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Short Communication

Advanced glycation end products evoke inflammatory reactions in proximal tubular cells via autocrine production of dipeptidyl peptidase-4

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ABSTRACT

We have previously shown that albuminuria and renal levels of advanced glycation end products (AGEs), receptor for AGEs (RAGE), and oxidative stress are suppressed in dipeptidyl peptidase-4 (DPP-4)-deficient diabetic rats, thus suggesting the crosstalk between AGE-RAGE axis and DPP-4 in experimental diabetic nephropathy. Therefore, we examined here the role of DPP-4 in AGE-evoked inflammatory reactions in human proximal tubular cells. Proteins were extracted from proximal tubular cells, and conditioned medium was collected, both of which were subjected to western blot analysis using anti-DPP-4 antibody. RAGE-aptamer was prepared using a systemic evolution of ligands by exponential enrichment. NF-κB p65 and monocyte chemoattractant protein-1 (MCP-1) gene expression was analyzed by reverse transcription-polymerase chain reaction. AGEs significantly increased DPP-4 expression and soluble DPP-4 production by tubular cells, the latter of which was attenuated by RAGE-aptamer or an anti-oxidant, *N*-acetylcysteine. AGEs or DPP-4 up-regulated NF-κB p65 or MCP-1 mRNA levels in tubular cells, which were suppressed by linagliptin, an inhibitor of DPP-4. AGEs stimulated NF-κB p65 gene expression in tubular cells isolated from control rats, but not from DPP-4-deficient rats. Our present results suggest that the AGE-RAGE-mediated oxidative stress could evoke inflammatory reactions in proximal tubular cells via autocrine production of DPP-4.

1. Introduction

Dipeptidyl peptidase-4 (DPP-4) inhibitors are one of the widely used oral anti-hyperglycemic agents in the world (Yamagishi et al., 2015). Since DPP-4 inhibitors can augment incretin effects by blocking the degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide, they not only enhance glucose-induced insulin secretion, but also suppress glucagon overproduction in diabetes, thereby ameliorating hyperglycemia (Yamagishi et al., 2015). DPP-4 is also involved in the cleavage of *N*-terminal amino acids from several chemokines and neuropeptides other than incretins (Ishibashi et al., 2013), and highly expressed in renal proximal tubular cells, whose expression is up-regulated in human diabetic nephropathy (Shakovska et al., 2014). In addition several papers have shown the protective role of DPP-4 inhibitors against diabetic nephropathy in type 1 diabetic animals, even though they did not ameliorate hyperglycemia (Avogaro and Fadini, 2014; Nakashima et al., 2014; Yamagishi and Matsui, 2011). These findings suggest the pleiotropic effects, *in other words*, glucose-lowering independent beneficial effects of DPP-4 inhibitors on experimental diabetic nephropathy.

There is accumulating evidence to show that advanced glycation end products (AGEs) and their receptor RAGE play a central role in the pathogenesis of diabetic nephropathy (Forbes and Cooper, 2012; Ramasamy et al., 2012; Yamagishi et al., 2015). We have previously shown that DPP-4-deficient type 1 diabetic rats exhibits decreased levels of AGEs, RAGE and oxidative stress in the kidneys, which is associated with reduction of albuminuria and suppression of glomerular area expansion (Matsui et al., 2015). Since there were no significant differences of blood glucose levels or lipid parameters between DPP-4-deficient diabetic rats and wild-type diabetic animals, lack of enzymatic

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activity of DPP-4 may confer renoprotection in these animals, which may be partly mediated by the inhibition of AGE-RAGE axis in the diabetic kidneys. However, a direct crosstalk between AGE-RAGE axis and DPP-4 in renal cellular constituents remains largely unknown. Therefore, in this study, we examined the pathological role of DPP-4 in AGE-evoked inflammatory reactions in human proximal tubular cells.

2. Materials and methods

2.1. Materials

N-acetylcysteine (NAC) and human DPP-4 were purchased from Sigma (St. Louis, MO) and R&D systems (Minneapolis, MN), respectively. Linagliptin, an inhibitor of DPP-4 was generously gifted from Boehringer Ingelheim (Ingelheim, Germany). Polyclonal or monoclonal antibody directed against human DPP-4 or β-actin, respectively were from Santa Cruz Biotechnology Inc. (Delaware, CA).

2.2. Preparation of AGEs and RAGE-aptamer

AGEs were prepared by incubating bovine serum albumin (BSA) with 0.1 M D-glyceraldehyde as described previously (Ishibashi et al., 2013). Non-glycated BSA was used as a control (Ishibashi et al., 2013). Preparation and section of DNA-aptamer raised against RAGE (RAGE-aptamer) were performed using systemic evolution of ligands by exponential enrichment; its sequence was 5′-TgTAgcccgAgTATcATTcTccATcgcccccAgATAcAAG-3′, where phosphorothioate nucleotides are indicated as capital letters (Matsui et al., 2017). We have already reported that RAGE-aptamer decreases the binding of AGEs to RAGE by about 30% (Matsui et al., 2017).

