学 位 論 文 の 要 旨

氏名　石原慎一郎

学 位 論 文 名	The PKM2 Activator TEPP-46 Suppresses Cellular Senescence in Hydrogen Peroxide-induced Proximal Tubular Cells and Kidney Fibrosis in CD-1$^{db/db}$ Mice.
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著 者 名	Shin-ichiro Ishihara, Md. Imrul Kayes, Hirofumi Makino, Hiroaki Matsuda, Asako Kumagai, Yoshihiro Hayashi, Sara Amelia Ferdaus, Emi Kawakita, Daisuke Koya, Keizo Kanasaki

論 文 内 容 の 要 旨

INTRODUCTION

The global rise in type 2 diabetes mellitus (T2DM) and chronic kidney disease (CKD) has led to intensified research aimed at addressing the widespread occurrence of diabetic kidney disease (DKD). The pathogenesis of DKD is not completely understood yet; cellular senescence associated with complex metabolic defects could play roles in the onset and progression of DKD. Increased cellular senescence has been reported in both podocytes and kidney tubular cells in individuals with type 2 diabetes and DKD, suggesting prominent cellular senescence-related pathways in people with DKD. Senescence is one of the most important pathogenic mechanisms in aging. Cellular senescence is a condition of irreversible cell cycle arrest and an altered secretory phenotype, referred to as the senescence-associated secretory phenotype (SASP). The SASP involves the release of proinflammatory cytokines such as interleukin (IL)-6, growth factors, and matrix metalloproteinases, contributing to chronic inflammation, tissue remodeling, and dysfunction. Senescent cells accumulate with age and are implicated in the progression of DKD through, inflammation, fibrosis, and impaired tissue repair. In DKD, chronic insults such as hyperglycemia and mitochondrial dysfunction play roles in increasing the production of reactive oxygen species, thereby further inducing oxidative stress. This oxidative stress induces cellular senescence.Senescent cells display aberrant glycolysis, and one of the essential enzymes in glycolysis, pyruvate kinase (PK), has been identified as a potential regulator of cellular senescence. PK activation influences cellular metabolism, oxidative stress, and inflammatory responses, all of which are closely related to the aging process. We and others have shown that the suppression of pyruvate kinase muscle isoform 2 (PKM2) activity is a key pathological

mechanism in the kidney damage associated with diabetes. PKM2 forms monomers, dimers, or tetramers, each of which has distinct pathophysiological functions; tetrameric PKM2 has high PK activity. TEPP-46 is a small-molecule activator that induces the formation of the PKM2 tetramer by stabilizing PKM2 subunit interactions and increasing PK activity. TEPP-46 has been shown to inhibit the SASP-like phenotype induced by SARS-CoV-2 proteins and is associated with the restoration of cellular metabolism. We hypothesized that PKM2 activation by TEPP-46 could suppress oxidative stress-induced renal tubular cell injury and cellular senescence.

MATERIALS AND METHODS

We used human primary renal proximal tubule epithelial cells (RPTECs), cultured in Renal Epithelial Cell Basal Media supplemented with components from the Renal Epithelial Cell Growth Kit. For certain experiments, TEPP-46 (100 μM) and the p38 inhibitor (10 μM) were exposed to the cells. Cellular senescence was analyzed using the β-galactosidase (β-Gal) Staining Kit. For western blot analysis, equal amounts of protein from cell lysates were separated on 4-20% MINI PROTEAN TGX gels and transferred to polyvinylidene fluoride membranes using the semidry method and ECL-visualized images were captured. Human or mouse IL-6 levels were measured using the Quantikine ELISA system. Cell viability was assessed using the MTS assay kit. For in vivo experiments, a previously established advanced kidney fibrosis model using type 2 diabetic CD-1$^{db/db}$ mice was utilized. At 12 weeks of age, mice were treated with TEPP-46 or vehicle via oral gavage for 4 weeks. Blood pressure and blood glucose levels were measured prior to euthanasia at 16 weeks of age. For fibrosis analysis, Picrosirius red staining for collagen was performed and visualized using a microscope. Oxidative stress was measured using the 8-OHdG ELISA kit. All experiments with animals in this study were approved by the Animal Care and Use Committee of Shimane University. Data are presented as the mean ± SD. Statistical analysis was conducted using GraphPad Prism software version 8.0. One-way analysis of variance (ANOVA) followed by Tukey's test was used for group comparisons, with a P value <0.05 considered statistically significant.

