

Research Paper

PPAR γ activation ameliorates PM2.5-induced renal tubular injury by inhibiting ferroptosis and epithelial–mesenchymal transition

Chien-Hung Lin ^{a,b,c}, Wen-Sheng Liu ^{b,c,d,e,f,1}, Chuan Wan ^{g,h,1}, Hsin-Hui Wang ^{a,b,i,1,*}

^a Division of Pediatric Immunology and Nephrology, Department of Pediatrics, Taipei Veterans General Hospital, Taipei 112201, Taiwan

^b School of Medicine, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan

^c College of Science and Engineering, Fu Jen Catholic University, New Taipei 242062, Taiwan

^d Division of Nephrology, Department of Medicine, Taipei City Hospital Zhongxing Branch, Taipei 10341, Taiwan

^e Institute of Food Safety and Health Risk Assessment, National Yang Ming Chiao Tung University, Hsinchu 300093, Taiwan

^f Department of Special Education, University of Taipei, Taipei 100234, Taiwan

^g Department of Pediatrics, Taipei City Hospital, Zhongxing Branch, Taipei 10341, Taiwan

^h Institute of Clinical Medicine, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan

ⁱ Institute of Emergency and Critical Care Medicine, National Yang Ming Chiao Tung University, Taipei 112304, Taiwan

ARTICLE INFO

Keywords:

PPAR γ

Ferroptosis

PM2.5

Epithelial–mesenchymal transition

Tubular injury

ABSTRACT

Exposure to fine particulate matter (PM2.5) has been associated with the development and progression of renal disease. Peroxisome proliferator-activated receptor gamma (PPAR γ), a key transcription factor involved in inflammation as well as lipid and glucose metabolism, helps maintain the integrity of tubular epithelial cells. However, the precise role of PPAR γ in PM2.5-induced tubular injury remains unclear. In this study, we investigated the regulatory effects of PPAR γ on PM2.5-induced ferroptotic stress and epithelial–mesenchymal transition (EMT) in tubular (HK-2) cells. We found that downregulation of PPAR γ expression was correlated with EMT in PM2.5-exposed cells. Pretreatment with the PPAR γ agonist 15d-PGJ2 protected the cells from EMT by reducing ferroptotic stress, whereas that with the PPAR γ antagonist GW9662 promoted EMT. Furthermore, pretreatment with ferrostatin-1 (Fer-1) significantly prevented PM2.5-induced EMT and downregulation of PPAR γ expression. Notably, overexpression of PPAR γ blocked PM2.5-induced downregulation of E-cadherin and GPX4 expression and upregulation of α -SMA expression. This study highlights the complex associations of PPAR γ with ferroptosis and EMT in PM2.5-exposed tubular cells. Our findings suggest that PPAR γ activation confers protection against PM2.5-induced renal injury.

1. Introduction

Epidemiological studies have demonstrated that ambient air pollution is closely associated with morbidity and mortality worldwide, particularly in developing countries (Boogaard et al., 2019; Cromar et al., 2022). Fine particulate matter (PM2.5) is a mixture of air pollutants with a diameter of < 2.5 μ m that can reach the alveoli, enter the bloodstream, and exert adverse systemic effects. PM2.5 exposure can cause various diseases, such as respiratory disorders, cardiovascular disease, and cancer (Li and Wang, 2023; Su et al., 2020; Wang et al., 2021a). Approximately 72.8 % of all cases of chronic kidney disease (CKD) worldwide are attributable to PM2.5 (Bowe et al., 2020). A 10 μ g/

m³ increase in the concentration of ambient PM2.5 results in a 1.28-fold increase in the risk of CKD (Li et al., 2021). Exposure to PM2.5 can cause CKD and end-stage renal disease by inducing oxidative stress, inflammatory responses, and cytotoxicity; thus, PM2.5 exposure is a key risk factor for renal function decline (Wen et al., 2023; Xu et al., 2023; Zhang et al., 2023).

Peroxisome proliferator-activated receptor gamma (PPAR γ) is a ligand-activated nuclear receptor that plays an essential role in several cellular processes, including the maintenance of glucose levels, management of lipid metabolism, regulation of inflammation, and control of extracellular matrix remodeling (Kokeny et al., 2021; Menendez-Gutierrez et al., 2012). PPAR γ activation protects renal cells from

* Corresponding author at: Division of Pediatric Immunology and Nephrology, Department of Pediatrics, Taipei Veterans General Hospital, No.201, Sec. 2, Shipai Rd., Beitou District, Taipei, 11201 Taiwan.

E-mail addresses: chlin5@vghtpe.gov.tw (C.-H. Lin), hhwang@vghtpe.gov.tw (H.-H. Wang).

¹ Equal contribution.

<https://doi.org/10.1016/j.crttox.2024.100189>

Received 10 February 2024; Received in revised form 21 July 2024; Accepted 23 July 2024

Available online 26 July 2024

2666-027X/© 2024 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

inflammation and oxidative stress—the hallmarks of acute kidney injury and contributors to CKD progression (Liu et al., 2020; Maquigussa et al., 2018).

Given that PM2.5 induces ferroptosis, understanding the role of PM2.5-induced ferroptotic stress in the pathogenesis and treatment of PM2.5-related diseases is imperative (Wang et al., 2021b). Tubular epithelial cells are susceptible to inflammation and ferroptosis induced by various stimuli (Giuliani et al., 2022; Ide et al., 2021). Interventions targeting ferroptotic stress have been demonstrated to prevent the occurrence of epithelial–mesenchymal transition (EMT) in tubular cells and decelerate the progression of renal diseases (Feng et al., 2022; Zhou et al., 2022). In this study, we investigated the regulatory effects of PPAR γ on ferroptotic stress and EMT in PM2.5-exposed tubular cells.

2. Materials and methods

2.1. Cell culture

The human proximal tubular epithelial cell line HK-2 was purchased from the American Type Culture Collection center and cultured in Dulbecco's Modified Eagle Medium/F12 supplemented with 10 % fetal bovine serum. The cells were cultured at 37 °C in a humidified environment with 5 % CO₂. After 24 h of incubation, the cells were subjected to serum starvation and subsequently treated with PM2.5.

2.2. PM2.5 and chemicals

PM2.5 was purchased from Sigma–Aldrich Chemical Reagent (St. Louis, MO, USA) and stored as a 10-mg/mL stock solution after ultrasonication. The viability of the HK-2 cells after PM2.5 exposure was examined. Ultimately, a PM2.5 concentration of 50 μ g/mL was used in subsequent experiments. The PPAR γ agonist 15d-PGJ2 and the PPAR γ antagonist GW9662 were purchased from Cayman Chemicals (Ann Arbor, MI, USA). The ferroptosis inhibitor ferrostatin-1 (Fer-1) was obtained from Santa Cruz Biotechnology (Paso Robles, CA, USA).

