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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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ABSTRACT

Aim/Introduction: Senescence is a key driver of age-related kidney dysfunction, including diabetic kidney disease. Oxidative stress activates cellular senescence, induces abnormal glycolysis, and is associated with pyruvate kinase muscle isoform 2 (PKM2) dysfunction; however, the mechanisms linking PK activation to cellular senescence have not been elucidated. We hypothesized that PKM2 activation by TEPP-46 could suppress oxidative stress-induced renal tubular cell injury and cellular senescence.

Materials and Methods: To investigate the effects of PKM2 activation on oxidative stress-induced cellular senescence, we conducted β-galactosidase staining and western blot analysis on human primary renal tubular cells (pRPTECs) treated with hydrogen peroxide with or without TEPP-46. IL-6 levels and glycolytic flux were measured. Cell viability and apoptosis were assessed via the MTS assay and caspase 3 cleavage. For in vivo experiments, we utilized CD-1$^{db/db}$ mice, a fibrotic type 2 diabetes model, which exhibit kidney fibrosis. After 4 weeks of TEPP-46 intervention, kidney fibrosis and the expression of senescence markers were analyzed.

Results: In pRPTECs, hydrogen peroxide increased the number of β-galactosidase-positive cells, the expression of senescence markers (p16, p21, p53), and p38 phosphorylation; co-incubation with TEPP-46 suppressed these alterations. Hydrogen peroxide reduced cell viability, induced apoptosis, mesenchymal alterations, and increased lactate production and IL-6 secretion; co-incubation with TEPP-46 or a p38 inhibitor mitigated these effects. 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.

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

INTRODUCTION

Noncommunicable diseases, such as diabetes, hypertension, chronic kidney disease (CKD), and cancer, are rising rapidly as the population ages. Data from national registries show that the prevalence of end-stage kidney disease (ESKD) in older people

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(>65 years) has steadily increased globally[1]. The global rise in type 2 diabetes mellitus (T2DM) and 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[2–4]. Increased cellular senescence has been reported in both podocytes and kidney tubular cells in individuals with type 2 diabetes and DKD[5], suggesting prominent cellular senescence-related pathways in people with DKD[6]. In particular, the increasing age of the population worldwide could facilitate the development of DKD in the kidney[2–4].

Senescence is a complex biological phenomenon that affects all multicellular organisms, including humans. It refers to the progressive and unavoidable decline in physiological performance and homeostasis over time. Understanding the molecular mechanisms underlying senescence and its impact on age-related disorders has become crucial as the population ages worldwide. Senescence is one of the most important pathogenic mechanisms in aging and may offer a more distinct aspect of age-related health beyond chronological age[7]. Nonetheless, the role of senescence in human health is not yet fully understood. Senescent cells exhibit a complex and diverse phenotype that significantly varies by source and cell type. DNA damage, inflammation, and other processes share several molecular characteristics with senescence[8], and there is no unique marker that consistently and accurately distinguishes senescence[9].

Cellular senescence is a condition of irreversible cell cycle arrest and an altered secretory phenotype, referred to as the senescence-associated secretory phenotype (SASP)[7,10]. 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[11,12]. 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[13,14]. This oxidative stress, in turn, dramatically induces cellular senescence[15,16].

Novel treatment strategies designed to control senescence have gained momentum in this setting. 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[17]. PK activation influences cellular metabolism, oxidative stress, and inflammatory responses, all of which are closely related to the aging process[18,19]. 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[20–22]. PKM2 forms monomers, dimers, or tetramers, each of which has distinct pathophysiological functions; tetrameric PKM2 has high PK activity[23]. TEPP-46 is a small-molecule activator that induces the formation of the PKM2 tetramer by stabilizing PKM2 subunit interactions and increasing PK activity[23]. 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[19].

Here, we found that a PKM2 activator could combat senescence-induced kidney proximal tubular cell damage under oxidative stress and kidney fibrosis in diabetic CD-1$^{db/db}$ mice.

MATERIALS AND METHODS

Method summary

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 SB203580 (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. The list of antibodies used is provided in the supplemental files. 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[24] 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[25] and blood glucose levels were measured prior to euthanasia at 16 weeks of age. The animal experiments were approved by the institutional animal care and use committee of Shimane University Faculty of Medicine (protocol numbers: IZ2-70, IZ5-7, and IZ3-63). 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. 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. Further details regarding the Materials and Methods are available in the Data S1.

