

Nestin⁺ Cells Isolated From The Peritoneum Attenuate Peritoneal Fibrosis Via Suppressing IL-33 /ST2 Signaling

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Research

Keywords: IL-33, Nestin, Peritoneal dialysis, Peritoneal fibrosis

Posted Date: August 13th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-46087/v1>

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Abstract

Background

The intermediate filament protein Nestin, a specific marker for multifunctional, multilineage progenitor cells, is regulated during the progression of reparative and reactive fibrosis in the liver, heart and kidney. However, whether Nestin regulates peritoneal fibrosis via suppressing IL-33/ST-2 signaling is unclear.

Methods

High-glucose peritoneal dialysis solution (PDS) or TGF- β 1 was administered to a mouse peritoneal dialysis (PD) model to induce peritoneal fibrosis (PF) in vivo and to human peritoneal mesothelial cells (HPMCs) in vitro to stimulate accumulation of extracellular matrix (ECM). Nestin⁺ cells isolated from the peritoneum of Nestin-green fluorescent protein (GFP) transgenic mice were transplanted in vivo, and adeno-associated virus (AAV)-RNAi was used to silence IL-33 in vitro.

Results

Notably, Nestin was found in both the mouse peritoneum and HPMCs derived from PD effluent (PDE) and HMrSV5. These Nestin⁺ PMCs showed extensive proliferation for more than 20 passages. The mice that received chronic PDS infusions showed typical features of PF, including substantially increased peritoneal thickness, excessive matrix deposition, increased peritoneal permeability, and increased expression levels of α -smooth muscle actin (α -SMA) and collagen I (Col I). Nestin⁺ cells isolated from the peritoneum could significantly ameliorate these pathological changes. A parallel decrease in interleukin-33 (IL-33) and ST-2 accumulation in the peritoneum of Nestin⁺ cell-transplanted mice was observed. In addition, downregulation of IL-33 expression increased TGF- β 1-induced ECM in the HPMCs.

Conclusion

Nestin⁺ cells isolated from the peritoneum had a clear protective effect on high-glucose PDS-induced PF by suppressing IL-33/ST-2 signaling.

Introduction

Peritoneal dialysis (PD) has been widely used as an effective treatment for end-stage renal disease (ESRD) in which the peritoneal mesothelium acts as a physical barrier with the capacity to trap bacteria and molecules [1, 2]. However, the prolonged exposure of peritoneal mesothelial cells (PMCs) in the peritoneal membrane to glucose-based PD solution (PDS) can cause PMC damage and loss, peritoneal fibrosis (PF), ultrafiltration failure and eventual discontinuation of PD [3–5]. Several therapeutic

strategies for the treatment of PF, including the use of pharmacological strategies and the transplantation of mesenchymal stem cells to inhibit the mesothelial-to-mesenchymal transition (EMT), have shown promise [6–8]. However, these therapies cannot prevent PMC loss, which plays a critical role in causing PF [9]. Previous studies have suggested that PMCs showed strong labeling with antibodies against Nestin, which is a specific marker for multifunctional, multilineage progenitor cells, indicating that these cells represent a “young”, not entirely differentiated cell population [10]. The intermediate filament protein Nestin is widely used as a marker of neural stem cells [11]. More importantly, Nestin is also expressed in some adult stem/progenitor cell populations, indicating that Nestin might be a common marker of multipotent stem cells [12]. Under specific damage conditions, upregulation of Nestin expression was found in tubular cells, podocytes, interstitial cells and fibrotic rat hearts [13–15]. More importantly, Nestin⁺ cells isolated from the kidney showed the characteristics of MSCs, which can repair acute kidney injury [16].

IL-33 is expressed in various tissues and cells in the human body, including endothelial cells, bronchial cells, and intestinal epithelial cells [17]. Recent reports have demonstrated that IL-33 promotes ST-2-dependent organ tissue fibrosis in several animal models, including lung [18], pancreas [19], and liver models [20].

However, the ability of Nestin⁺ cells isolated from the peritoneum to regulate PF has not been evaluated to our knowledge. In this study, cells isolated from the peritoneum of Nestin-GFP transgenic mice were transplanted into C57BL/6 wild-type mice, and the adeno-associated virus (AAV)-RNAi-IL-33 was used to infect to HPMCs. The effects and mechanism of Nestin⁺ cells were investigated in an established PF model.