2.3. Measurement of iron content

The iron content in the PM2.5-exposed HK-2 cells was evaluated using a colorimetric iron assay kit (Abcam, Cambridge, UK) in accordance with the manufacturer's protocol. Absorbance was measured at 593 nm by using a microplate reader.

2.4. Reduced and oxidized glutathione assay

The levels of reduced glutathione (GSH) and oxidized glutathione (GSSG) in the HK-2 cells were measured using the GSH/GSSG Ratio Detection Assay Kit (Abcam) in accordance with the manufacturer's instructions. The GSH/GSSG ratio was determined through linear regression of values obtained from the standard curve.

2.5. Measurement of malondialdehyde (MDA) levels

The level of MDA, a representative end product of lipid peroxidation, in the HK-2 cells was evaluated through a colorimetric assay of lipid reactive oxygen species (ROS) production. The assay was performed using a lipid peroxidation (MDA) assay kit (Sigma–Aldrich) in accordance with the manufacturer's instructions.

2.6. Assessment of lipid peroxidation

Lipid peroxidation in the live HK-2 cells was studied using the Image-iT Lipid Peroxidation Kit (Invitrogen, Eugene, USA) in accordance with the manufacturer's protocol. The fluorescence density of lipid peroxidation products was detected using the BODIPY 581/591 reagent, a fluorescent sensor that shifts from red to green fluorescence upon

oxidation. Images were taken using a fluorescence microscope (Leica DM 4000B).

2.7. Cell migration assay

The migration of the HK-2 cells was assessed using 24-well Transwell plates with polycarbonate filter membranes (pore size: 8.0 μ m). The cells were seeded at a density of 50,000 cells per well and incubated for 24 h. Subsequently, the cells were treated with PM2.5 under the appropriate conditions. After removal of nonmigratory cells, the cells on the lower surface of the membrane were fixed with methanol for 10 min and then stained with 0.5 % crystal violet for 30 min. The total count of stained cells in five randomly selected fields was determined.

2.8. Quantitative real-time polymerase chain reaction

Total RNA was extracted from the HK-2 cells by using TRIzol (Invitrogen) and was reverse-transcribed into complementary DNA by using the PrimeScript RT reagent kit in accordance with the manufacturer's instructions. Next, the levels of target gene expression were measured through quantitative real-time polymerase chain reaction (PCR), which was performed using the SYBR Green Master Mix and the ABI PRISM 7900 system (Applied Biosystems, Foster City, CA, USA). The mRNA levels of the target genes were normalized to the mRNA level of glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

The sequences of the primers used in the PCR were as follows.

GAPDH: 5'-ACGGGAAGCTCACTGGCATGG-3' (forward) and 5'-GGTCCACCACCCTGTGCTGTA-3' (reverse).

PPAR γ : 5'-TCGCTGATGCACTGCCTATG-3' (forward) and 5'-GAGAGGTCCACAGAGCTGATT-3' (reverse).

E-cadherin: 5'-TGCCAGAAAATGAAAAGG-3' (forward) and 5'-GTGTATGTGGCAATGCGTTC-3' (reverse).

α -smooth muscle actin (α -SMA): 5'-CTATGCTCTGGACGCACAAC-3' (forward) and 5'-CAGATCCAGACGCATGATGGCA-3' (reverse).

2.9. Western blotting

Protein extracts from the HK-2 cells were separated on a 10 % sodium dodecyl sulfate polyacrylamide gel. The resultant bands were transferred onto a polyvinylidene fluoride membrane. The membrane was incubated with primary antibodies—rabbit polyclonal anti-PPAR γ (Abcam), rabbit monoclonal anti-glutathione peroxidase 4 (GPX4) antibodies (Abcam), rabbit polyclonal anti-E-cadherin (Sigma), mouse monoclonal anti- α -SMA (Sigma), and anti-GAPDH (loading control). After washing, the membrane was incubated with corresponding enzyme-linked secondary antibodies. Immunoreactivity was detected using an enhanced chemiluminescence detection system.

2.10. Transfection

A pcDNA3.1-PPAR γ plasmid (Thermo Fisher, Waltham, MA, USA) was used for the overexpression of PPAR γ . PPAR γ was amplified through PCR and inserted into the expression vector, which was transfected into *E. coli* DH5a cells by using Lipofectamine 2000 (Invitrogen) following the manufacturer's instructions. Then, the cells were streaked onto Luria–Bertani agar plates containing 100 mg/mL ampicillin and incubated overnight at 37 °C. A single colony from a plate was selected for PCR and validated through sequencing.

2.11. Statistical analysis

Data were normalized to the data obtained from control cells and are presented in terms of mean \pm standard deviation values of at least three independent experiments. The experimental and control groups were compared using the one-way and two-way analysis of variance tests. A *p* value of < 0.05 indicated significance.

3. Results

3.1. PM2.5 exposure inhibits expression of PPAR γ in HK-2 cells

The effect of PM2.5 on the expression of PPAR γ in HK-2 cells was evaluated through quantitative PCR and Western blotting. After 24 h of PM2.5 (50 $\mu\text{g}/\text{mL}$) exposure, a significant reduction was noted in the mRNA level of PPAR γ compared with the level in control cells (Fig. 1A). Such a reduction was also observed in the protein level of PPAR γ and occurred in a dose-dependent manner (25, 50, and 100 $\mu\text{g}/\text{mL}$; Fig. 1B, C). These findings strongly suggest that PM2.5 downregulates the expression of PPAR γ in HK-2 cells.

3.2. PPAR γ agonist and antagonist modulate the effects of PM2.5 on HK-2 cells

PM2.5-induced changes in the phenotype of HK-2 cells toward EMT were blocked by pretreatment with 15d-PGJ2 but enhanced by that with GW9662 (Fig. 2A). PM2.5 increased the level of HK-2 cell migration; this change was prevented by pretreatment with 15d-PGJ2 and enhanced by that with GW9662 (Fig. 2B). As presented in Fig. 2C, 15d-PGJ2 significantly blocked PM2.5-induced downregulation of PPAR γ expression, whereas GW9662 partially exacerbated PM2.5-induced downregulation of PPAR γ expression. In addition, the PM2.5-induced increase in the mRNA level of α -SMA and reduction in the mRNA level of E-cadherin

