db/db mice. *J Am Soc Nephrol* 2007;**18**:1227–38.
27. Ishibashi Y, Nishino Y, Matsui T, Takeuchi M, Yamagishi S. Glucagon-like peptide-1 suppresses advanced glycation end product-induced monocyte chemoattractant protein-1 expression in mesangial cells by reducing advanced glycation end product receptor level. *Metabolism* 2011;**60**:1271–7.
28. Li W, Cui M, Wei Y, Kong X, Tang L, Xu D. Inhibition of the expression of TGF-beta1 and CTGF in human mesangial cells by exendin-4, a glucagon-like peptide-1 receptor agonist. *Cell Physiol Biochem* 2012;**30**:749–57.
29. Vargas-Sanchez K, Vargas L, Urrutia Y, Beltran I, Rossi AB, Lozano HY, et al. PPARalpha and PPARbeta/delta are negatively correlated with pro-inflammatory markers in leukocytes of an obese pediatric population. *J Inflamm (Lond)* 2020;**17**:35.
30. Jensen JK, Binderup T, Grandjean CE, Bentsen S, Ripa RS, Kjaer A. Semaglutide reduces vascular inflammation investigated by PET in a rabbit model of advanced atherosclerosis. *Atherosclerosis* 2022;**352**:88–95.
31. Daoudi M, Hennuyer N, Borland MG, Touche V, Duhem C, Gross B, et al. PPARbeta/delta activation induces enteroendocrine L cell GLP-1 production. *Gastroenterology* 2011;**140**:1564–74.

32. Yang Y, Tong Y, Gong M, Lu Y, Wang C, Zhou M, et al. Activation of PPARbeta/delta protects pancreatic beta cells from palmitate-induced apoptosis by upregulating the expression of GLP-1 receptor. *Cell Signal* 2014;**26**:268–78.
33. Sathyanarayana P, Jogi M, Muthupillai R, Krishnamurthy R, Samson SL, Bajaj M. Effects of combined exenatide and pioglitazone therapy on hepatic fat content in type 2 diabetes. *Obesity (Silver Spring)* 2011;**19**:2310–5.
34. Chomanicova N, Gazova A, Adamickova A, Valaskova S, Kyselovic J. The role of AMPL/mTOR signaling pathway in anticancer activity of metformin. *Physiol Res* 2021;**70**:501–8.
35. Bourouh M, Marignani PA. The tumor suppressor kinase LKB1: metabolic nexus. *Front Cell Dev Biol* 2022;**10**:881297.
36. Zaidi S, Gandhi J, Joshi G, Smith NL, Khan SA. The anticancer potential of metformin on prostate cancer. *Prostate Cancer Prostatic Dis* 2019;**22**:351–61.
37. Nie X, Tang W, Zhang Z, Yang C, Qian L, Xie X, et al. Procyanidin B2 mitigates endothelial endoplasmic reticulum stress through a PPARdelta-dependent mechanism. *Redox Biol* 2020;**37**:101728.
38. Chen M, Jing D, Ye R, Yi J, Zhao Z. PPARbeta/delta accelerates bone regeneration in diabetic mellitus by enhancing AMPK/mTOR pathway-mediated autophagy. *Stem Cell Res Ther* 2021;**12**:566.
39. Aguilar-Recarte D, Palomer X, Wahli W, Vazquez-Carrera M. The PPARbeta/delta-AMPK connection in the treatment of insulin resistance. *Int J Mol Sci* 2021;**22**:8555.
40. Barroso E, Eyre E, Palomer X, Vazquez-Carrera M. The peroxisome proliferator-activated receptor beta/delta (PPARbeta/delta) agonist GW501516 prevents TNF-alpha-induced NF-kappaB activation in human HaCaT cells by reducing p65 acetylation through AMPK and SIRT1. *Biochem Pharmacol* 2011;**81**:534–43.
41. Song S, Guo R, Mehmood A, Zhang L, Yin B, Yuan C, et al. Liraglutide attenuate central nervous inflammation and demyelination through AMPK and pyroptosis-related NLRP3 pathway. *CNS Neurosci Ther* 2022;**28**:422–34.
42. Ma G, Liu Y, Wang Y, Wen Z, Li X, Zhai H, et al. Liraglutide reduces hyperglycemia-induced cardiomyocyte death through activating glucagon-like peptide 1 receptor and targeting AMPK pathway. *J Recept Signal Transduct Res* 2020;**40**:133–40.