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LABORATORY STUDY

Non-steroidal mineralocorticoid receptor antagonist finerenone inhibits peritoneal fibrosis induced by high-glucose dialysate via regulating enhancer of zeste homolog 2 (EZH2) expression

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ABSTRACT

Finerenone, a novel high-selective aldosterone receptor antagonist, exhibits powerful anti-inflammatory and antifibrotic effects in previous researches. The aim of our study was to investigate of it on peritoneal fibrosis. In our current research, we found that high glucose could induce epithelial mesothelial transformation (EMT) of peritoneal mesothelial cells (HPMCs). Under high glucose stimulation, the addition of finerenone could alleviate high glucose induced EMT and disordered cytoskeleton rearrangement in HPMCs. Moreover, finerenone decreased the expression of enhancer of zeste homolog 2 (EZH2). Results of rescue experiment showed that after overexpression of EZH2 in the presence of finerenone, the protective effect of finerenone on EMT, migration capacity and cytoskeleton rearrangement was counteracted by EZH2 overexpression. The above results have also been demonstrated in *in vivo* experiments. These findings imply that finerenone could alleviate EMT and peritoneal fibrosis *via* regulating EZH2. More studies are needed to validate it and explore further mechanism.

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Peritoneal fibrosis (PF); peritoneal dialysis; epithelial-mesenchymal transition (EMT); finerenone; enhancer of zeste homolog 2 (EZH2)

1. Introduction

Statistics show that approximately 11% of the world's kidney failure patients receive peritoneal dialysis (PD) [1], one of the renal replacement treatments available for patients with chronic kidney disease (CKD) progressing to end-stage renal disease (ESRD). However, prolonged PD can lead to peritoneal fibrosis (PF) due to the non-biocompatible peritoneal dialysis fluid with a low pH, high glucose concentration and high permeability [2,3], which may compromise the therapeutic effect of PD. Therefore, inhibiting PF during PD is a crucial clinical challenge. Finerenone is a selective, non-steroidal mineralocorticoid receptor antagonist (MRA). It can prevent damage caused by overactivation of the mineralocorticoid receptors (MR), including inflammation and fibrosis [4]. In this paper, the mechanism by which finerenone inhibits PF is investigated.

Epithelial-mesenchymal transition (EMT) occurs during PF. During this process, quiescent epithelial cells transform into motile mesenchymal cells, which are characterized by the absence of epithelial cell markers such as cytokeratins and E-cadherin, and the upregulated expression of mesenchymal cell markers such as N-cadherin, vimentin, and α-SMA. This results in the deposition of extracellular matrix (ECM) and subsequent peritoneal fibrosis [5]. Glucose is used as an osmotic agent in peritoneal dialysate. Studies have shown that high concentrations of glucose can promote peritoneal fibrosis by upregulating the transforming growth factor-β1 (TGF-β1) [6]. TGF-β1 is the primary signaling factor that induces EMT [7,8]. Following EMT, mesothelial cells lose cell polarity, adhesion and tight junctions, and acquire the shape and invasive ability of fibroblasts, thereby contributing to peritoneal fibrosis [9].

Mineralocorticoid receptors (MR) exist in many tissues that mediate many normal physiological functions, including tissue repair and electrolyte and fluid homeostasis [10]. However, a number of studies have shown that overactivation of the MR leads to inflammation and fibrosis in the heart, kidneys and blood vasculature [11–13]. The overactivation of MR can be inhibited by two different classes of MR antagonists (MRAs). Steroidal MRAs such as spironolactone and eplerenone can have significant side effects in daily

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clinical practice, such as increased risk of hyperkalemia and gynecomastia, which potentially limits dosages [14]. The second class of MRA is non-steroidal MRA, which is designed to target the MR. Finerenone [15] is a novel, selective, non-steroidal MRA that blocks MR-mediated sodium reabsorption and overactivation of MR, and has anti-inflammatory and anti-fibrosis effects [16]. Current evidence suggests that finerenone may inhibit cardiac fibrosis and delay the progression of kidney disease [13,17]. However, there are few articles discussing the effect of finerenone on PF. Therefore, our research in this paper focuses on investigating the effect of finerenone on PF and its mechanism.

Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase that catalyzes the trimethylation of the lysine residue at position 27 of histone H3 and is involved in cell cycle regulation, differentiation and transcriptional silencing of related target genes [18]. Research has demonstrated that EZH2 can suppress the expression of E-Cadherin by trimethylating H3K27, thereby promoting EMT [19–21]. EZH2 is highly expressed in aggressive tumors and can regulate EMT by targeting specific gene subsets [22]. Fatemeh Nourmohammadi et al. found that EZH2 can induce EMT in esophageal squamous carcinoma by upregulating the expression of miR-200c [23]. In addition, many studies have shown that EZH2 is closely linked to fibrosis. The overexpression of EZH2 in fibroblasts and endothelial cells in scleroderma is known to promote profibrosis [24]. In patients with idiopathic pulmonary fibrosis, EZH2 is required for TGF-β1-induced fibroblast differentiation to myofibroblasts, and inhibiting EZH2 reduces pulmonary fibrosis [25]. EZH2 has also been found to promote hepatic stellate cell (HSC) activation and fibrosis in the liver [26]. Inhibition of EZH2 can suppress the differentiation of atrial fibroblasts into myofibroblasts, as well as inhibiting the activation and migration of fibroblasts, thus inhibiting atrial fibrosis [27]. Genetic or pharmacological inhibition of EZH2 has been shown to reduce PF [28], for example, EZH2 inhibition with 3-DZnep effectively improves PF in patients with long-term PD. Therefore, we hypothesized that finerenone could inhibit PF by inhibiting the expression of EZH2.

This study hypothesized that finerenone could inhibit PF in long-term peritoneal dialysis patients and that finerenone exerts its effect in inhibiting PF by suppressing the expression of EZH2. To this end, we performed a series of *in vivo* and *in vitro* experiments to demonstrate that finerenone can reduce peritoneal fibrosis by inhibiting the expression of EZH2.

2. Materials and methods

2.1. Chemicals and antibodies

Finerenone was purchased from Biochem Safebuy Bio-Technology company. A 10 mM stocking solution was dissolved with DMSO and stored at −80 °C. The EZH2 overexpression lentiviral vector was purchased from Biobiogenesis (Shanghai, China) and stored at-20 °C. Dulbecco's modified Eagle's medium(DMEM F12/1:1), Fetal bovine serum (FBS), trypsin (EDTA) were purchased from Epizyme Biomedical Technology (Shanghai, China). The primary antibodies were as follows:anti-α-SMA (USA, Abcam), anti-E-cadherin (China, PTG), anti-vimentin (China, PTG), anti-EZH2 (USA, Abcam), anti-GAPDH (China, PTG) were used. Goat anti-rabbit and goat anti-mouse antibodies combined with horseradase markers were purchased from Beyotime Biotechnology (Shanghai, China). The CCK8 reagent was purchased from Epizyme Biomedical Technology (Shanghai, China).