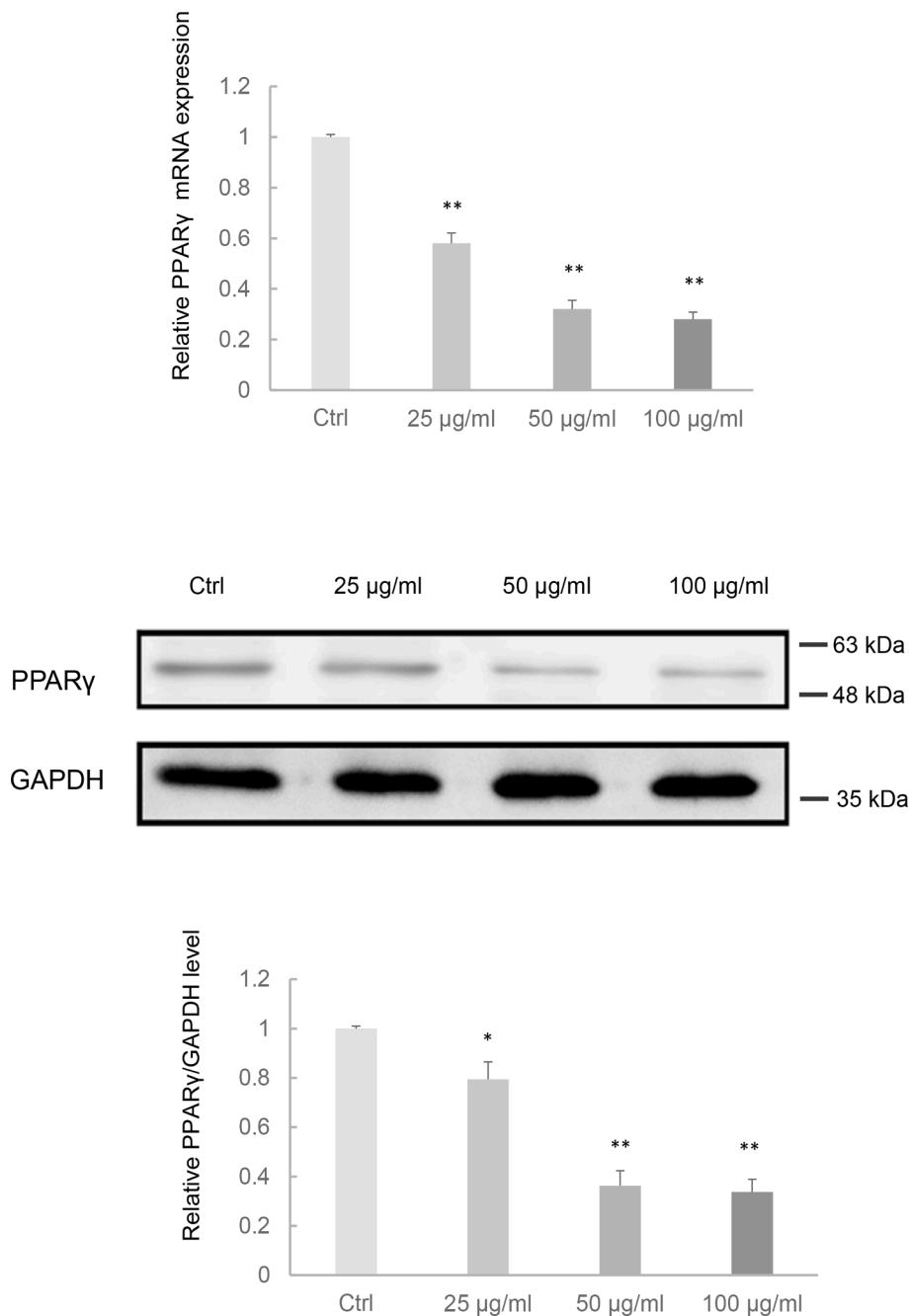


Fig. 1. PPAR γ expression in HK-2 cells exposed to different concentrations of PM2.5. The mRNA and protein levels of PPAR γ were measured through quantitative real-time polymerase chain reaction and Western blotting. (A) Level of PPAR γ expression in PM2.5-exposed HK-2 cells. (B) Immunoblots depicting PPAR γ levels after PM2.5 exposure. (C) Densitometric data on the protein level of PPAR γ . Data are presented in terms of mean \pm standard deviation values. * $p < 0.05$ and ** $p < 0.01$, compared with the respective controls.