2.3. Cell culture

Human proximal tubular cells or isolated rat tubular cells were treated with or without 100 μg/ml AGEs, 100 μg/ml non-glycated BSA or 100 ng/ml DPP-4 in the presence or absence of 1 mM NAC, 100 nM RAGE-aptamer, or 10 nM linagliptin for 4 h (real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis) or 24 h (western blot analysis) (Tahara et al., 2013).

2.4. RT-PCR

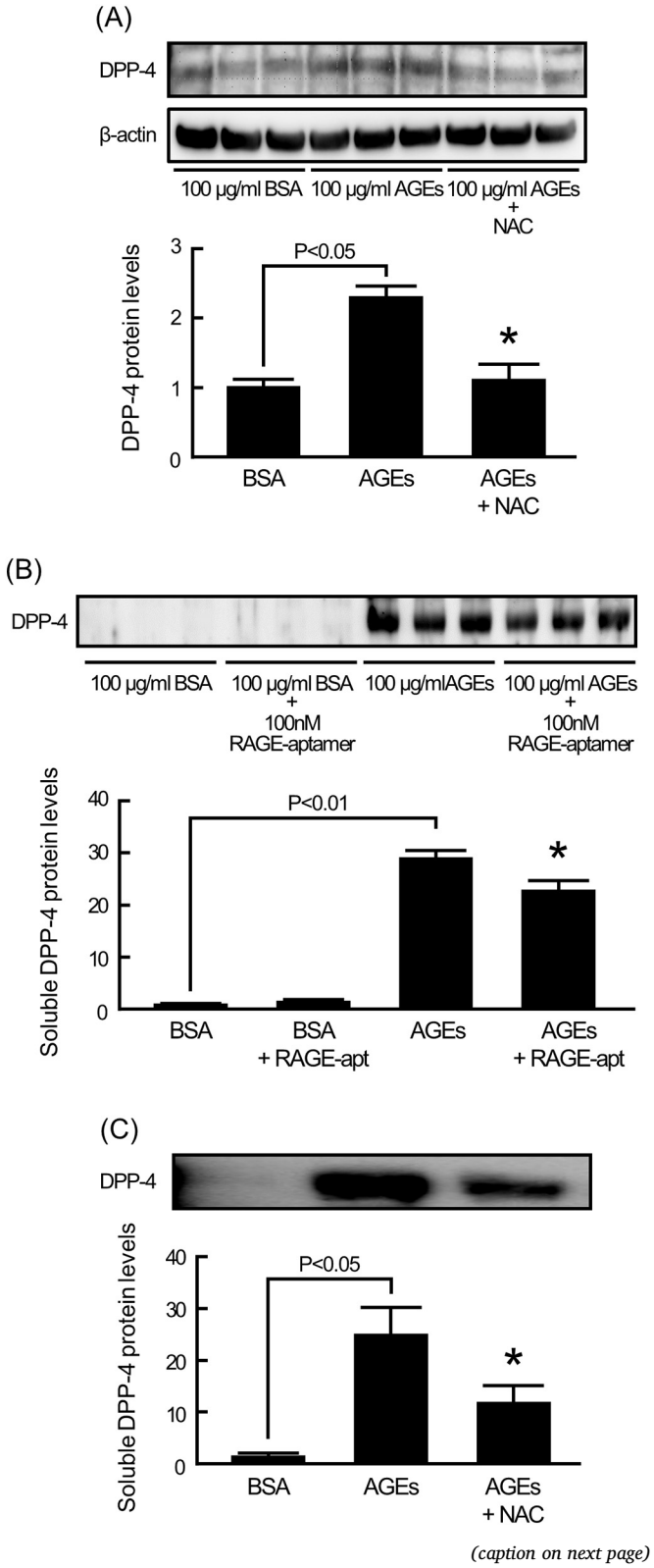
RT-PCR analysis was performed as described previously; IDs of primers for human and rat NF-κB p65, monocyte chemoattractant protein-1 (MCP-1), human and rat GAPDH, and 18S rRNA gene were Hs01042010_m1 and Rn01502266_m1, Hs00234140_m1, Hs99999905_m1 and Rn99999916_s1, and Hs99999901_s1, respectively (Ishibashi et al., 2013).

2.5. Western blot analysis

Tubular cell lysates or conditioned medium were subjected to western blot analysis using anti-DPP-4 polyclonal antibody as previously reported (Tahara et al., 2013).

2.6. Isolation of tubular cells from animals

Eight week-old male Sprague-Dawley (SD) rats and DPP-4-deficient F344/DuCrl/Crlj rats (Charles River Japan) were used in the present experiments. Proximal tubular cells were isolated form each rat kidney according to the method as previously described (Vinay et al., 1981). More than 95% of the cells were identified as proximal tubular cells by their characteristic morphology and positive staining for alkaline phosphatase.



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2.7. Statistical analysis

All values were presented as mean ± standard error. Comparisons between two groups were analyzed by using Student's *t*-test. ANOVA followed by Tukey's test was performed for statistical comparisons of > 2 groups; $p < 0.05$ was considered significant.