RESULS AND DISCUSSION

In pRPTECs, hydrogen peroxide increased the number of β-gal-positive cells, the expression of senescence markers (p16, p21, p53), and p38 phosphorylation; co-incubation with TEPP-46 suppressed these alterations. When apoptosis was analyzed by caspase 3 cleavage, hydrogen peroxide-induced caspase 3 cleavage was significantly inhibited by co-incubation with TEPP-46. TEPP-46 did not influence basal apoptosis. Furthermore, the MTS assay revealed that hydrogen peroxide suppressed cell viability; however, TEPP-46 restored cell viability after incubation with hydrogen peroxide. In addition, TEPP-46 co-incubation inhibited the induction

of mesenchymal marker α smooth muscle actin (αSMA) in kidney tubular cells. In the presence of the p38MAPK inhibitor, hydrogen peroxide-induced increases in p16 and p21 levels were inhibited. Additionally, the p38MAPK inhibitor suppressed hydrogen peroxide-induced caspase 3 activation. β-gal staining clearly demonstrated that the p38MAPK inhibitor inhibited hydrogen peroxide-induced cellular senescence. We analyzed the levels of IL-6, the primary cytokine involved in SASP. Compared with those of the control cells, the medium of hydrogen peroxide-exposed primary tubular cells presented significant increases in IL-6 levels; both TEPP-46 and p38MAPK inhibitors significantly suppressed the hydrogen peroxide-induced increase in IL-6 levels. The lactate levels of the cells exposed to hydrogen peroxide were significantly elevated. Both TEPP-46 and the p38MAPK inhibitor significantly suppressed lactate levels. These data suggested that TEPP-46 and p38MAPK inhibitor suppressed hydrogen peroxide-induced cellular senescence and apoptosis in pRPTECs.

Next, we performed a preliminary investigation to confirm the in vivo influence of TEPP-46 administration on fibrotic kidney type 2 diabetic mouse model, the CD-1$^{db/db}$ model. both male and female CD-1$^{db/db}$ mice present a fibrotic phenotype in the kidney. Compared with vehicle, TEPP-46 significantly suppressed the fibrotic phenotype in the kidneys of CD-1$^{db/db}$ mice. The randomly harvested blood glucose levels tended to be lower in TEPP-46-treated animals. Western blot analysis revealed that the levels of senescence-associated molecules, such as p53, p21, and p16 in kidney, were not significantly altered by TEPP-46; however, p53 and p21 levels tended to decrease with TEPP-46 intervention. Cleaved caspase 3 levels were inhibited in TEPP-46-treated CD-1$^{db/db}$ mice. In support of the antifibrotic effects of TEPP-46, αSMA levels were significantly suppressed by TEPP-46. The plasma IL-6 levels tended to decrease but not yet significant in the CD-1$^{db/db}$ mice treated with TEPP-46. Plasma 8-OHdG levels were not different in the CD-1$^{db/db}$ mice compared to the CD-1$^{db/m}$ mice. In contrast, urinary 8-OHdG concentration and daily output was significantly higher in the CD-1$^{db/db}$ mice compared to that of CD-1$^{db/m}$ mice; TEPP-46 administration did not significantly alter the level of urinary 8-OHdG levels. Taken together, in CD-1$^{db/db}$ mice, TEPP-46 intervention suppressed apoptosis, fibrosis and tended to reduce the levels of senescence-associated molecules in the kidney without significant suppression of oxidative stress.

CONCLUSION

PKM2 activation could be a molecular target for protection against senescence-associated organ damage, including diabetic kidney disease.