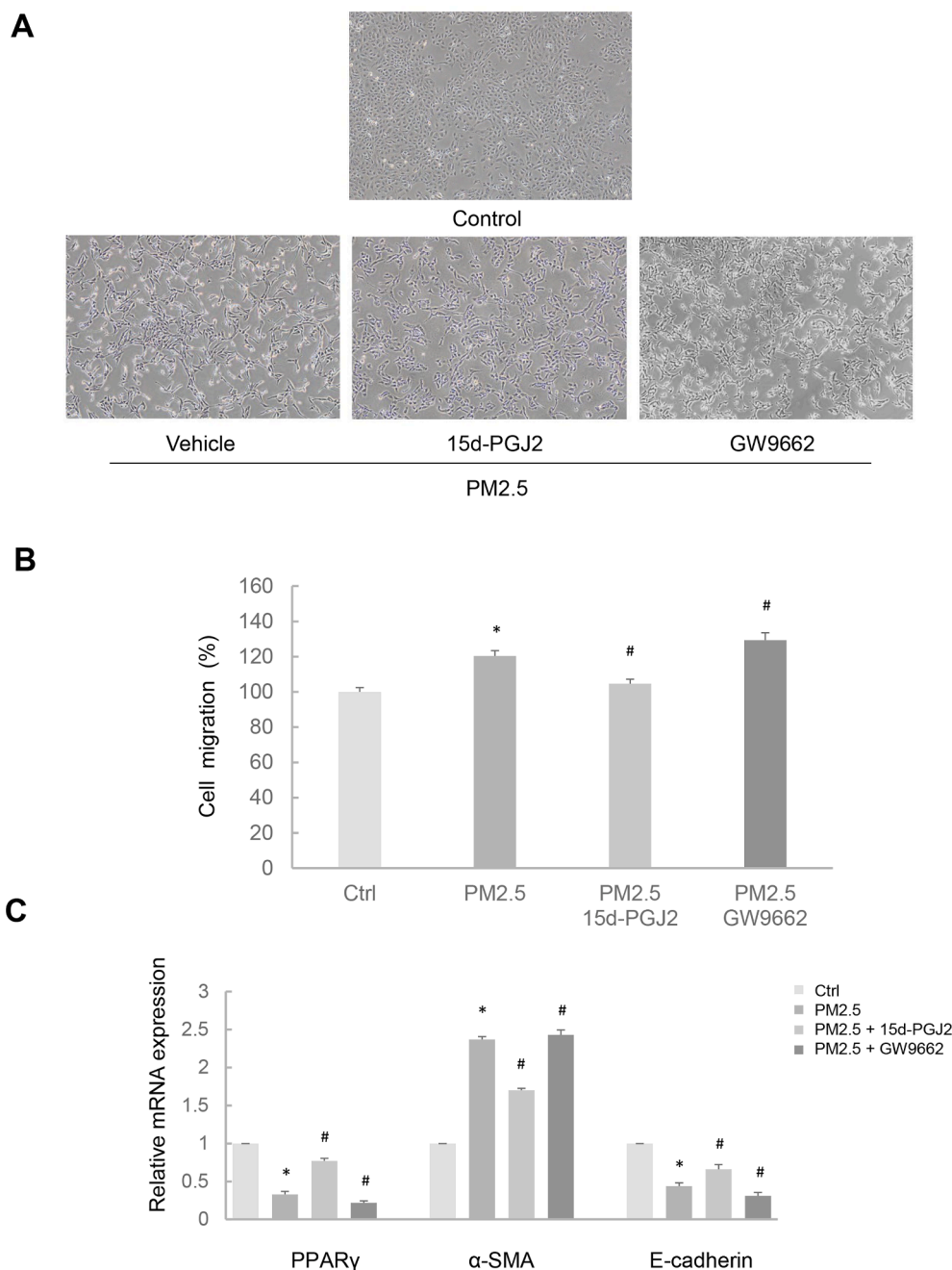


Fig. 2. Pretreatments with 15d-PGJ2 and GW9662 altered the EMT-related changes in PM2.5-exposed HK-2 cells. HK-2 cells were treated with 15d-PGJ2 or GW9662 24 h before PM2.5 exposure. (A) Cell morphology observed under a microscope (magnification, 100 \times). (B) Cell migration was assayed using the Transwell migration assay. (C) PM2.5-induced changes in the mRNA levels of EMT-related markers were mitigated or exacerbated by 15d-PGJ2 and GW9662, respectively. Data are presented in terms of mean \pm standard deviation values. * $p < 0.05$, compared with the control group; # $p < 0.05$, compared with the PM2.5-exposed group.

were blocked by pretreatment with 15d-PGJ2 but enhanced by that with GW9662. Therefore, aberrant PPAR γ expression may contribute to this pathological process.

3.3. PPAR γ regulates ferroptosis susceptibility of PM2.5-exposed HK-2 cells

Ferroptotic stress is pivotal for various physiological and pathological responses, and its association with PM2.5-induced cellular toxicity has been noted. We investigated the potential role of PPAR γ in regulating PM2.5-induced ferroptotic stress. This was examined by assessing iron levels, the ratio of GSH to GSSG, MDA levels, and the fluorescence of lipid peroxidation in cells subjected to 15d-PGJ2 or GW9662

pretreatment followed by PM2.5 exposure. PM2.5 markedly increased the iron production in the HK-2 cells, and pretreatment with 15d-PGJ2 or GW9662 did not significantly change the iron status (Fig. 3A). Furthermore, PM2.5 reduced the GSH/GSSG ratio and increased the MDA level in the HK-2 cells; these effects were blocked by pretreatment with 15d-PGJ2 and exacerbated and enhanced, respectively, by pretreatment with GW9662 (Fig. 3B,C). In addition, PM2.5 exposure increased the level of lipid ROS; this effect was blocked by pretreatment with 15d-PGJ2 and exacerbated by pretreatment with GW9662 (Fig. 3D). These findings indicate that induction of ferroptotic stress is associated with modulation of PPAR γ expression in PM2.5-exposed HK-2 cells.