2.2. Cell culture

The human peritoneal mesothelial cell line HMrSV5 was obtained from Prof Xueqing Yu, The First Affiliated Hospital of Sun Yat-sen University. Human peritoneal mesothelial cells (HPMCs) were cultured in DMEM/F12 in medium containing 10% fetal bovine serum (FBS) and placed in incubator containing 5%CO2 and 95% air at 37 °C. When the number of HPMCs in the culture flask reached suitable density, the cells were digested with EDTA and passaged. In the following experiments, cells were treated with high concentration of glucose (1.25%, 2.5%), 2.5% mannitol and high glucose plus finerenone for 48 h.

2.3. Establishment of rat peritoneal fibrosis models and finerenone administration

All research involving animals was conducted according the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments). Animal experiments were approved by the Animal Ethics Committee of Shandong Provincial Hospital affiliated to Shandong First Medical University (approval number: No.2022-143). 6-8 weeks old male Sprague-Dawley rats (130–140 g body weight, $n = 24$) were purchased from Animal Center of Shandong University (Jinan, China) to construct a peritoneal fibrosis model. Rats were divided into 3 groups of 8 each, as detailed control group, PDF group, finerenone group. The PDF group was injected with 4.25% peritoneal dialysis fluid with 100 mL/kg daily for 28 days, and the control group also received an equal volume of 0.9% saline. To test the effect of finerenone on peritoneal fibrosis, 10 mg/kg of finerenone was administered daily by gavaged after PDF injection. After 28 days, parietal and visceral peritoneum were collected for subsequent experiments.

2.4. Western blot

Total protein from HPMCs was extracted using protein lysates containing 1% protease inhibitors. Proteins were separated by electrophoresis by adding 20 ug of total protein in a 10% SDS-PAGE gel, and the proteins were transferred to a PVDF membrane. They were then blocked with 5% skim milk for 1 h at room temperature and treated overnight at 4 °C with the following primary antibody: anti-α-SMA (1:1000, abcam), anti-E-cadherin (1: 20000, PTG), anti-vimentin (1: 1000, PTG), anti-EZH2 (1: 250, Abcam), anti-GAPDH (1: 1000, PTG). The membranes were washed three times with TBST for 10 min

each the next day before being incubated for one hour at room temperature with anti-mouse IgG and anti-rabbit IgG (1:5000) with secondary antibodies. After three further washes with TBST, the protein bands were detected with an ECL system and a Bio-Rad electrophoresis image analyzer.

2.5. Wound-healing assay

HPMCs were inoculated in six-well plates and incubated with common medium for 24 h, followed by high glucose (1.25%, 2.5%), 2.5% mannitol stimulation, and stimulated for 48 h in the presence or absence of finerenone (1 μM, 2 μM). When cells were grown to 90% confluence, scratches were formed on the cell surface with a microtubule tip. After washing with PBS three times, the cells were cultured in serum-free DMEM/F12. The breadth of the scratch wounds was compared using 40x microscope images of the migrating cells under each group at 0 and 12 h.

2.6. Transwell migration assay

Transwell cell culture inserts (pore size 8 μm; Corning Costar Corp.) was placed in DMEM/F12 supplemented with 10% fetal bovine serum. Trypsin was used to harvest HPMCs with various pretreatments, and the resulting suspension was then put back into serum-free DMEM/F12. The upper chambers were seeded with $1*10^5$ cells/mL, which were allowed to attach at 37 °C for 12 h. Then, the cells were fixed with 4% paraformaldehyde and washed three times with PBS. Unmigrated cells on the membrane were removed with a cotton swab, the cells on the membrane were stained with 1% crystal violet for 5 min, and images of the migrating cells were collected under the phase con-trast microscopy.

2.7. Cellular immunofluorescence

HPMCs with different pretreatments were blocked with 5% BSA at 37 °C and incubated overnight with primary antibodies (rabbit anti-vimentin 1:200, rabbit anti-EZH2 1:250, and mouse anti-α-SMA 1:200) at 4 °C. The appropriate secondary antibodies were applied to the cells the following day at 37 °C for 45 min. All sections were counterstained with 4′-6-diamidino-2-phenylindole (DAPI, Invitrogen). Specific fluorescence was obtained by positive fluorescence microscope.

2.8. Phalloidin staining

Cultured HPMCs were fixed with 4% paraformaldehyde for 15 min and washed three times with PBS before being treated with 0.5% TritonX-100 solution for 5 min. After washing three times with PBS again, the ghost cyclotide working solution was added and incubated for 30 min at room temperature. After addition of anti-fluorescence quenching sealing tablets, they were visualized by positive fluorescence microscope.