RESULTS

The PKM2 activator inhibited hydrogen peroxide-induced senescence

Hydrogen peroxide has been shown to inactivate PKM2 enzymatic activity via the formation of dimers[26]. The lower activity dimer is essential for aberrant glycolysis[23], which is a key molecular mechanism in cellular senescence[27]. After the cells were subjected to oxidative stress conditions, we analyzed their senescence status via β-gal staining. Here, we found that hydrogen peroxide-induced senescence (Figure 1a,b,d) was significantly inhibited by the PKM2 activator TEPP-46 (Figure 1c,d). Oxidative stress induces the p16^{INk4a} and p53/p21^{Cip1} pathways,

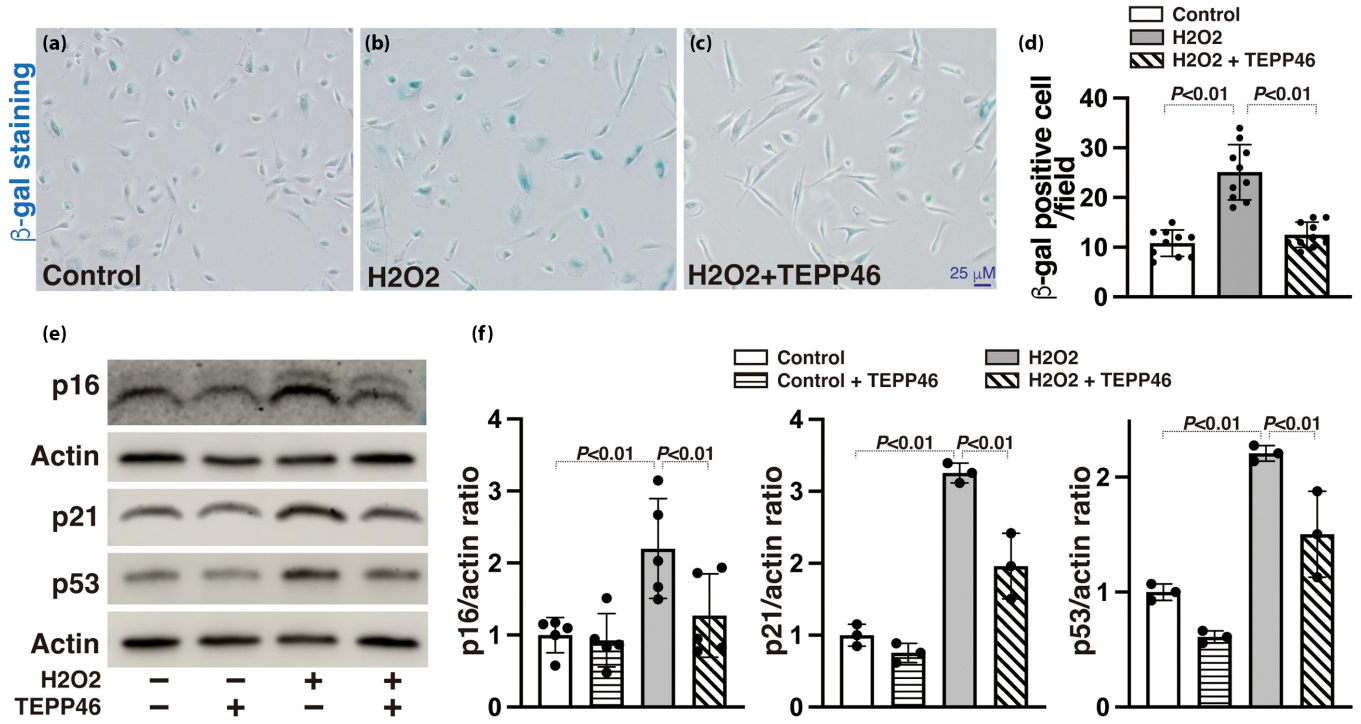

Figure 1 | TEPP-46 suppressed hydrogen peroxide-induced cellular senescence in proximal tubular cells. (a–c) RPTECs were exposed to hydrogen peroxide (100 μM) for 48 h with or without TEPP-46 (100 μM). (d) The graph shows the number of β-gal-positive cells per field at 200× magnification from 6 independent images. Scale bar: 25 μm. The data are presented as the mean ± SD of independent experiments (n = 10). (e) Western blot analysis of senescence marker proteins (p16, p21, and p53) in RPTECs treated with TEPP-46 for 48 h. The protein lysate (10 μg) was separated on polyacrylamide gels and transferred onto a PVDF membrane. The immunoreactive bands were analyzed via the ECL method. Representative blots from three or four independent experiments are shown. (f) Quantification via densitometry revealed a significant reduction in the level of senescence marker proteins in the cells after treatment with TEPP-46 (100 μM). One-way ANOVA followed by Tukey's test was performed. $P < 0.05$ was interpreted as statistically significant. Hydrogen peroxide is designated as H_2O_2 in the figure.

causing cell cycle arrest and cellular senescence[28]. Western blot analysis revealed that senescence-associated molecules, such as p16 ^{INk4a}, p21^{Cip1}, and p53, were all induced by hydrogen peroxide (Figure 1e,f); TEPP-46 co-incubation inhibited these senescence-associated molecules induced by hydrogen peroxide (Figure 1e,f).