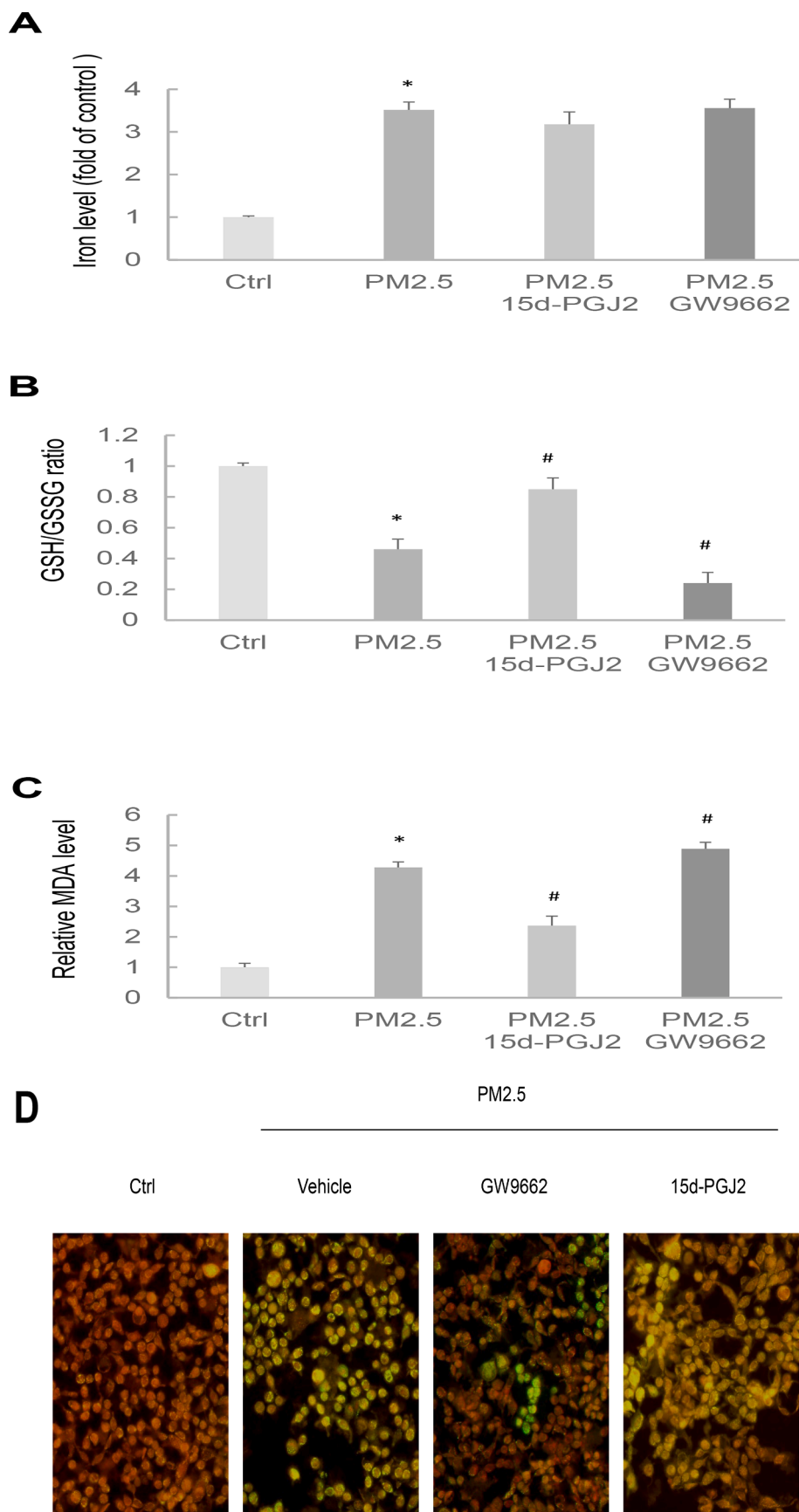


Fig. 3. Effects of 15d-PGJ2 and GW966 on the ferroptosis susceptibility of PM2.5-exposed HK-2 cells. HK-2 cells were treated with 15d-PGJ2 or GW9662 24 h before PM2.5 exposure. (A) Iron contents. (B) Reduced glutathione level. (C) MDA level. (D) The detection of lipid peroxidation in live cells.

3.4. Alleviation of ferroptotic stress blocks PM2.5-induced EMT and downregulation of PPAR γ expression in HK-2 cells

To elucidate the associations between ferroptotic stress, PPAR γ activity, and EMT, HK-2 cells were treated with the ferroptosis inhibitor fer-1 4 h before being exposed to PM2.5. As indicated in Fig. 4A, fer-1 significantly mitigated PM2.5-induced downregulation of PPAR γ expression as well as concurrent upregulation and downregulation of α -SMA and E-cadherin expression, respectively. Moreover, fer-1 effectively prevented PM2.5-induced activation of EMT (Fig. 4B). These results were corroborated by those of the cell migration assay, confirming that fer-1 reduces PM2.5-induced migration of HK-2 cells (Fig. 4C). Thus, the inhibitory effect of fer-1 on ferroptotic stress effectively mitigates PM2.5-induced downregulation of PPAR γ and alteration of the cellular phenotype in HK-2 cells.

3.5. PPAR γ overexpression restores GPX4 expression and suppresses EMT in PM2.5-exposed HK-2 cells

To understand the role of PPAR γ in PM2.5-induced alterations in the epithelial cell phenotype, we designed a pcDNA3.1-PPAR γ plasmid that selectively expresses PPAR γ . HK-2 cells were transfected with either the pcDNA3.1-PPAR γ plasmid or an empty vector. The expression of PPAR γ was evaluated 48 h after transfection. Real-time PCR revealed an approximately 3.3-fold increase in the mRNA level of PPAR γ in the cells transfected with the pcDNA3.1-PPAR γ plasmid compared with the level in those transfected with the empty vector (Fig. 5A). Additionally, Western blotting revealed a significant increase in the protein level of PPAR γ in the cells transfected with the pcDNA3.1-PPAR γ plasmid (Fig. 5B,C), indicating successful overexpression. Next, we measured the

protein levels of E-cadherin, α -SMA, and GPX4 in PM2.5-exposed cells with or without PPAR γ overexpression (Fig. 5D,E). A substantial increase and reduction were noted in the levels of E-cadherin and α -SMA, respectively, in the cells transfected with the pcDNA3.1-PPAR γ plasmid compared with the levels in those transfected with the empty vector. Notably, PPAR γ overexpression blocked PM2.5-induced downregulation of GPX4 expression in the HK-2 cells, perhaps by alleviating PM2.5-induced ferroptotic stress. These results indicate that PPAR γ confers protection against PM2.5-induced EMT and ferroptotic stress in tubular cells. Thus, PPAR γ activation may emerge as a potential strategy for alleviating PM2.5-induced tubular injury.

4. Discussion

Ambient air pollution is a major risk factor for poor health worldwide. PM2.5 negatively affects human health by causing various diseases (Liao et al., 2023; Thangavel et al., 2022), and exposure to PM2.5 increases the risks of renal disease and CKD progression (Chang et al., 2022; Politis et al., 2024; Wathanavasin et al., 2024). PM2.5 exposure enhances inflammation and oxidative stress, thereby reducing cells' antioxidant capacity and increasing lipid peroxidation; PM2.5 exposure also influences some signaling pathways related to EMT and organ fibrosis (Fu et al., 2019; Lee et al., 2020; Lin et al., 2021).

In the present study, PM2.5 exposure reduced the mRNA and protein levels of PPAR γ and altered the cellular phenotype toward EMT in HK-2 cells. The trend toward EMT was associated with PM2.5-induced downregulation of PPAR γ expression. Exposure to PM2.5 results in aberrant PPAR γ activity, which increases the risks of lung and hepatic fibroses (Jeong et al., 2021; Zheng et al., 2015). We found that PM2.5 exposure inhibited PPAR γ expression, causing tubular injury and