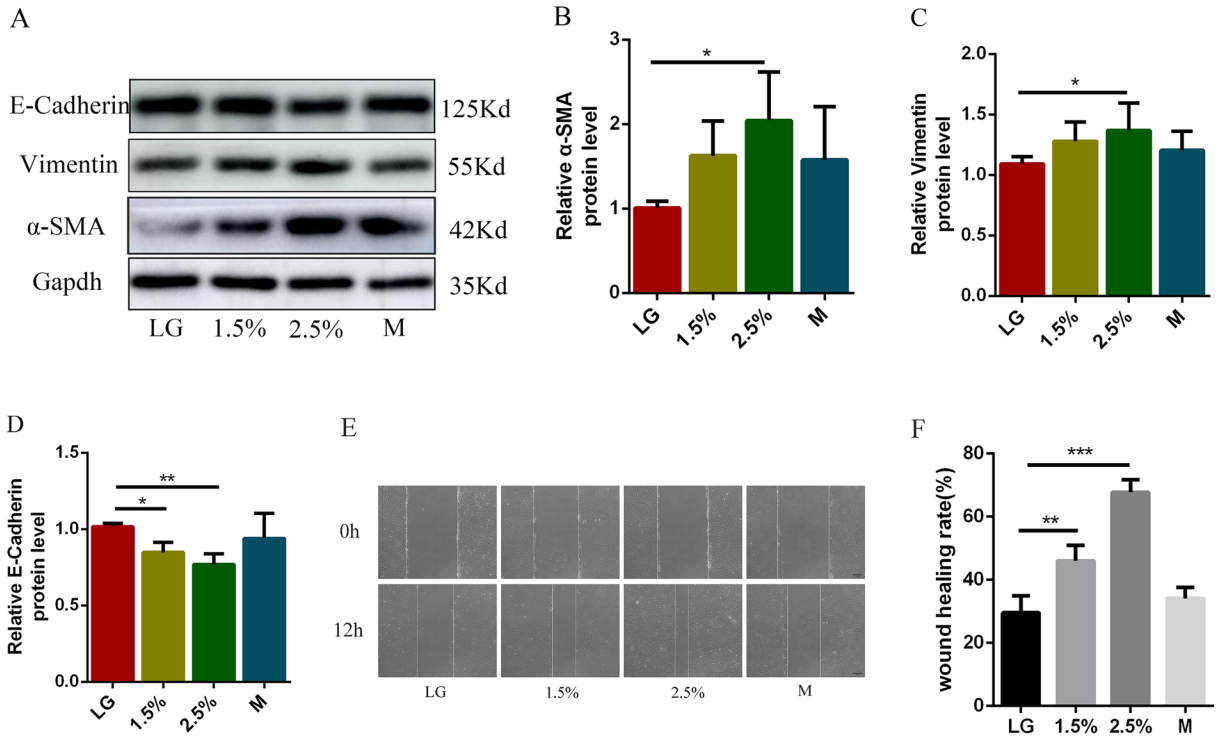


Figure 1. EMT and stronger migration capacity were induced in high glucose circumstance while high osmotic invalid.
HPMCs were treated with a medium containing various concentrations of glucose (1.5%, 2.5%) and a medium containing 2.5% mannitol for 48 h. (A) Representative immunoblots of α-SMA, vimentin and E-cadherin in HPMCs under various treatments are shown. GAPDH served as a loading control. (B–D) The relative expression of α-SMA, vimentin and E-cadherin (Data are the mean ± SD; $*P<0.05$, $**P<0.01$, n = 3). (E) A wound healing assay showed that high glucose could accelerate the migration of HPMCs (scale bar =100 μm). (F) Quantitative analysis of the wound-healing assay (Data are the mean ± SD; $*P<0.05$, $**P<0.01$, $***P<0.001$, n = 3).

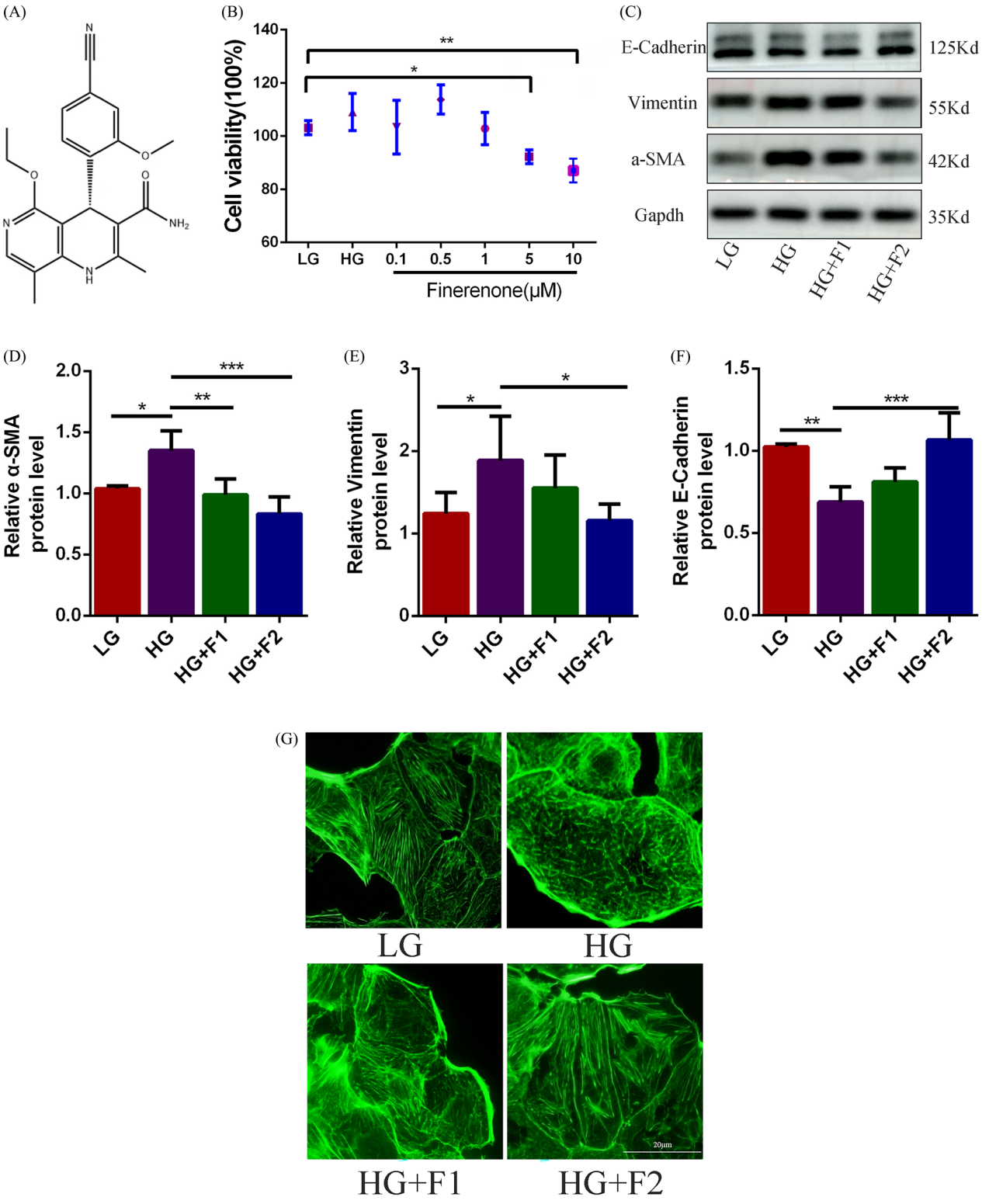


Figure 2. Finerenone could ameliorate EMT, cytoskeleton rearrangement and migration capacity induced by high glucose.
(A) The chemical structure of the finerenone. (B) Cell viability was measured by CCK-8 assay and expressed as percent cell survival relative to low glucose controls. (**$P < 0.01$ vs LG, ****$P < 0.0001$ vs LG, n = 3). HPMCs were treated with various concentrations of finerenone (1&2 μm) after being stimulated by high glucose. (C) Representative immunoblots of α-SMA, vimentin and E-cadherin in HPMCs under various treatments are shown. GAPDH served as a loading control. (D–F) The relative expression of α-SMA, vimentin and E-cadherin. (Data are the mean ± SD; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 3). (G) The effect of finerenone on cell cytoskeleton of HPMCs was assessed using phalloidin staining (scale bar = 20 μm). (H) The effect of finerenone on the migration of HPMCs was assessed using a transwell assay, and the migrated cells were detected by crystal violet staining (scale bar = 100 μm). (I) The effect of finerenone on the migration of HPMCs was assessed using a wound-healing assay (scale bar = 100 μm). (J) Quantitative analysis of the wound-healing assay. (Data are the mean ± SD; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 3).