TEPP-46 attenuates the effects of hydrogen peroxide on cell viability and apoptosis

Cellular senescence could be a cell protection system to combat insults to cells[15]; when considering therapeutic interventions for DKD, the inhibition of senescence should be accompanied by healthy homeostasis of cells and viability but should not be associated with the cell death program. When apoptosis caused by caspase 3 cleavage was analyzed, hydrogen peroxide-induced caspase 3 cleavage was significantly inhibited by co-incubation with TEPP-46 (Figure 2a,b). TEPP-46 did not influence basal apoptosis (Figure 2a,b). Furthermore, the MTS assay revealed that hydrogen peroxide suppressed cell viability; however, TEPP-46 restored cell viability after incubation with hydrogen peroxide (Figure 2c). In addition, hydrogen peroxide-induced caspase 3 cleavage was associated with the induction of mesenchymal marker α smooth muscle actin (αSMA) in kidney tubular cells; TEPP-46 co-incubation inhibited this (Figure 2a,d).

The PKM2 activator TEPP-46 can counteract the hydrogen peroxide-induced activation of the p38MAPK pathway

Hydrogen peroxide is known to activate the p38MAPK pathway[29]. In the cells exposed to hydrogen peroxide, TEPP-46 inhibited the phosphorylation of p38MAPK (Figure 3a). To confirm the role of p38MAPK activation in hydrogen peroxide-induced cellular senescence, we utilized a selective inhibitor of p38MAPK (Figure 3b,c). In the presence of the p38MAPK inhibitor, hydrogen peroxide-induced increases in p16 ^{INk4a} and p21^{Cip1} levels were inhibited (Figure 3d,e). Additionally, the p38MAPK inhibitor suppressed hydrogen peroxide-induced caspase 3 activation (Figure 3d,e). β-gal staining clearly demonstrated that the p38MAPK inhibitor inhibited hydrogen peroxide-induced cellular senescence (Figure 3f–i). These data suggest that hydrogen peroxide-induced activation

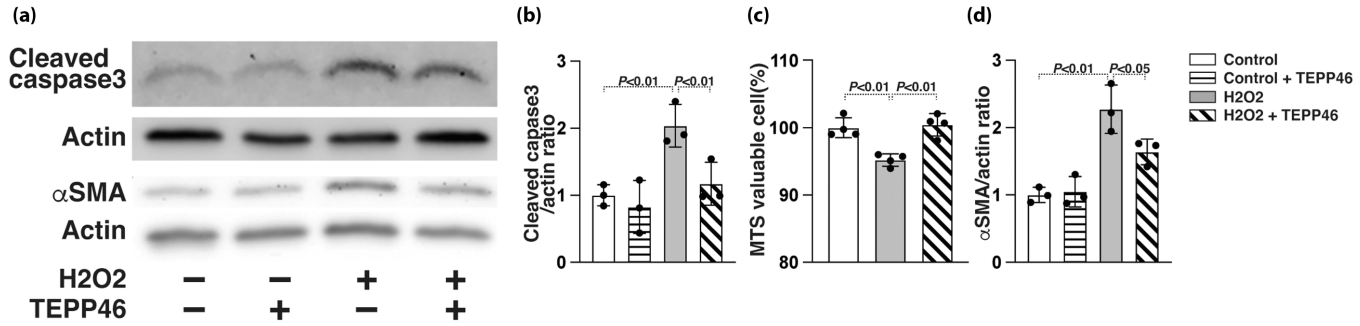

Figure 2 | TEPP-46 inhibited hydrogen peroxide-induced apoptosis in proximal tubular cells. (a) Effects of TEPP-46 on the level of cleaved caspase 3 in RPTECs treated with hydrogen peroxide (100 μM). The same blot was reanalyzed for caspase 3, as shown in Figure 1, for p16. Therefore, the same Actin blot was used here. In addition, αSMA and corresponding Actin were shown (b) Densitometric analysis of cleaved caspase 3. The data were normalized to those of β-Actin. $N = 3$ experiments were analyzed. (c) Cell viability was assessed by the MTS assay. (d) Densitometric analysis of αSMA in RPTECs treated with TEPP-46 for 48 h. The deta were normalized to those of Actin. The data are expressed as the mean ± SD. Statistical analysis was performed by one-way ANOVA followed by Tukey's test. $P < 0.05$ was interpreted as statistically significant. Hydrogen peroxide is designated as H_2O_2 in the figure.

of p38MAPK contributes to both cellular senescence and cell death signaling.