Materials And Methods

The mice were purchased from Wenzhou Medical University Laboratory Animal Center (Wenzhou, China) and all of the animal work had taken place there. The mice had free access to a standard rodent diet and tap water and were anaesthetized with 40 mg/kg pentobarbital sodium through an intraperitoneal injection before sacrifice. This study has been carried out in accordance with the World Medical Association Declaration of Helsinki, and that all subjects provided written informed consent.

Animal studies

Homozygous transgenic mice that expressed enhanced GFP under the control of a Nestin promoter (Nestin-GFP, on the C57BL/6 genetic background) were provided by Cyagen Biosciences. We used male C57BL/6 mice at 8 weeks of age. C57BL/6 mice were divided into three groups: the control group, which received intraperitoneal administration of normal saline; the PD model mouse group without treatment, which received an intraperitoneal administration of PDS (4.25% Dianeal; Deerfield, IL, Baxter, USA) at 10 ml/100 g⁻¹·d⁻¹ for 4 weeks only; and the treatment group, PD model mice transplanted with Nestin⁺ cells.

A peritoneal equilibration test (PET) was applied to evaluate the peritoneal permeability function 28 days after modeling. The mice were instilled with PDS at 10 ml/100 g body weight before being killed. After 30 min, the peritoneal fluid was removed [8], and orbital sinus blood samples were collected. Peritoneal solute transport was calculated by D_{30}/D_0 and D/P_{urea} . D_{30} was defined as the glucose concentration in the dialysate sample at 30 min, and D_0 was defined as the initial dialysate glucose concentration. D indicates the dialysate urea concentration, and P_{urea} indicates the plasma urea concentration. Then, the parietal peritoneum, omentum, and diaphragm were carefully dissected for use in western blots and immunofluorescence staining.

Isolation and Nestin-GFP⁺ cell transplantation

The peritoneum was dissected from 2-week-old Nestin-GFP mice and cut into small pieces. The PMCs were then dissociated from the visceral peritoneum by digestion with 0.25% pancreatin at 37 °C for 15 min, and Eagle's medium (DMEM)/F12 (Invitrogen) containing 10% fetal bovine serum (FBS, Gibco) was added to stop the pancreatin activity. The samples were centrifuged at 1000 rpm for 5 min at 4 °C, and the supernatant was aspirated. The centrifugal sediment was resuspended and passed through a 70 μm strainer, which resulted in single cells. Flow cytometry was used to sort these PMCs expressing GFP from the suspension.

In the treatment group, after 1 week of intraperitoneal injection of PDS, Nestin-GFP⁺ PMCs (2×10^6 cells) were suspended in 2.5 ml of 4.25% PDS and injected intraperitoneally. At 28 days of injection, the mice were sacrificed, and the PDE, parietal peritoneum and omentum were collected.

Culture of PMCs from PDE and PMC lines

Human PMCs (HPMCs) harvested from PDE from patients with ESRD who underwent placement of PD catheters for less than 1 month were used for the culture. PDE was drained into a 50 ml centrifuge tube and centrifuged at 1000 rpm for 5 min. Cell pellets were suspended in Dulbecco's modified Eagle's medium/F12 (DMEM/F12, Gibco, NY, USA) with 10% fetal bovine serum (FBS, Gibco, NY, USA) and antibiotics (100 U/ml penicillin and 100 mg/ml streptomycin, Gibco, NY, USA). Then, suspended cells were seeded into a 25-cm² culture flask (Corning, Armonk, NY, USA).

Several antibodies were used to examine every batch of initially isolated mesothelial cells to ensure that they were positive for the mesothelial markers cytokeratin 18 (CK 18, Abcam, Cambridge, MA, USA) and vimentin (Abcam, Cambridge, MA, USA). HPMCs were used at passages 3–5. The HPMC line (HMrSV5) was cultured in the same medium mentioned above.

The HPMCs were divided into four groups: a normal control group, with a normal glucose concentration (17.5 mM); a group treated with transforming growth factor β1 (TGF-β1, MCE, NJ, UAS) for varying durations (6, 12, 24 and 48 h) to identify the optimal time, after which the most effective concentrations were determined (1, 5, and 10 nM); and a group with AAV-RNAi-Vector and AAV-RNAi-IL-33 infection. At

the end of the treatments, the cells were collected and subjected to western blot analyses and immunofluorescence staining.