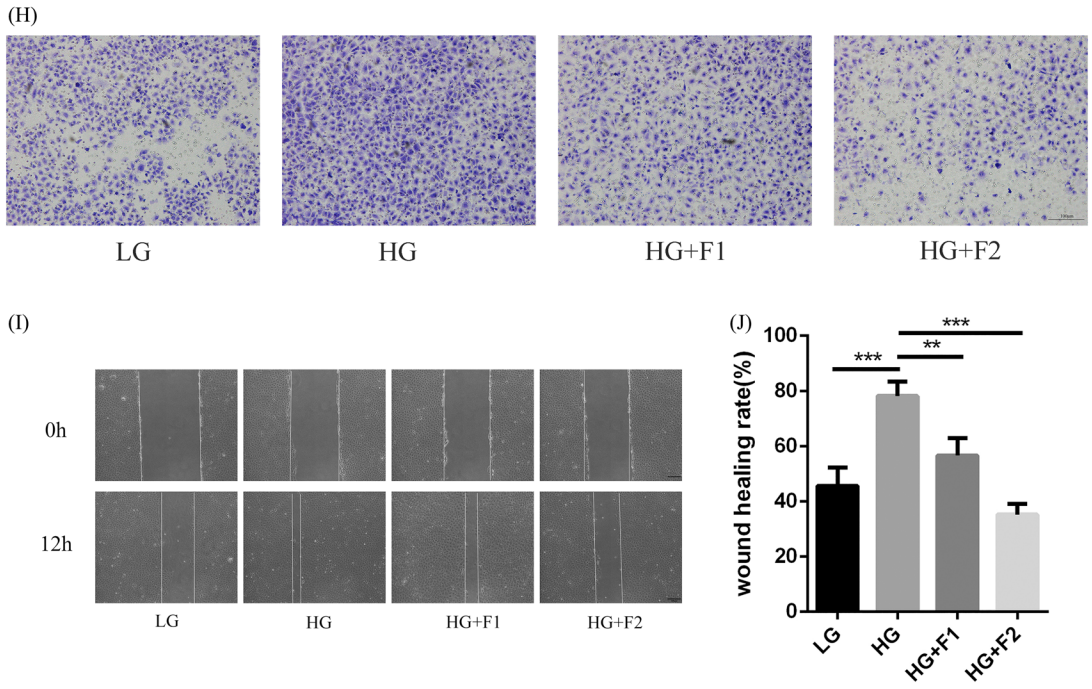


Figure 2. Continued.

2.9. Histopathological and immunohistochemical analyses

Parietal peritoneal tissues were fixed in 10% neutral formalin and embedded in paraffin to obtain 3-4-μm-thick serial tissue sections. The sections were stained with hematoxylin and eosin (H & E) and Masson tricolor solutions to analyze histopathological features characteristics. Before immunohistochemistry, tissue sections were heated using citrate buffer (pH 6.0) to reveal the antigen. After being treated with hydrogen peroxide and peroxidase, the cells were incubated for an hour with primary antibodies to α-SMA, vimentin, and EZH2 (PTG, China, dilution 1:200).

Samples were incubated with the secondary antibodies for 30 min at room temperature. DAB is used to visualize color, followed by hematoxylin staining. The sections were observed under the microscope after being dehydrated and sealed.

2.10. Statistical analysis

Data are presented as the mean ± SD unless stated otherwise. One-way ANOVA was used to determine significant differences between groups. Dunnett's test was used to perform multiple comparisons between groups. Two-tailed $p < 0.05$ indicated statistical significance.

3. Results

3.1. The EMT-related indicators were highly expressed in HPMCs under high-glucose stimulation

To determine whether EMT occurred in HPMCs upon high-glucose stimulation, we cultured HPMCs cells with DMEM/F12 medium containing high glucose. Western blot showed that the HPMCs treated with high glucose significantly increased the levels of the mesenchymal cell markers α-SMA, vimentin, and decreased the expression of the epithelial cell marker E-cadherin (Figure 1A–D). Wound-healing assay also found that high glucose could generate enhanced migration capacity (Figure 1E,F). The above results illustrated that EMT was induced in HPMCS stimulated with high glucose.

3.2. Finerenone could alleviate high glucose induced EMT and disordered cytoskeleton rearrangement in HPMCs

The chemical structure of finerenone is shown in Figure 2A. To determine the optimal concentration of finerenone treated with HPMCs, CCK-8 was conducted. As exhibited in Figure 2B, finerenone with 1-2 μM was the optimal concentration without cytotoxicity. We then destimulated the HPMCs with both 1 μM and 2 μM finerenone, on the addition of high glucose to explore the effect of it. The results of Western blot showed that the increased expression of mesenchymal markers α-SMA and vimentin and the decreased expression of epithelial cell marker E-cadherin which were induced by high glucose could be released by finerenone (Figure 2C–F). Besides, the results of phalloidin staining showed that the cytoskeleton of HPMCs became disordered under high-glucose stimulation, while became clear again after the application of finerenone (Figure 2G). The results of wound-healing assay and the transwell migration assay exhibited that the increased cell migration ability at high glucose was inhibited by the addition of finerenone (Figure 2H–J).

3.3. Finerenone inhibited the expression of EZH2 induced by high glucose

In our previous research, EZH2 was found to be a vital molecule that involved in PF [29,30]. To explore whether it was participated in the effect of finerenone, the following

experiments was done. Results showed the expression of EZH2 was elevated in high glucose stimulation. After the addition of finerenone, and the increased expression of EZH2 was significantly decreased (Figure 3A,B). Also, the results of cellular Immunofluorescence showed that EZH2 expression was significantly increased under high-glucose stimulation with a stronger fluorescent signal. After the addition of finerenone, the fluorescent signal became weaker (Figure 3C).

3.4. The effect of finerenone on inhibiting peritoneal fibrosis decreased after overexpression of EZH2

To explore whether EZH2 mediated the effect of finerenone, rescue experiment was conducted. Lentivirus was transfected into HPMCs to overexpress EZH2 (Figure 4A,B). Western blot results showed that the inhibitory effect of finerenone on EMT was attenuated under high glucose after EZH2 overexpression (Figure 4C,D). Also, cellular immunofluorescence results showed that overexpression of EZH2 increased the expression of vimentin and α-SMA in HPMCs (Figure 4I). Besides, overexpression of EZH2 reduces the protective effect of finerenone on the cytoskeleton under high glucose, as shown by phalloidin staining (Figure 4E). Transwell migration assay and wound-healing assay showed that inhibitory effect of finerenone on cell migration ability was attenuated under high glucose after EZH2 overexpression (Figure 4F–H).