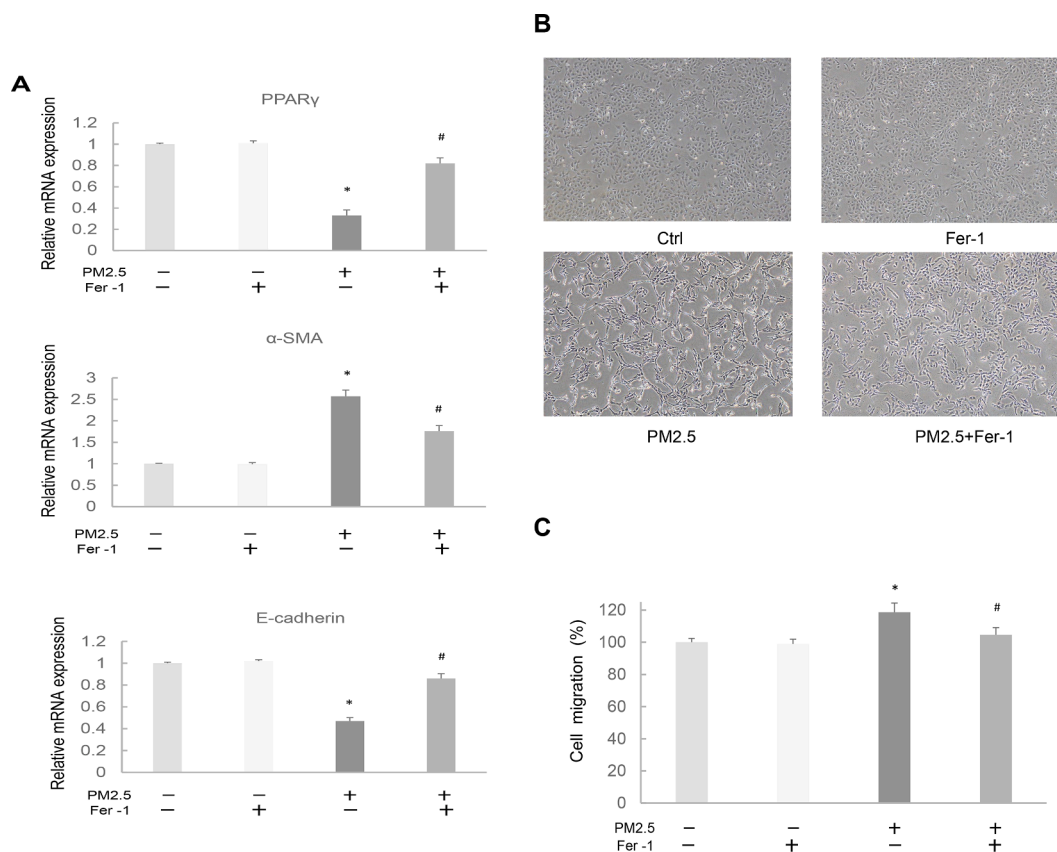


Fig. 4. Fer-1 mitigates downregulation of PPAR γ expression and alteration of the cellular phenotype in PM2.5-exposed HK-2 cells. HK-2 cells were treated with fer-1 (10 μ g/mL) 4 h before PM2.5 exposure. (A) Expression levels of PPAR γ , α -SMA, and E-cadherin. (B) Morphology of control and experimental cells. (C) Cell migration assessed through Transwell migration assay. Data are presented in terms of mean \pm standard deviation values. * p < 0.05, compared with the control group; # p < 0.05, compared with the PM2.5-exposed group.

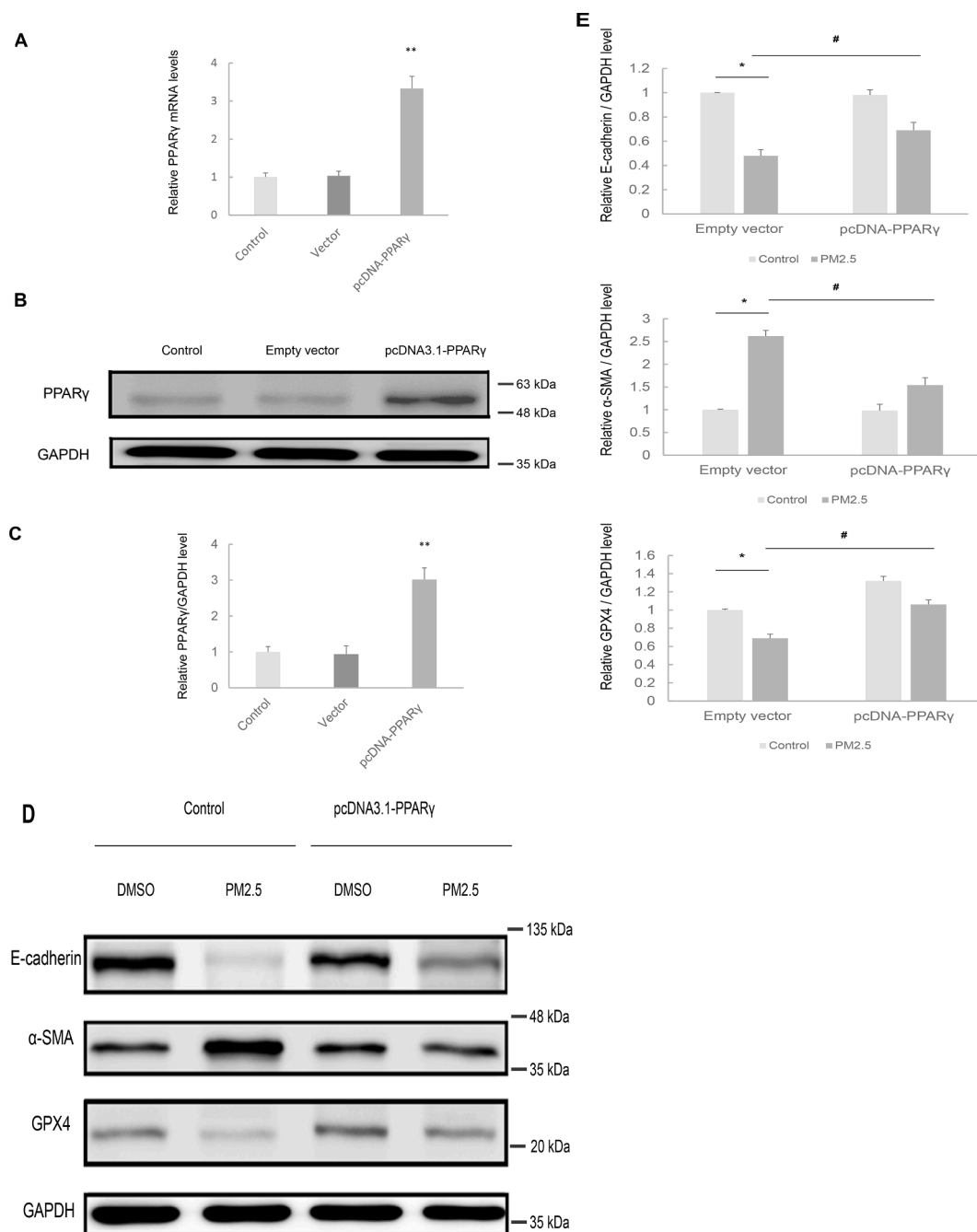


Fig. 5. PPAR γ overexpression blocked the PM2.5-induced changes in EMT and GPX4 expression in HK-2 cells. (A) Expression level of PPAR γ was estimated as the ratio of the control. Data are presented in terms of mean \pm standard deviation values. ** $p < 0.01$, compared with the vector group. (B) Levels of PPAR γ in control cells, cells transfected with an empty vector, and cells transfected with the pcDNA3.1-PPAR γ plasmid. (C) Densitometric ratios of PPAR γ were normalized. ** $p < 0.01$, compared with the vector group. (D) Levels of E-cadherin, α -SMA, and GPX4 in cells transfected with the empty vector or the pcDNA3.1-PPAR γ plasmid and then exposed to PM2.5. (E) Densitometric ratios of E-cadherin, α -SMA, or GPX4 were normalized. Data are presented in terms of mean \pm standard deviation values. * $p < 0.05$, compared with the control group; # $p < 0.05$, compared with the PM2.5-exposed group.

inducing ferroptotic stress, as evidenced by the elevated iron content, MDA level, and lipid ROS production and the reduced GSH/GSSG ratio. The alterations in PPAR γ expression by its agonist and antagonist might explain the different susceptibility levels of HK-2 cells to PM2.5-induced ferroptosis. EMT-related changes were observed in PM2.5-exposed cells: the cells lost cell–cell adhesion ability and exhibited enhanced motility; in addition, the expression of α -SMA was upregulated, whereas that of E-cadherin was downregulated. However, pretreatment with 15d-PGJ2 or GW9662 suppressed or promoted EMT in these cells. Fer-1 can prevent downregulation of PPAR γ , block changes in α -SMA and E-cadherin

levels, and suppress phenotypic switching in PM2.5-exposed cells. These findings highlight the associations among ferroptotic stress, aberrant PPAR γ activity, and EMT in PM2.5-exposed cells.