Immunofluorescence

Histology and immunofluorescence staining of 4- μ m paraffin sections from the anterior or posterior peritoneal tissues were performed as described previously [21]. For identification of the HPMCs, the cells from the PDE were stained for CK 18 and vimentin. For confirmation of the expression of Nestin in the peritoneum, human peritoneum samples obtained from patients undergoing abdominal surgery were assessed by immunofluorescence staining. The following primary antibodies were used: rabbit monoclonal anti-vimentin antibody (Abcam, Cambridge, MA, USA), mouse monoclonal anti-CK 18 antibody (Abcam, Cambridge, MA, USA), rabbit polyclonal anti-collagen I (Col-I) antibody (Abcam, Cambridge, MA, USA), and anti- α -smooth muscle actin (α -SMA) antibody (Abcam, Cambridge, MA, USA).

Hematoxylin-eosin staining and Masson's staining.

The parietal peritoneum specimens from the 3 groups of mice were soaked in paraformaldehyde solution and then prepared for hematoxylin-eosin and Masson's staining as previously described [22].

Western blot analysis

Sample collection and immunoblotting were performed as previously described [21]. The primary antibodies used were rabbit polyclonal anti-Nestin antibody (Abcam, Cambridge, MA, USA), anti- α -SMA (Abcam, Cambridge, MA, USA), anti-Col I (ABclonal, Wuhan, China), anti-ST-2 (ABclonal, Wuhan, China), IL-33 (ABclonal, Wuhan, China) and mouse monoclonal anti- β -actin (Thermo Scientific, Waltham, USA). The secondary antibodies used in this study were horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin G (Cell Signaling Technology, Danvers, MA, USA) antibody and goat anti-mouse immunoglobulin G (Cell Signaling Technology, Danvers, MA, USA).

RNA extraction and quantitative real-time reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA from the peritoneum samples collected from different week-old Nestin-GFP mice was extracted using a Transcriptor First Strand cDNA Synthesis Kit (Roche, Basel, Switzerland) according to the manufacturer's instructions. Real-time PCR for Nestin and β -actin mRNA expression was performed using the FastStart Universal SYBR Green Master (ROX) kit (Roche, Basel, Switzerland) with an ABI Prism 7900HT Sequence Detection System (Life Technology, Carlsbad, CA, USA) as previously described [21].

The mRNA expression levels of Nestin were measured using β -actin as an internal reference gene. The primers included the following: mouse-*Nestin* forward 5'-TCG CCA GGG AGG AGG CCA TT-3' and reverse 5'-CTC CCC AGC CCT CCC CAG AC-3'; mouse- β -actin forward 5'-AGA GGG AAA TCG TGC GTG AC-3' and reverse 5'-CAA TAG TGA TGA CCT GGC CGT-3'. Relative expression was calculated using the $2^{-\Delta\Delta CT}$ method.

Cytokine assays

IL-33 concentrations were determined in cell culture supernatants and PDS by ELISAs following the manufacturer's instructions (Elabscience Biotechnology, Wuhan, China).

Statistical analysis

The results are presented as the mean \pm SD for continuous variables. Comparisons were made using analysis of variance followed by Tukey's *post hoc* test. SPSS 20.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the data. A *p*-value < 0.05 was considered statistically significant.

Results

Identification of cells isolated from PDE as HPMCs

PDE was derived from 4 uremic patients who underwent peritoneal dialysis therapy in one month. Among these 4 patients, 1 had gout, 1 had chronic glomerulonephritis, and 2 showed hypertensive nephropathy. In PDE-derived cultures, PMCs detached from the PDE generally grew well in DMEM/F12 medium containing 10% FBS. Suspended cells adhered to the dish within 1 day of seeding. Active proliferation occurred if the cell number was sufficient for the neighboring adherent cells to be in contact with each other. A confluent monolayer was reached at approximately 1 week, comprising passage 1. Cells isolated from the PDE presented a stellate or fusiform appearance with good transparency prior to confluency (Figure 1A) and developed a typical cobblestone appearance after 3 passages (Figure 1B). The phenotype of the PMCs was confirmed by immunofluorescence staining. The immunofluorescence study revealed that a proportion of the cells derived from the PDE showed positive staining in their cytoplasm with both the anti-vimentin and anti-CK 18 antibodies (Figure 1C). PDE-derived HPMCs could be passaged for more than 20 generations.