3.5. Finerenone alleviated PD-related peritoneal fibrosis via *EZH2*

The experiments *in vivo* demonstrated decreased expression of epithelial cell marker E-Cadherin, and increased expression of mesenchymal markers α-SMA and vimentin in the peritoneal tissue of SD rats injected with long-term peritoneal

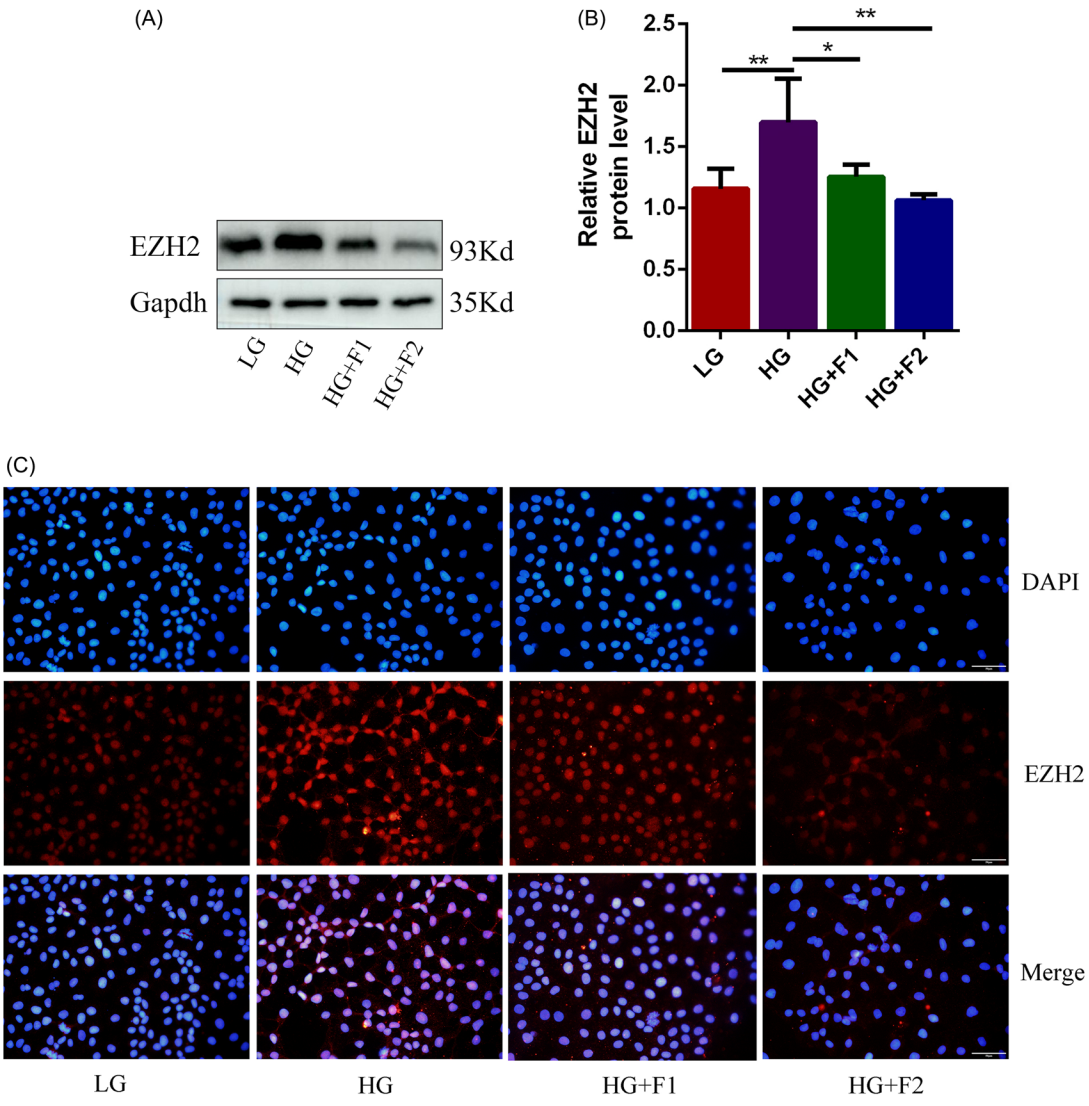


Figure 3. Finerenone inhibited the expression of EZH2 induced by high glucose.
Cells were treated with various concentrations of finerenone (1&2 μm) after being stimulated by high glucose. (A) Representative immunoblots of EZH2 in HPMCs under various treatments are shown. GAPDH served as a loading control. (B) The relative expression of EZH2. (Data are the mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, n = 3). (C) Cellular immunofluorescence was used to assess the effect of finerenone on EZH2 expression in HPMCs. EZH2 was stained red and the nuclei were stained blue with DAPI (scale bar = 50 μm).