Ferroptotic stress, which plays a crucial role in pathological responses, has been implicated in PM2.5-induced cellular toxicity (Gu et al., 2023; Gu et al., 2022; Yan et al., 2022). We found that expression PPAR γ was negatively correlated with that of ferroptosis markers in PM2.5-exposed cells. The activation of PPAR γ reduced the cells' ferroptotic susceptibility and thus suppressed EMT. Furthermore, overexpression of PPAR γ prevented inhibition of GPX4 expression and

blocked alterations in E-cadherin and α -SMA expression in PM2.5-exposed cells. PPAR γ exerts anti-inflammatory effects and can indirectly alleviate oxidative stress and ROS production (Anderson et al., 2014; Croasdell et al., 2015; Fan et al., 2022). We found that PPAR γ upregulates expression of GPX4, a key antioxidant enzyme essential for protecting cells from the negative effects of lipid peroxidation and ferroptosis. These findings support the notion that the upregulation of PPAR γ expression could potentially mitigate PM2.5-induced tubular injury by suppressing ferroptosis and EMT.

5. Conclusion

This study revealed that PM2.5-induced impairment of PPAR γ activity results in ferroptotic stress and EMT in tubular cells. PPAR γ activation holds potential as a preventive strategy for PM2.5-induced renal injury.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Not applicable.

Funding

National Science and Technology Council - Grant numbers: 107-2314-B-075-036, 108-2314-B-075-043-MY2, 110-2314-B-075-017, 111-2314-B-075-046-MY2, 111-2314-B-075-048, 113-2314-B-075-070. Taipei Veterans General Hospital - Grant numbers: V111C-177, V112C-177, V113C-062. Yen Tjing Ling Medical Foundation Grant numbers: CI-111-21, CI-113-24. Taipei Institute of Pathology - Grant numbers: TIP-112-004, TIP-113-002.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