Influence of hydrogen peroxide on the secretory phenotype

Given the significance of SASP in the context of cellular senescence-induced organ damage, we investigated whether TEPP-46-mediated inhibition of cellular senescence could also inhibit the SASP phenotype in primary cultured tubular cells.

First, we analyzed the levels of interleukin (IL)-6, the primary cytokine involved in SASP[30]. 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 (Figure 4a).

Lactate is considered not only a waste of glycolytic flux but also a variable signaling molecule. Lactate has been shown to stimulate p38MAPK phosphorylation[31]. Additionally, lactate by itself may induce senescence[32]. Therefore, we measured medium lactate levels and glucose levels in cells exposed to hydrogen peroxide and test molecules using a Live Cell Metabolic Analyzer system. In all of the groups, the medium glucose levels were not altered; however, the lactate levels of the cells exposed to hydrogen peroxide were significantly elevated (Figure 4b). Both TEPP-46 and the p38MAPK inhibitor significantly suppressed lactate levels (Figure 4b). Next, we exposed primary tubular cells to lactate (5 mM). However, unlike previous reports[32], lactate did not induce the expression of senescence-associated molecules, such as p53, p16^{INk4a} or p21^{Cip1} (Figure 4c).

Preliminary analysis of the effects of TEPP-46 on the fibrotic kidney phenotype in diabetic animals

Diabetes is associated with prominent features of premature senescence in the kidney[33], and a senescent phenotype in kidney tubular cells induces kidney fibrosis[34]. Therefore, 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[24]. Although the magnitude of this effect differed, both male and female CD-1$^{db/db}$ mice present a fibrotic phenotype in the kidney (Figure 5a). Compared with the vehicle, TEPP-46 significantly suppressed the fibrotic phenotype in the kidneys of CD-1$^{db/db}$ mice (Figure 5a–c). The physiological parameters and body and organ weights were not markedly altered; however, randomly harvested blood glucose levels tended to be lower in TEPP-46-treated animals (Figure 5d), supporting a previous report on the role of a PKM2 activator in insulin secretion and insulin-mediated glucose metabolism[35,36]. Western blot analysis revealed that the levels of senescence-associated molecules, such as p53, p21^{Cip1}, and p16^{INK4a} in kidney were not significantly altered by TEPP-46; however, p53 and p21^{Cip1} levels tended to decrease with TEPP-46 intervention (Figure 5e,f). Cleaved caspase 3 levels were inhibited in TEPP-46-treated CD-1$^{db/db}$ mice (Figure 5f). In support of the antifibrotic effects of TEPP-46, αSMA levels were significantly suppressed by TEPP-46 (Figure 5e,f). The plasma IL-6 levels tended to decrease but were not yet significant in the CD-1$^{db/db}$ mice treated with TEPP-46 (Figure 5g). Unexpectedly, plasma 8-OHdG levels were not different in the CD-1$^{db/db}$ mice compared with the CD-1$^{db/m}$ mice. In contrast, urinary 8-OHdG concentration and daily output were 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 (Figure 5h).

DISCUSSION

In this study, we aimed to counteract progressive kidney disease related to aging, particularly by inhibiting cellular senescence,