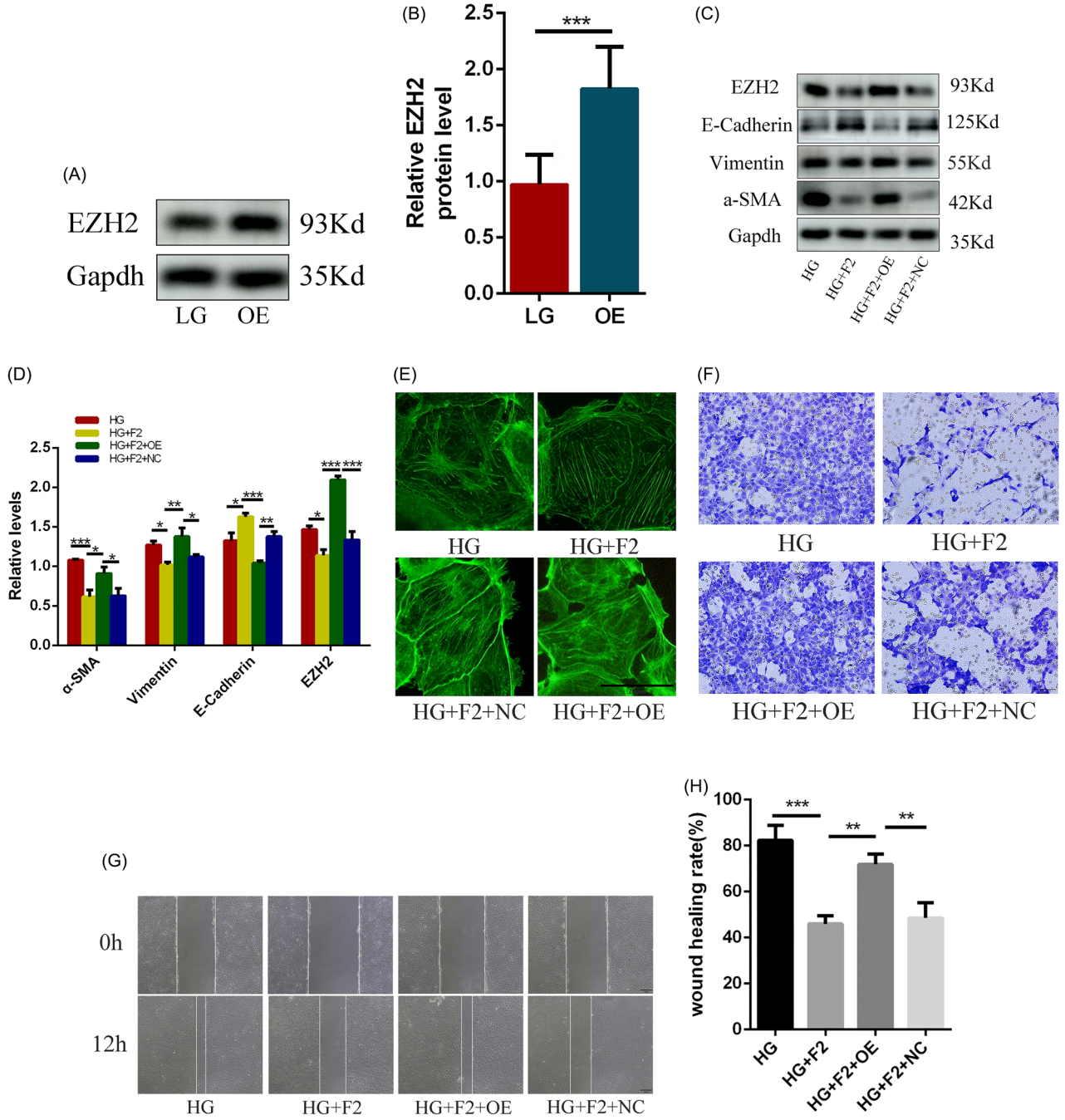


Figure 4. The effect of finerenone on inhibiting peritoneal fibrosis decreased after overexpression of EZH2.
Lentivirus was transfected into HPMCs to overexpress EZH2. (A) The overexpression efficiency of EZH2 by lentivirus was detected by Western blot. GAPDH served as a loading control. (B) The relative expression of EZH2. (Data are the mean ± SD; ***$P < 0.001$, n = 3). HPMCs were treated with high glucose (HG group) or high glucose plus finerenone of 2 μm (HG+F2 group). After transfection with lentivirus (HG+F2 + OE group) and blank vehicle (HG+F2 + NC group), the HPMCs were were treated with high glucose plus finerenone of 2 μm. (C) Representative immunoblots of α-SMA, vimentin, E-cadherin, and EZH2 in HPMCs under various treatments are shown. GAPDH served as a loading control. (D) The relative expression of α-SMA, vimentin, E-cadherin, and EZH2. (Data are the mean ± SD; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 3). (E) The effect of finerenone on HPMCs cell cytoskeleton was assessed using a phalloidin staining after overexpression of EZH2 (scale bar = 20 μm). (F) The effect of finerenone on the migration of HPMCs was assessed using a transwell assay after overexpression of EZH2, and the migrating cells were detected by crystal violet staining (scale bar = 100 μm). (G) The effect of finerenone on the migration of HPMCs was assessed using a wound-healing assay after overexpression of EZH2 (scale bar = 100 μm). (H) Quantitative analysis of the wound-healing assay. (Data are the mean ± SD; *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, n = 3). (I) Cellular immunofluorescence was used to assess the expression of α-SMA and vimentin in HPMCs. α-SMA was stained green, vimentin was stained red and the nuclei were stained blue with DAPI (scale = 50 μm).

dialysis fluid. Furthermore, the expression of EZH2 was also elevated. These changes were suppressed after the addition of finerenone (Figure 5A–E). HE and Masson staining showed thicker peritoneal tissue and higher collagen fiber content in peritoneal tissue from SD rats treated with long-term peritoneal dialysis fluids. After the addition of finerenone, the degree of peritoneal fibrosis is reduced (Figure 5F). Immunohistochemistry showed that peritoneal fibrosis was

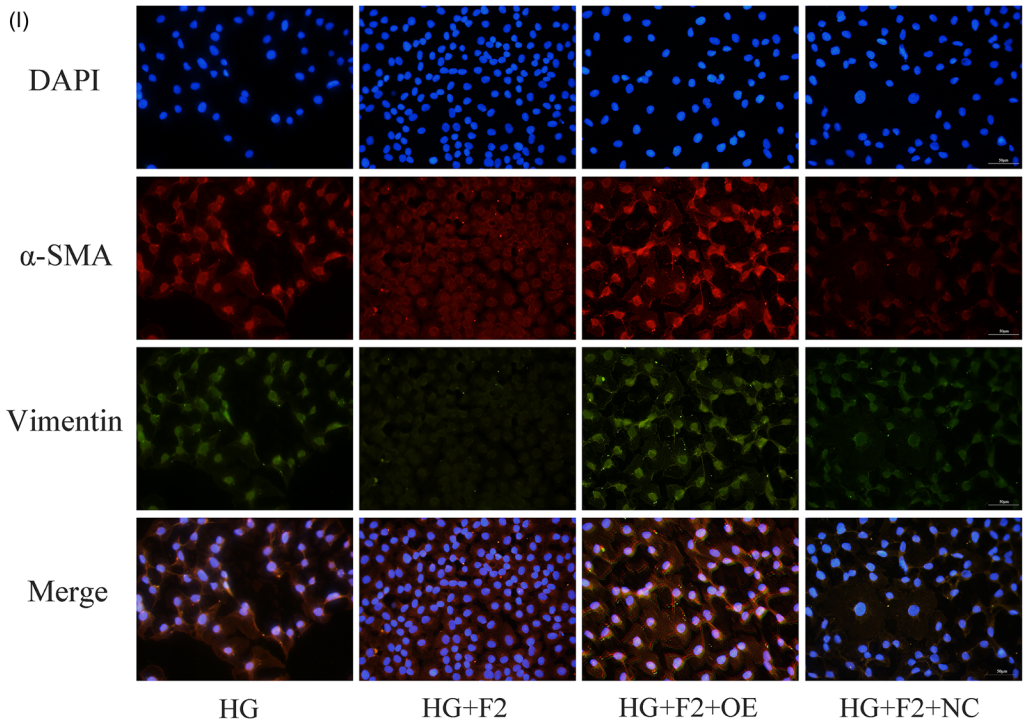


Figure 4. Continued.

significantly reduced, and the expression of α-SMA, vimentin and EZH2 was also significantly decreased after the addition of finerenone, compared with the PDF group (Figure 5G–J).