Nestin is expressed in the PMCs and the peritoneum

As a representative of Nestin expression, GFP fluorescence was observed in the peritoneum of Nestin-GFP transgenic mice via microscopic examination (Figure 1D). Nestin was expressed on the HPMCs derived from the PDE (Figure 1E) and HMrSV5, as shown by immunofluorescence (Figure 1F). The Nestin mRNA levels were also observed using RT-PCR analyses of the peritoneum of 1-, 2-, 4-, and 12-week-old mice (Figure 1G). Thus, Nestin is continuously present in neonates and adults in the peritoneum.

Treatment with Nestin⁺ cells isolated from the peritoneum attenuates peritoneal fibrosis in mice

Hematoxylin-eosin staining (Figure 2A) was used to evaluate changes in cell density, and Masson's staining (Figure 2B) was used to assess peritoneal thickening. We found that an intraperitoneal administration of 4.25% PDS at 10 ml/100 g⁻¹·d⁻¹ for 4 weeks induced an increase in the cellularity of the submesothelial compact zone (84.67 ± 6.51 vs. 23.33 ± 6.11 cells $\times 100/\text{mm}^2$, $P < 0.001$) and thickness of this zone until day 28 (127.07 ± 20.94 vs. 42.93 ± 9.19 μm , $P < 0.001$). Both the cell density of the

collagenous compact zone (52.00 ± 6.08 vs. 84.67 ± 6.51 cells $\times 100/\text{mm}^2$, $P < 0.001$) and the thickness of this zone (88.97 ± 7.52 vs. 127.07 ± 20.94 μm , $P = 0.015$) were significantly decreased in mice treated with Nestin⁺ cells (2×10^6 cells) injected intraperitoneally 1 week after 4.25% PDS stimulation compared with mice given the PDS only.

Nestin⁺ cells isolated from the peritoneum suppress the expression of Col I and α -SMA in mice

After intraperitoneal administration of 4.25% PDS, both α -SMA and Col-I were increased in the submesothelial compact zone on day 28 (Figure 3). However, treatment with Nestin⁺ cells isolated from the peritoneum significantly reduced the area in which α -SMA and Col-I accumulated on day 28 compared with treatment with PDS only, as shown by western blots.

Nestin⁺ cells isolated from the peritoneum reduce the functional impairments of the peritoneal membrane in mice with PF

A PET was performed on day 28 to assess the functional alteration of the peritoneal membrane. The absorption rate of glucose from the dialysate (0.20 ± 0.04 vs. 0.46 ± 0.05 , $P < 0.001$) (Figure 4A) and the transport rate of blood urea from the plasma (1.12 ± 0.15 vs. 0.62 ± 0.06 , $P = 0.001$) (Figure 4B) were significantly higher in mice given the PDS only than in control mice, whereas these parameters were significantly improved in PDS-injected mice treated with Nestin⁺ cells isolated from the peritoneum (Figure 4A, B) (0.42 ± 0.04 vs. 0.20 ± 0.04 , $P = 0.001$; 0.75 ± 0.07 vs. 1.12 ± 0.15 , $P = 0.004$).

Nestin⁺ cells isolated from the peritoneum suppress IL-33/ST-2 signaling in mice with PF

The fibrosis-related IL-33 was significantly increased in the PDE of mice with PDS-induced PF by day 28 compared with that of the control mice (116.50 ± 16.97 vs. 11.83 ± 3.25 pg/ml, $p < 0.001$) (Figure 4C). Meanwhile, the expression of ST-2 was increased obviously in the mice with PDS only (Figure 3). Treatment with Nestin⁺ cells isolated from the peritoneum significantly suppressed the secretion of IL-33 (49.33 ± 9.98 vs. 116.50 ± 16.97 pg/ml, $p = 0.001$) and expression of ST-2 compared with treatment with PDS only.