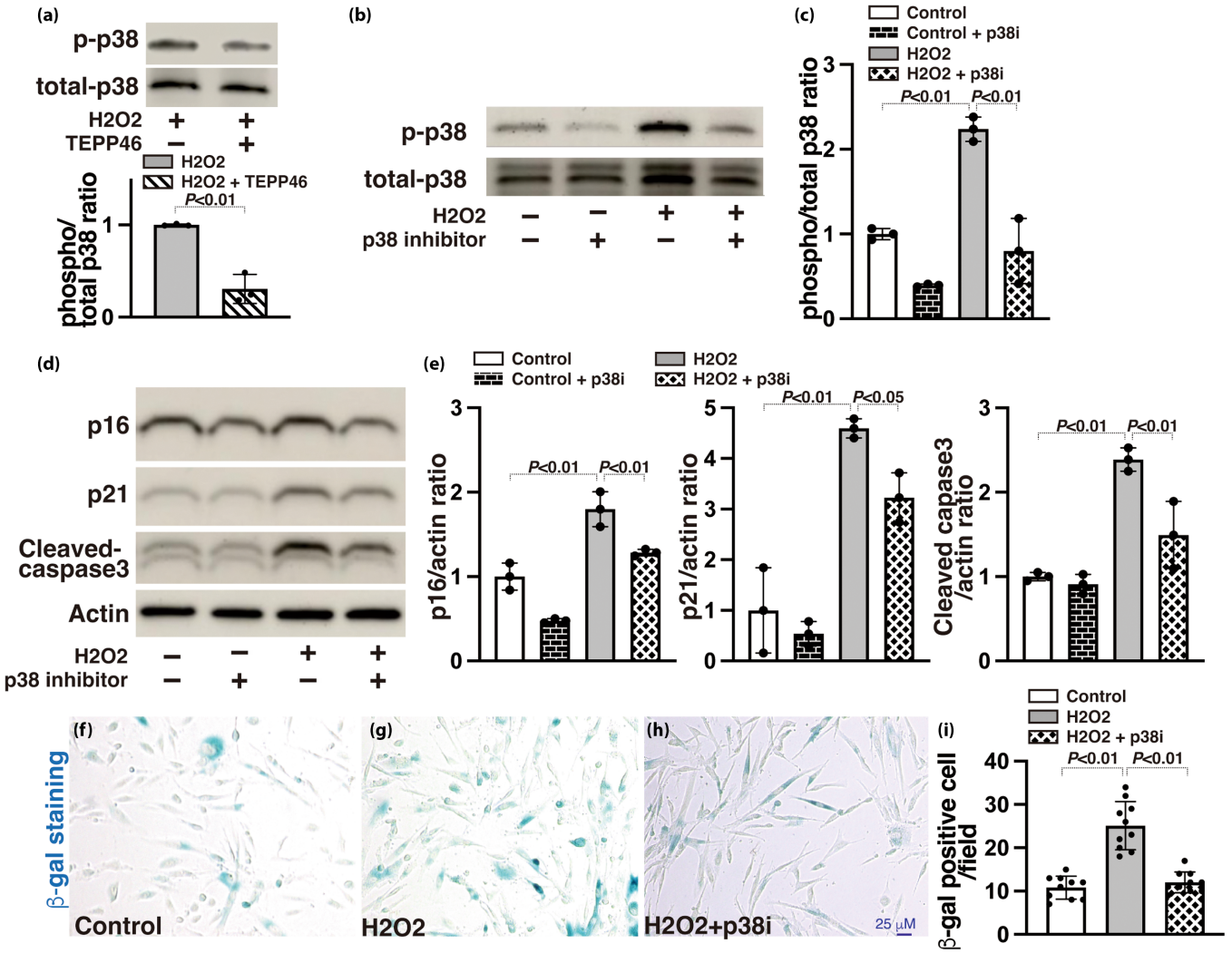

Figure 3 | P38MAPK Activation is involved in hydrogen peroxide-induced senescence in proximal tubular cells. (a) Effect of TEPP-46 on phosphor p38MAPK levels in RPTECs exposed to hydrogen peroxide (100 μM). The data were normalized to the total p38 expression. (b) Selective inhibition of p38MAPK by SB 203580 (10 μM) for 48 h. Western blot analysis of p-p38 levels and (c) densitometric analysis of the p-p38/total p38 ratio. $N = 3$. (d) Immunoblot analysis of the levels of the senescence marker proteins p16, p21, and caspase 3 in cells exposed to hydrogen peroxide with or without the p38MAPK inhibitor SB 203580. (e) Densitometric analysis of the p16, p21, and caspase 3 proteins normalized to β-Actin. $N = 3$. (f–h) RPTECs were exposed to hydrogen peroxide for 48 h with or without p38 inhibitors (10 μM). β-gal staining was performed. (i) The graph shows the β-gal-positive cells per field at 200× magnification from 10 independent images. Scale bar: 25 μm. The data are presented as the mean ± SD of independent experiments ($n = 10$). One-way ANOVA followed by Tukey's test was performed. $P < 0.05$ was interpreted as statistically significant. Hydrogen peroxide is designated as H_2O_2 in the figure.

which is a form of cell cycle arrest that occurs when cells are under stress or injury. Patients with type 2 diabetes are at increased risk of developing kidney disease and experiencing its progression. The final common pathway of any kind of progressive kidney disease, including DKD, is kidney fibrosis. SASP is a collection of proinflammatory and profibrotic factors produced mainly by senescent cells that drive kidney fibrosis. Therefore, our main focus in this study was to target senescent cells and combat kidney fibrosis to halt the progression of kidney disease.