4. Discussion

Peritoneal dialysis (PD) is a form of renal replacement therapy. Prolonged use of PD can result in peritoneal fibrosis (PF), which ultimately leads to PD failure. Peritoneal dialysate is a kind of solution with low pH, high glucose concentration, and high osmolarity. Among these components, glucose is widely recognized as a key factor leading to structural and functional alterations in the peritoneum [31–34]. In this investigation, we discovered that finerenone can mitigate PF produced by long-term PD, suggesting that finerenone may be an effective treatment for PF. Furthermore, we showed that finerenone could relieve peritoneal fibrosis by downregulating EZH2 which means EZH2 is a vital molecule involved in PF.

In the process of PF, the epithelial mesenchymal transition (EMT) of HPMCs is considered as a critical step of PF [9]. EMT is characterized by the disruption of intercellular connections and the conversion of mesothelial cells to a mesenchymal phenotype. These altered mesothelial cells have the ability to secrete pro-fibrotic and angiogenic cytokines, such as TGF-β1, which stimulate excessive production of extracellular matrix (ECM) material, induce peritoneal angiogenesis, and trigger infiltration of mononuclear cells, ultimately leading to PF.

It is well known that the renin-angiotensin-aldosterone system (RAAS) plays an important role in regulating blood pressure and body fluid balance, and aldosterone is one of the bioactive products of the RAAS. Aldosterone is a mineralocorticoid whose biological effects are mostly mediated *via* mineralocorticoid receptors (MR). The binding of aldosterone to MR enhances the reabsorption of sodium ions by renal distal tubules and the excretion of potassium ions. It will also promote some pathophysiological processes such as inflammation, myocardial fibrosis and kidney fibrosis [35,36]. Therefore, MR overactivation can cause inflammation and fibrosis in the heart and the kidney both.

Several MR antagonists (MRA), including eplerenone [37], and spironolactone [38], have been shown to effectively inhibit TGF-β1-induced fibrosis. Furthermore, several studies have suggested that using MRA during PD may help reduce cardiovascular inflammation and fibrosis [39]. Finerenone [15], as a selective MRA, can block MR overactivation and inhibit the effects of aldosterone, which may reduce fibrosis in the heart and kidney. It was found that finerenone significantly reduces the probability of cardiovascular outcomes and renal failure in patients with chronic kidney disease or type II diabetes [40]. Furthermore, studies have shown that finerenone can reduce leukocyte recruitment and the inflammatory response after vascular damage, thereby preventing smooth muscle cell proliferation and neointima development in wounded arteries [41]. In this study, it was found that high glucose caused EMT in HPMCs. Additionally, EMT was observed in the peritoneal tissue of rats undergoing long-term peritoneal dialysis, as shown by increased expression of α-SMA and vimentin and decreased expression of E-Cadherin. However, finerenone maintained the expression of E-cadherin while suppressing the increased expression of α-SMA and vimentin. Furthermore, we found that the addition of finerenone reduced the enhanced cell migratory ability and rearranged the disordered cytoskeleton under high