References

- Anderson, E.J., Thayne, K.A., Harris, M., Shaikh, S.R., Darden, T.M., Lark, D.S., Williams, J.M., Chitwood, W.R., Kypson, A.P., Rodriguez, E., 2014. Do fish oil omega-3 fatty acids enhance antioxidant capacity and mitochondrial fatty acid oxidation in human atrial myocardium via PPARgamma activation? *Antioxid. Redox Signal.* 21, 1156–1163.
- Boogaard, H., Walker, K., Cohen, A.J., 2019. Air pollution: the emergence of a major global health risk factor. *Int. Health* 11, 417–421.
- Bowe, B., Artimovich, E., Xie, Y., Yan, Y., Cai, M., Al-Aly, Z., 2020. The global and national burden of chronic kidney disease attributable to ambient fine particulate matter air pollution: a modelling study. *BMJ Glob. Health* 5, e002063.
- Chang, P.Y., Li, Y.L., Chuang, T.W., Chen, S.Y., Lin, L.Y., Lin, Y.F., Chiou, H.Y., 2022. Exposure to ambient air pollutants with kidney function decline in chronic kidney disease patients. *Environ. Res.* 215, 114289.
- Croasdell, A., Duffney, P.F., Kim, N., Lacy, S.H., Sime, P.J., Phipps, R.P., 2015. PPARgamma and the Innate Immune System Mediate the Resolution of Inflammation. *PPAR Res.* 2015, 549691.
- Cromar, K.R., Gladson, L.A., Hicks, E.A., Marsh, B., Ewart, G., 2022. Excess Morbidity and Mortality Associated with Air Pollution above American Thoracic Society Recommended Standards, 2017–2019. *Ann. Am. Thorac. Soc.* 19, 603–613.
- Fan, X., Xu, M., Ren, Q., Fan, Y., Liu, B., Chen, J., Wang, Z., Sun, X., 2022. Downregulation of fatty acid binding protein 4 alleviates lipid peroxidation and oxidative stress in diabetic retinopathy by regulating peroxisome proliferator-activated receptor gamma-mediated ferroptosis. *Bioengineered.* 13, 10540–10551.
- Feng, Q., Yu, X., Qiao, Y., Pan, S., Wang, R., Zheng, B., Wang, H., Ren, K.D., Liu, H., Yang, Y., 2022. Ferroptosis and Acute Kidney Injury (AKI): Molecular Mechanisms and Therapeutic Potentials. *Front. Pharmacol.* 13, 858676.
- Fu, Y., Lu, R., Cui, J., Sun, H., Yang, H., Meng, Q., Wu, S., Aschner, M., Li, X., Chen, R., 2019. Inhibition of ATP citrate lyase (ACLY) protects airway epithelia from PM2.5-induced epithelial-mesenchymal transition. *Ecotoxicol. Environ. Saf.* 167, 309–316.
- Giuliani, K.T.K., Grivei, A., Nag, P., Wang, X., Rist, M., Kildey, K., Law, B., Ng, M.S., Wilkinson, R., Ungerer, J., Forbes, J.M., Healy, H., Kassianos, A.J., 2022. Hypoxic human proximal tubular epithelial cells undergo ferroptosis and elicit an NLRP3 inflammasome response in CD1c(+) dendritic cells. *Cell Death Dis.* 13, 739.
- Gu, Y., Hao, S., Liu, K., Gao, M., Lu, B., Sheng, F., Zhang, L., Xu, Y., Wu, D., Han, Y., Chen, S., Zhao, W., Lou, X., Wang, X., Li, P., Chen, Z., Yao, K., Fu, Q., 2022. Airborne fine particulate matter (PM2.5) damages the inner blood-retinal barrier by inducing inflammation and ferroptosis in retinal vascular endothelial cells. *Sci. Total Environ.* 838, 156563.
- Gu, W., Hou, T., Zhou, H., Zhu, L., Zhu, W., Wang, Y., 2023. Ferroptosis is involved in PM2.5-induced acute nasal epithelial injury via AMPK-mediated autophagy. *Int. Immunopharmacol.* 115, 109658.
- Ide, S., Kobayashi, Y., Ide, K., Strausser, S.A., Abe, K., Herbek, S., O'Brien, L.L., Crowley, S.D., Barisoni, L., Tata, A., Tata, P.R., Souma, T., 2021. Ferroptotic stress promotes the accumulation of pro-inflammatory proximal tubular cells in maladaptive renal repair. *Elife* 10.
- Jeong, J., Bae, S.Y., Choi, J., 2021. Identification of toxicity pathway of diesel particulate matter using AOP of PPARgamma inactivation leading to pulmonary fibrosis. *Environ. Int.* 147, 106339.
- Kokeny, G., Calvier, L., Hansmann, G., 2021. PPARgamma and TGFbeta-Major Regulators of Metabolism, Inflammation, and Fibrosis in the Lungs and Kidneys. *Int. J. Mol. Sci.* 22.
- Lee, H., Hwang-Bo, H., Ji, S.Y., Kim, M.Y., Kim, S.Y., Park, C., Hong, S.H., Kim, G.Y., Song, K.S., Hyun, J.W., Choi, Y.H., 2020. Diesel particulate matter2.5 promotes epithelial-mesenchymal transition of human retinal pigment epithelial cells via generation of reactive oxygen species. *Environ. Pollut.* 262, 114301.
- Li, G., Huang, J., Wang, J., Zhao, M., Liu, Y., Guo, X., Wu, S., Zhang, L., 2021. Long-Term Exposure to Ambient PM(2.5) and Increased Risk of CKD Prevalence in China. *J Am Soc Nephrol* 32, 448–458.
- Li, W., Wang, W., 2023. Causal effects of exposure to ambient air pollution on cancer risk: Insights from genetic evidence. *Sci. Total Environ.* 912, 168843.
- Liao, Q., Li, Z., Li, Y., Dai, X., Kang, N., Niu, Y., Tao, Y., 2023. Specific analysis of PM (2.5)-attributed disease burden in typical areas of Northwest China. *Front. Public Health* 11, 1338305.
- Lin, C.H., Wan, C., Liu, W.S., Wang, H.H., 2021. PM2.5 Induces Early Epithelial Mesenchymal Transition in Human Proximal Tubular Epithelial Cells through Activation of IL-6/STAT3 Pathway. *Int. J. Mol. Sci.* 22.
- Liu, J., Zhao, N., Shi, G., Wang, H., 2020. Geniposide ameliorated sepsis-induced acute kidney injury by activating PPARgamma. *aging (Albany NY)* 12, 22744–22758.
- Maquigussa, E., Paterno, J.C., de Oliveira Pokorny, G.H., da Silva Perez, M., Varela, V.A., da Silva Novaes, A., Schor, N., Boim, M.A., 2018. Klotho and PPAR Gamma Activation Mediate the Renoprotective Effect of Losartan in the 5/6 Nephrectomy Model. *Front. Physiol.* 9, 1033.
- Menendez-Gutierrez, M.P., Roszer, T., Ricote, M., 2012. Biology and therapeutic applications of peroxisome proliferator-activated receptors. *Curr. Top. Med. Chem.* 12, 548–584.
- Politis, M.D., Gutierrez-Avila, I., Just, A., Pizano-Zarate, M.L., Tamayo-Ortiz, M., Greenberg, J.H., Tellez-Rojo, M.M., Sanders, A.P., Rosa, M.J., 2024. Recent ambient temperature and fine particulate matter (PM2.5) exposure is associated with urinary kidney injury biomarkers in children. *Sci. Total Environ.* 907, 168119.
- Su, X., Tian, J., Li, B., Zhou, L., Kang, H., Pei, Z., Zhang, M., Li, C., Wu, M., Wang, Q., Han, B., Chu, C., Pang, Y., Ning, J., Zhang, B., Niu, Y., Zhang, R., 2020. Ambient PM2.5 caused cardiac dysfunction through FoxO1-targeted cardiac hypertrophy and macrophage-activated fibrosis in mice. *Chemosphere* 247, 125881.
- Thangavel, P., Park, D., Lee, Y.C., 2022. Recent Insights into Particulate Matter (PM (2.5))-Mediated Toxicity in Humans: An Overview. *Int. J. Environ. Res. Public Health* 19.
- Wang, F., Chen, T., Chang, Q., Kao, Y.W., Li, J., Chen, M., Li, Y., Shia, B.C., 2021a. Respiratory diseases are positively associated with PM2.5 concentrations in different areas of Taiwan. *PLoS One* 16, e0249694.
- Wang, Y., Zhong, Y., Liao, J., Wang, G., 2021b. PM2.5-related cell death patterns. *Int. J. Med. Sci.* 18, 1024–1029.
- Wathanavasin, W., Banjongjit, A., Phannajit, J., Eiam-Ong, S., Susantitaphong, P., 2024. Association of fine particulate matter (PM2.5) exposure and chronic kidney disease outcomes: a systematic review and meta-analysis. *Sci. Rep.* 14, 1048.
- Wen, F., Xie, Y., Li, B., Li, P., Qi, H., Zhang, F., Sun, Y., Zhang, L., 2023. Combined effects of ambient air pollution and PM(2.5) components on renal function and the potential mediation effects of metabolic risk factors in China. *Ecotoxicol. Environ. Saf.* 259, 115039.
- Xu, C., Zhang, Q., Huang, G., Huang, J., Zhang, H., 2023. The impact of PM2.5 on kidney. *J. Appl. Toxicol.* 43, 107–121.
- Yan, K., Hou, T., Zhu, L., Ci, X., Peng, L., 2022. PM2.5 inhibits system Xc- activity to induce ferroptosis by activating the AMPK-Beclin1 pathway in acute lung injury. *Ecotoxicol. Environ. Saf.* 245, 114083.
- Zhang, X., Tao, J., Lei, F., Sun, T., Lin, L., Huang, X., Zhang, P., Ji, Y.X., Cai, J., Zhang, X. J., Li, H., 2023. Association of the components of ambient fine particulate matter

- (PM_{2.5}) and chronic kidney disease prevalence in China. *J. Environ. Manage.* 339, 117885.
- Zheng, Z., Zhang, X., Wang, J., Dandekar, A., Kim, H., Qiu, Y., Xu, X., Cui, Y., Wang, A., Chen, L.C., Rajagopalan, S., Sun, Q., Zhang, K., 2015. Exposure to fine airborne particulate matters induces hepatic fibrosis in murine models. *J. Hepatol.* 63, 1397–1404.
- Zhou, L., Xue, X., Hou, Q., Dai, C., 2022. Targeting ferroptosis attenuates interstitial inflammation and kidney fibrosis. *Kidney Dis (basel)*. 8, 57–71.