Here, we found that (1) TEPP-46 inhibited hydrogen peroxide-induced senescence in proximal tubular cells; (2) hydrogen peroxide-stimulated apoptosis and reduction in cell viability associated with the induction of mesenchymal program were all inhibited by TEPP-46; (3) TEPP-46 inhibited hydrogen peroxide-induced p38MAPK activation, a key signaling pathway involved in cellular senescence; (4) TEPP-46 inhibited hydrogen peroxide-induced SASP phenotypes such as IL-6 and lactate production, although lactate did not play a pathogenic role in our in vitro system; and (5) preliminary analysis in a small

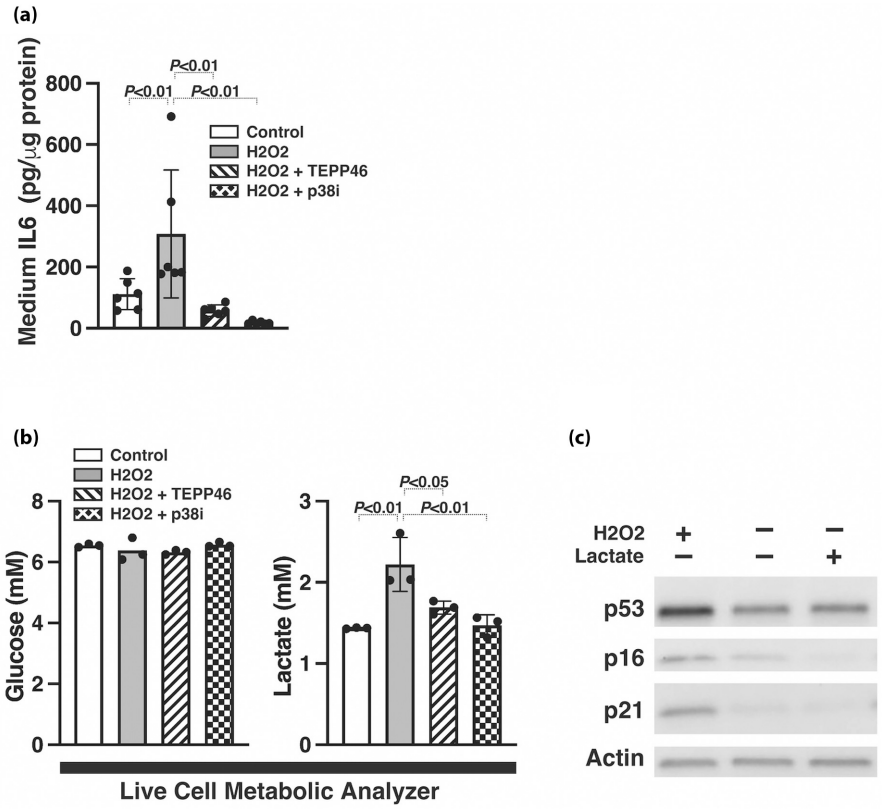

Figure 4 | SASP phenotype analysis and effects of TEPP-46 and p38 inhibitors. (a) Enzyme-linked immunosorbent assay for IL-6. RPTECs were exposed to hydrogen peroxide with or without TEPP-46 (100 μM) or the p38MAPK inhibitor SB 203580 (10 μM). The IL-6 concentration was normalized to the corresponding protein concentration. The data are expressed as the means ± SDs. $N = 6$. (b) The levels of glucose and lactate were analyzed using a live-cell metabolic analyzer. The data are expressed as the mean ± SD. $N = 3$. (c) Western blot analysis of the senescence markers p53, p16, and p21 in cells treated with lactate (5 mM) for 48 h. One-way ANOVA followed by Tukey's test was performed. $p < 0.05$ was interpreted as statistically significant. Hydrogen peroxide is designated as H_2O_2 in the figure.

number of CD-1$^{db/db}$ mice, a fibrotic type 2 diabetic model, revealed that TEPP-46 intervention suppressed kidney fibrosis, with a trend toward the suppression of certain senescence markers. (6) 8-OHdG meseaurements revealed the advanced oxidative stresses in CD-1$^{db/db}$ mice; yet, TEPP-46 did not exhibit remarkable anti-oxidative stress effects in CD-1$^{db/db}$ mice. These data, along with our previous reports, suggest that PKM2 activation could be an attractive therapeutic target to combat residual risk in the management of DKD.

The PKM2 activator TEPP-46 works to activate PK activity by inducing PKM2 tetramer formation while suppressing dimer formation. The PKM2 dimer has been shown to be associated with aberrant glycolysis in cancer, designated the "Warburg effect,"[37] which is characterized by aerobic glycolysis. Proximal tubular cells in the kidney are known to have a minimal rate of glycolysis under normal physiological conditions. However, recent reports, including our studies, suggest that under conditions of kidney insult, aerobic glycolysis significantly contributes to kidney pathogenesis[20,21,23,38–41], Low PK activity associated with the PKM2 dimer has been implicated in various pathogenic conditions, including cellular senescence in SARS-CoV-2-infected lung epithelial cells[19]. Here, we demonstrated for the first time that a deficiency in PK enzymatic activity in proximal tubular cells contributes to the induction of cellular senescence.