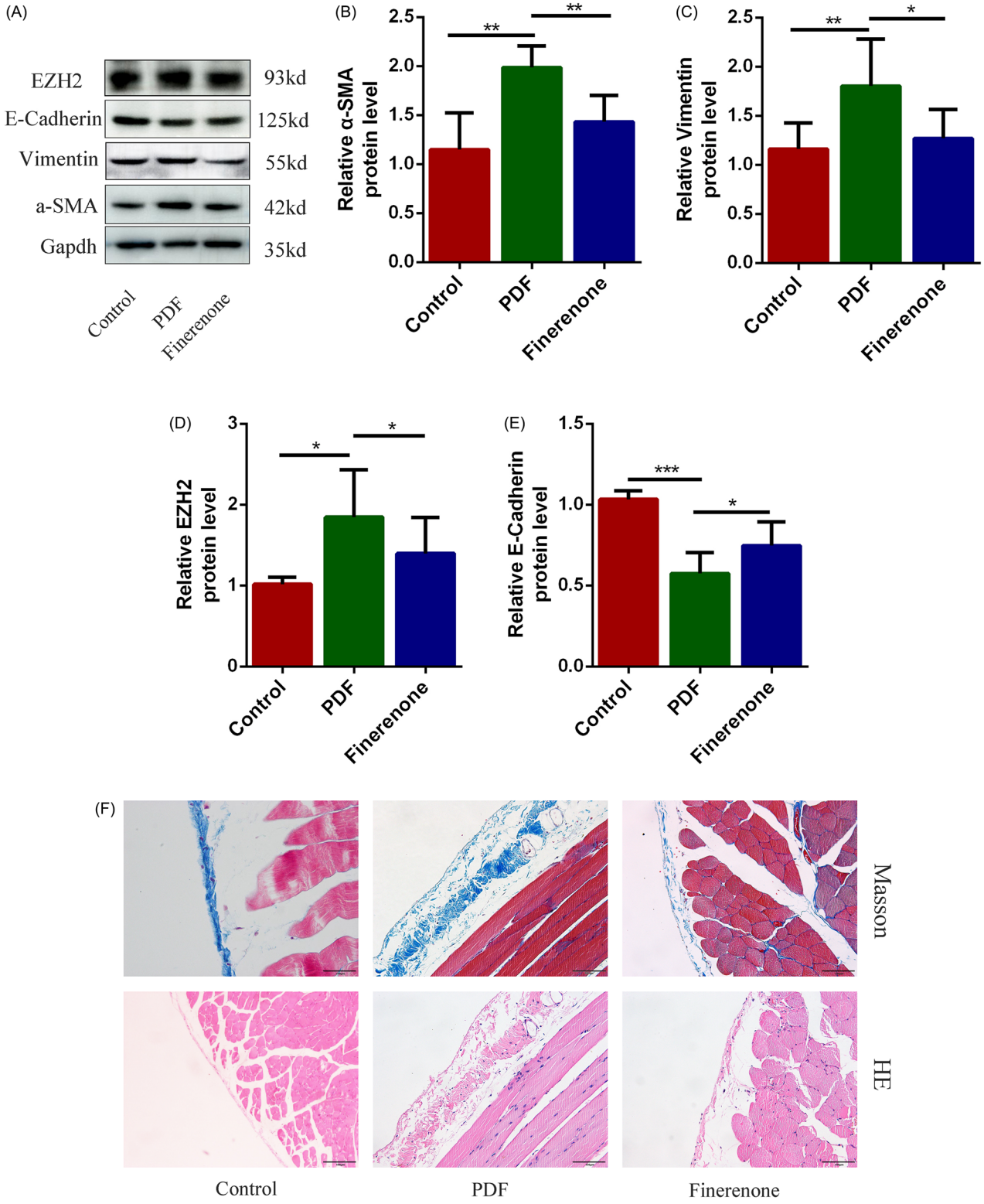
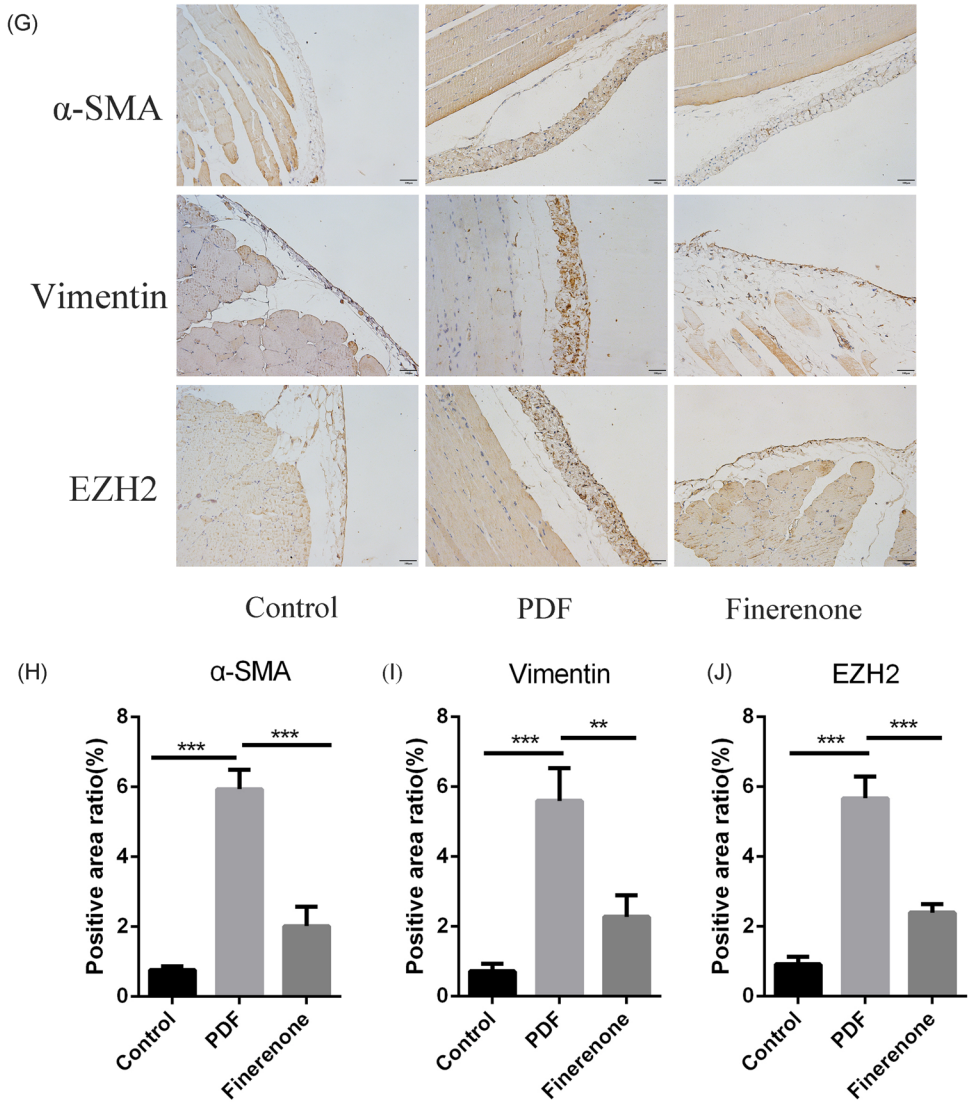


Figure 5. Finerenone alleviated PD-related peritoneal fibrosis *via* EZH2.
Rats were divided into 3 groups of 8 each, as detailed control group, PDF group, finerenone group. The PDF group was injected with 4.25% peritoneal dialysis fluid with 100 ml/kg daily for 28 days, and the control group also received an equal volume of 0.9% saline. (A) Representative immunoblots of α-SMA, vimentin, E-cadherin, and EZH2 in peritoneal tissue of rat under various treatments are shown. GAPDH served as a loading control. (B–E) The relative expression of α-SMA, vimentin, E-cadherin, and EZH2. (Data are the mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3). (F) HE staining and Masson staining in peritoneal tissue of rat (scale = 100 μm). (G) Immunohistochemical analysis of α -SMA, vimentin, and EZH2 in peritoneal tissue of rat (scale = 100 μm). (H–J) The relative expression of α-SMA, vimentin, and EZH2. (Data are the mean ± SD; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, n = 3).



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glucose. Therefore, finerenone inhibited PF in HPMCs induced by high glucose.

EZH2 is a transcriptional repressor, and numerous studies have demonstrated that EZH2 is crucial for carcinogenesis. For example, increased EZH2 expression is crucial in promoting the development of breast cancer [42]. Moreover, EZH2 can activate NF-κB signaling during the progression of breast cancer [43]. EZH2 promotes EMT by suppressing the expression of E-calbronectin *via* H3K27 trimethylation (H3K27me3) [13]. Furthermore, it has been discovered that PF can be avoided by pharmacologically inhibiting EZH2 or silencing it [20]. In this investigation, we found that the expression of EZH2 in HPMCs was increased by high glucose, but this effect was inhibited by addition of finerenone. Therefore, we hypothesized that finerenone would inhibit peritoneal fibrosis by suppressing EZH2 expression. In subsequent research, It was found that the effects of finerenone on inhibiting EMT, delaying cell migration and the protecting the cytoskeleton were attenuated after overexpression of EZH2 in HPMCs stimulated with high glucose. Therefore, we found that the ability of finerenone to inhibit PF was suppressed after overexpression of EZH2. Based on the aforementioned results, we speculated that finerenone exerts its effect in inhibiting PF by suppressing EZH2.

This work provides evidence for finerenone's anti-fibrotic activity in PF and identifies that the underlying mechanism of finerenone-induced peritoneal protection may be related to EZH2 inhibition. However, further analysis of EZH2 and its upstream pathways was not performed. This may be considered in future research. In conclusion, our results point to finerenone's potential therapeutic value in avoiding peritoneal fibrosis.

Authors' contributions

All authors contributed to the study conception and design. Material preparation were performed by Jingshu Sun, Xinyu Zhang and Simeng Wang. Data collection and analysis were performed by Dandan Chen, Jianqiang Shu, Zhikang Sun, Nannan Chong and Qinglian Wang. The first draft of the

manuscript was written by Dandan Chen and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Disclosure statement

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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