Effects of different TGF- β 1 concentrations and durations on IL-33 secretion in HPMCs

HPMCs were stimulated by different concentrations of TGF- β 1 (0, 1, 5, and 10 nM) for 24 h. The levels of IL-33 increased significantly when HPMCs (both from PDE and HMrSV5) were stimulated with 5 nM and 10 nM (Figure 5A, B) compared with those of the control group. Then, HPMCs were stimulated with TGF- β 1 for different durations (6, 12, 24 and 48 h) at a concentration of 5 nM. The levels of IL-33 showed the strongest changes in HPMCs stimulated with TGF- β 1 at 5 nM for 48 h compared with those of the control group (Figure 5C, D). Thus, a concentration of 5 nM and a duration of 48 h were selected for further use.

Downregulation of IL-33 expression increases TGF- β 1-induced EMT of HPMCs

After HPMCs were stimulated with 5 ng/ml TGF- β 1 for 48 h, western blot (Figure 6) and immunofluorescence analyses (Figure 7A, B and C) showed that the levels of α -SMA and Col-I were significantly increased compared with those of the control group. Furthermore, in the AAV-RNAi-IL-33 group, α -SMA and Col-I were decreased significantly compared with those of the AAV-RNAi-vector group. There were no significant changes in the levels of α -SMA and Col-I between the HPMCs incubated with TGF- β 1 only and cells incubated with the AAV-RNAi-vector.

Downregulation of IL-33 expression upregulates TGF- β 1 induced IL-33/ST2 signaling in HPMCs

HPMCs from PDE were infected with AAV-RNAi-vector and AAV-RNAi-IL-33 and then stimulated with TGF- β 1 at 5 ng/ml for 48 h. Compared to the control group, the levels of IL-33 (Figure 6, 7B) and ST-2 (Figure 6) were significantly increased following TGF- β 1 stimulation. There were no significant changes in the levels of IL-33 and ST-2 between the HPMCs incubated with TGF- β 1 only and the cells incubated with the AAV-RNAi-vector. AAV-RNAi-IL-33 resulted in a stronger decrease in these levels than AAV-RNAi-vector (Figure 6).

Discussion

This study provided the first evidence that Nestin⁺ cells isolated from the peritoneum have a clear protective effect on high-glucose PDS-induced PF through suppression of IL-33 secretion. Intraperitoneal administration of Nestin⁺ cells isolated from the peritoneum of Nestin-GFP mice through flow cytometric sorting ameliorated PDS-induced PF in mice, as evidenced by a significant reduction in peritoneal thickening and collagen deposition. In addition, Nestin⁺ cells significantly decreased IL-33 secretion in mice with PF, indicating that PF was reduced through suppression of IL-33. Moreover, transplanted Nestin⁺ cells obviously reduced the functional impairments of the peritoneal membrane in mice with PF. Furthermore, downregulation of IL-33 expression could decrease the expression of ST-2, α -SMA and Col-I in HPMCs, indicating that Nestin delays the course of EMT in HPMCs induced by TGF- β 1. These results indicate that the delivered Nestin⁺ cells isolated from the peritoneum could protect mice from PF impairment and against peritoneal function damage after PDS injury by suppressing IL-33/ST-2 signaling.

Nestin is widely known as a marker of neural stem cells [12]. More importantly, this protein labels adult proliferating and stem/progenitor cell populations, indicating that Nestin is a specific marker of multifunctional progenitor cells [12]. Rats PMCs showed strong labeling with this antibody [10]. Similarly, in the present study, both HPMCs and mouse peritoneum strongly expressed Nestin, indicating that these cells represent a 'young', not entirely differentiated cell population. We also confirmed that the cultured PMCs from the PDE grew well even after 20 passages, which implied that these cells possessed a strong proliferative ability. Nestin expression was upregulated in cardiac, [15] renal [23] and lung ([24]) fibrosis. However, few studies have investigated the effects of Nestin⁺ PMCs on PF. Furthermore, we prospectively isolated Nestin⁺ cells from the peritoneum based on the GFP fluorescence intensity of Nestin-GFP mice. Excitingly, Nestin⁺ cells could suppress the expression of Col I and α -SMA and reduce the functional

impairments of the peritoneal membrane in mice with PF, eventually leading to delayed PF progression. Therefore, Nestin⁺ cells isolated from the peritoneum can exert a protective effect on high-glucose PDS-induced PF.