SASP is an important phenotype in cellular senescence-induced organ damage. In our analysis, hydrogen peroxide-exposed proximal tubular cells secreted more IL-6. TEPP-46 or a p38 inhibitor could suppress the senescence phenotype in proximal tubular cells and reduce IL-6 levels. Lactate production in senescent cells may facilitate the acquisition of the pathogenic SASP phenotype[42]. Urine lactate is a strong indicator of poorer prognosis in DKD patients, and kidney-derived lactate via aberrant glycolysis in DKD patients has been suggested[38]. Our analysis also revealed that either TEPP-46 or a p38 inhibitor could suppress lactate production, as determined by a live-cell metabolic analyzer. However, we found that lactate alone in the media did not induce a senescent phenotype, suggesting that lactate may facilitate a

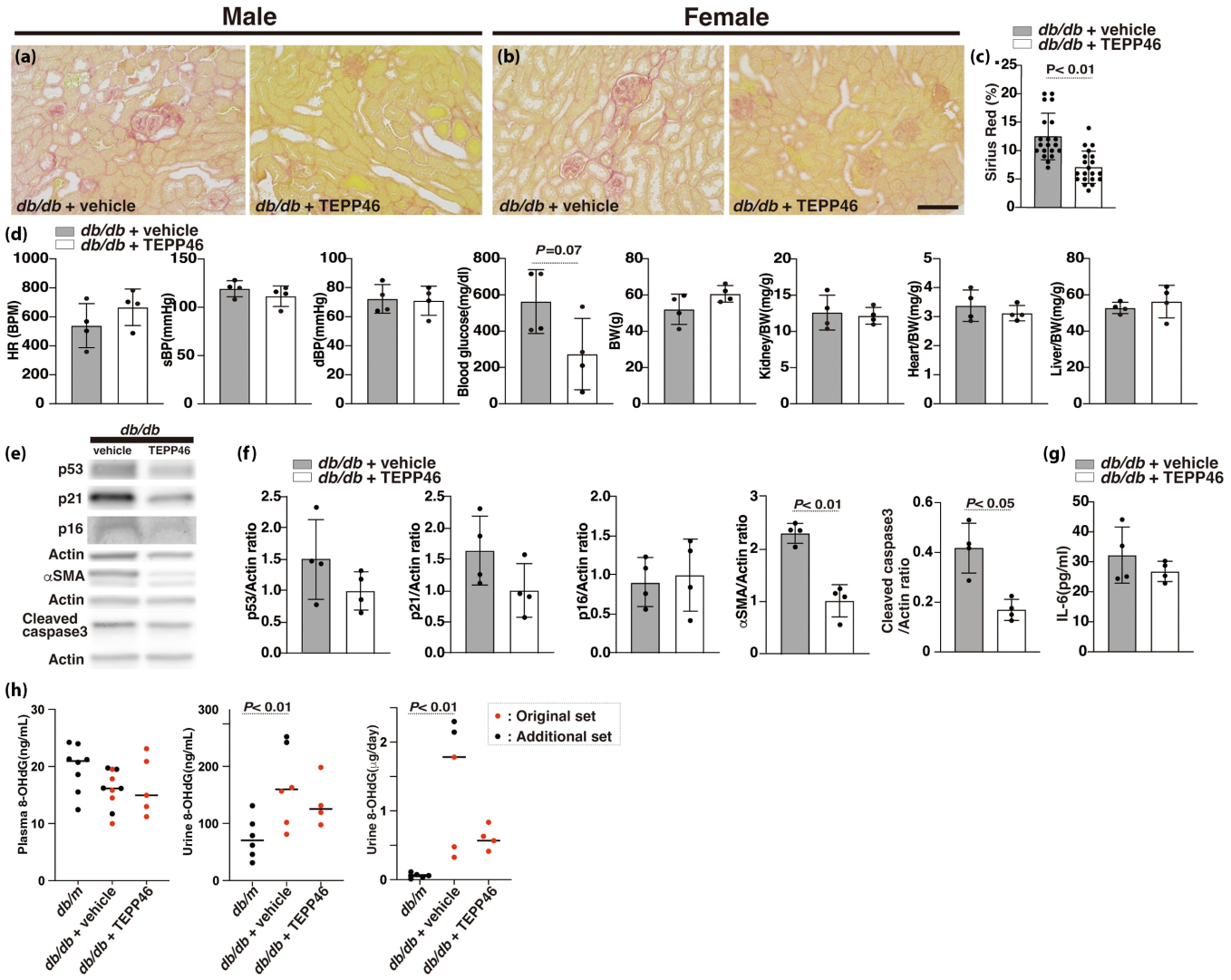

Figure 5 | TEPP-46 administration in CD-1$^{db/db}$ mice has antifibrotic effects. (a,b) Picrosirius red staining of renal tissue sections from control CD1$^{db/db}$ mice treated with vehicle or TEPP-46. Scale bar: 25 μm. (c) The percentage of Sirius red (fibrotic) positive area obtained from 5 areas per mouse was captured and calculated via a BZ-X810 microscope. (d) Heart rate (HR), systolic blood pressure (sBP), diastolic blood pressure (dBP), fasting plasma glucose level (FPG), body weight (BW), kidney-to-BW ratio, heart-to-BW ratio, and liver-to-BW ratio are shown. (e) Representative blots for p53, p21, p16, αSMA, and cleaved caspase 3 in kidney lysates from indicated animals. (f) Densitometric analysis of p53, p21, p16, αSMA, and cleaved caspase 3 normalized to the corresponding Actin level. (g) Enzyme-linked immunosorbent assay (ELISA) of mouse plasma IL-6 levels. $N = 4$ (two males and two females) in each group. (h) plasma or urine concentration and daily urine output of 8-OHdG. Red-filled circle indicated the original experimental set and black-filled circle indicated additional experimental sets. The data are shown as the mean ± SD. One-way ANOVA followed by Tukey's test was performed. $P < 0.05$ was interpreted as statistically significant.

pathogenic phenotype induced by other insults rather than being the sole contributor.

TEPP46, a PKM2 activator, did not significantly reduce elevated urinary or plasma 8-OHdG levels in CD-1$^{db/db}$ mice. This outcome was unexpected, as the observed amelioration of the kidney phenotype suggested a likely association with metabolic normalization. Moreover, TEPP46 intervention appeared to trend toward suppression of blood glucose levels. In this context, TEPP46 has been reported to inhibit NK cell proliferation and cytokine production linked to increased oxidative stress[43]. In neutrophils, TEPP46 was shown to increase reactive oxygen species production[44]. Notably, TEPP46 also reduced oxidative stress associated with NLRP3 inflammasome-mediated IL-1β secretion in a thoracic aortic aneurysm and dissection model[45]. These findings, combined with our current data, suggest that PKM2 activation by TEPP46 exerts cell type-specific

and pathogenic condition-dependent effects on oxidative stress regulation. Further research is essential to elucidate the cell-specific effects of PKM2 activation and the influence of TEPP46.

The greatest limitation of our analysis was the insufficient number of animals due to budget constraints, which prevented us from achieving statistically significant levels of senescence-relevant molecules in vivo. However, we confirmed the sufficient antifibrotic effect of the PKM2 activator and the suppression of αSMA levels via western blot analysis. Apoptosis was also inhibited by TEPP-46. Additionally, we analyzed both male and female CD-1$^{db/db}$ mice, revealing the sex-independent influence of PKM2 activation. Despite significant advances in the study of cellular senescence, reliably interpreting senescent cells in vivo remains challenging[9]. Unlike cell cultures, where cell states can be more easily controlled and observed, most cells in vivo are either quiescent or terminally differentiated. This makes some of the markers used to identify senescent cells in vitro, such as the absence of DNA synthesis or proliferation, potentially invalid for in vivo identification[9]. Even with such limitations, molecules associated with senescence tend to be suppressed by intervention with TEPP-46. In vivo, blood glucose levels also tended to be suppressed by TEPP-46. PKM2 activation has been shown to induce robust catabolic activity and ameliorate insulin resistance[35,36]. Additionally, it activates the Wnt/CTNNB1 pathway in β cells, promoting their proliferation and insulin secretion, thereby lowering blood glucose levels[35,36]. Notably, the amelioration of insulin resistance and β-cell function influences the kidney phenotype, including senescence. Adjusting such conditions, including managing both insulin resistance and β-cell function with other control interventions, is technically challenging. Therefore, we cannot exclude the possibility that the improvement in the kidney phenotype and the trend toward senescence could be mediated through the systemic restoration of the metabolic phenotype in leptin-deficient diabetic *db/db* mice. Even with such limitations, the inhibition of kidney fibrosis by TEPP-46 in CD-1$^{db/db}$ mice, similar to our previous publication reporting the antifibrotic effects of TEPP-46 in albumin-injected STZ-induced type 1 diabetic mice[20], demonstrates its potential as an antifibrotic therapy for kidneys affected by either type 1 or type 2 diabetes, with possible antisenescence activity.

In conclusion, our findings clearly indicate that PKM2 activation could be a target mechanism to combat senescence-associated organ damage, including DKD.

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DISCLOSURE

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Approval date of the registry and the registration no. of the study/trial: N/A.
Animal studies: Animal experiments were approved by the institutional animal care and use committee of Shimane University Faculty of Medicine (protocol numbers: IZ2-70, IZ5-7, and IZ3-63).

DATA AVAILABILITY STATEMENT

KK is responsible for all of the data in the manuscript, which can be provided upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Data S1. Materials and Methods.