IL-33 is a major cytokine in the modulation of fibrosis [25]. Furthermore, recent reports have confirmed that IL-33 promotes ST-2-dependent organ tissue fibrosis, such as lung [26], liver [27], and kidney fibrosis [28]. Conversely, IL-33 prevented global myocardial periarteriolar fibrosis in pressure-overloaded left ventricles [29]. However, whether and how this cytokine can modulate PF remains unclear. To address the possible mechanism of PF induced by PDS, we detected the levels of IL-33 in different groups and found that secretion of IL-33 was obviously increased in mice with PF, indicating that IL-33 positively correlates with the extent of PF. Furthermore, Nestin⁺ cells could reduce the secretion of IL-33, eventually leading to the delayed progression of PF. In addition, downregulation of Nestin expression decreased TGF- β 1-induced EMT in HPMCs. Therefore, Nestin⁺ PMCs could protect against high-glucose PDS-induced PF by depressing IL-33 secretion.

Conclusion

Nestin expression and IL-33 secretion are increased in mice with PF, and Nestin⁺ cells isolated from the peritoneum induced Col I and α -SMA expression and IL-33 secretion in mice with PF, eventually leading to delayed PF progression. In addition, downregulation of IL-33 decreased the expression of Col I and α -SMA and ST-2 in HPMCs. These findings suggest that Nestin⁺ cells isolated from the peritoneum can exert a protective effect on high-glucose PDS-induced PF by suppressing IL-33/ST-2 signaling.

Abbreviations

PDS: peritoneal dialysis solution; PD: peritoneal dialysis; PF: peritoneal fibrosis; HPMCs: human peritoneal mesothelial cells; ECM: extracellular matrix; PDE: peritoneal dialysis effluent; α -SMA: α -smooth muscle actin; Col I: collagen I; IL-33:interleukin-33; ESRD: end-stage renal disease; PMCs: peritoneal mesothelial cells; PDS: peritoneal dialysis solution; EMT: mesothelial-to-mesenchymal transition; MSCs: multipotent stem cells; PET: peritoneal equilibration test; DMEM: Dulbecco's modified Eagle medium; FBS: fetal bovine serum; CK 18:cytokeratin 18; PCR: polymerase chain reaction; FBS: fetal bovine serum; TGF- β :transforming growth factor- β .

Declarations

Acknowledgements

We thank the First Affiliated Hospital of Wenzhou Medical University for providing the PDE. The authors are also grateful to this article's reviewers and editor.

Author's contributions

Performed the experiments and analyzed the data: YZ, YPST and SQM. Participated in manuscript preparation: YZ, YPST and SMY. Detection of immunofluorescence, western blot and real-time PCR: YZ, YPST, NJ and ZYZ. Detection of HE staining and Masson's staining: XYL, HC, DCY and HCJ. Analyzed and interpreted the data: CSC, CFZ and YHB. Participated in the experimental design and coordination: CSC and CFZ. Conceived the project, supervised the experiments, and analyzed data: CSC and CFZ. Wrote the manuscript: YZ, YPST and SQM.

Funding

This work was supported by grants from the Wenzhou Municipal Science and Technology Bureau (ZS2017008 and Y20180159), the Natural Science Foundation of Zhejiang Province (LQ19H050002), and the National Natural Science Foundation of China (31900685 and 81772264).

Conflict of interests

None.

Ethics approval and consent to participate

The use of laboratory animals and human PD effluent (PDE) in this study was approved by the Ethical Committee of Wenzhou Medical University and Laboratory Animal Center, Wenzhou Medicine University, according to the licenses for use of experimental animals issued by the Zhejiang Ministry of Justice.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated for this study are available on request from the corresponding author.

Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Mehrotra R, Devuyst O, Davies SJ and Johnson DW. The Current State of Peritoneal Dialysis. *J Am Soc Nephrol.* 2016; 27:3238-3252.
2. Isaza-Restrepo A, Martin-Saavedra JS, Velez-Leal JL, Vargas-Barato F and Riveros-Duenas R. The Peritoneum: Beyond the Tissue - A Review. *Front Physiol.* 2018; 9
3. Wu J, Xing C, Zhang L, Mao H, Chen X, Liang M, Wang F, Ren H, Cui H, Jiang A, Wang Z, Zou M and Ji Y. Autophagy promotes fibrosis and apoptosis in the peritoneum during long-term peritoneal dialysis.

- J Cell Mol Med. 2018; 22:1190-1201.
4. Wang L, Balzer MS, Rong S, Menne J, von Vietinghoff S, Dong L, Gueler F, Jang MS, Xu G, Timrott K, Tkachuk S, Hiss M, Haller H and Shushakova N. Protein kinase C alpha inhibition prevents peritoneal damage in a mouse model of chronic peritoneal exposure to high-glucose dialysate. *Kidney Int.* 2016; 89:1253-1267.
 5. Zhou Y, Fan J, Zheng C, Yin P, Wu H, Li X, Luo N, Yu X and Chen C. SGLT-2 inhibitors reduce glucose absorption from peritoneal dialysis solution by suppressing the activity of SGLT-2. *Biomed Pharmacother.* 2019; 109:1327-1338.
 6. Loureiro J, Aguilera A, Selgas R, Sandoval P, Albar-Vizcaino P, Perez-Lozano ML, Ruiz-Carpio V, Majano PL, Lamas S, Rodriguez-Pascual F, Borrás-Cuesta F, Dotor J and Lopez-Cabrera M. Blocking TGF-beta1 protects the peritoneal membrane from dialysate-induced damage. *J Am Soc Nephrol.* 2011; 22:1682-1695.
 7. Fan, Y.P., Hsia, et al. The Therapeutic Potential of Human Umbilical Mesenchymal Stem Cells From Wharton's Jelly in the Treatment of Rat Peritoneal Dialysis-Induced Fibrosis. *Stem Cells Transl Med.* 2016;5:235-247.
 8. Ueno T, Nakashima A, Doi S, Kawamoto T, Honda K, Yokoyama Y, Doi T, Higashi Y, Yorioka N, Kato Y, Kohno N and Masaki T. Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-beta1 signaling. *Kidney Int.* 2013; 84:297-307.
 9. Liu Y, Dong Z, Liu H, Zhu J, Liu F and Chen G. Transition of mesothelial cell to fibroblast in peritoneal dialysis: EMT, stem cell or bystander? *Perit Dial Int.* 2015; 35:14-25.
 10. Katz S, Balogh P and Kiss AL. Mesothelial cells can detach from the mesentery and differentiate into macrophage-like cells. 2011; 119:782-793.
 11. Calderone A. The Biological Role of Nestin(+) Cells in Physiological and Pathological Cardiovascular Remodeling. *Front Cell Dev Biol.* 2018; 6
 12. Wiese C, Rolletschek A, Kania G, Blyszczuk P, Tarasov KV, Tarasova Y, Wersto RP, Boheler KR and Wobus AM. Nestin expression—a property of multi-lineage progenitor cells? *Cell Mol Life Sci.* 2004; 61:2510-2522.
 13. Patschan D, Michurina T, Shi HK, Dolff S, Brodsky SV, Vasilieva T, Cohen-Gould L, Winaver J, Chander PN, Enikolopov G and Goligorsky MS. Normal distribution and medullary-to-cortical shift of Nestin-expressing cells in acute renal ischemia. *Kidney Int.* 2007; 71:744-754.
 14. Wen D, Ni L, You L, Zhang L, Gu Y, Hao CM and Chen J. Upregulation of nestin in proximal tubules may participate in cell migration during renal repair. *Am J Physiol Renal Physiol.* 2012; 303:F1534-F1544.
 15. Hertig V, Tardif K, Meus MA, Duquette N, Villeneuve L, Toussaint F, Ledoux J and Calderone A. Nestin expression is upregulated in the fibrotic rat heart and is localized in collagen-expressing mesenchymal cells and interstitial CD31(+) cells. *Plos One.* 2017; 12
 16. Jiang MH, Li G, Liu J, Liu L, Wu B, Huang W, He W, Deng C, Wang D, Li C, Lahn BT, Shi C and Xiang AP. Nestin(+) kidney resident mesenchymal stem cells for the treatment of acute kidney ischemia injury.

- 2015; 50:56-66.
17. Moussion C, Ortega N and Girard JP. The IL-1-like cytokine IL-33 is constitutively expressed in the nucleus of endothelial cells and epithelial cells in vivo: a novel 'alarmin'? *Plos One*. 2008; 3:
 18. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, Graham GJ, Kurowska-Stolarska M, Liew FY, McSharry C and Xu D. IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J Allergy Clin Immunol*. 2014; 134:1422-1432.
 19. Nishida A, Andoh A, Imaeda H, Inatomi O, Shiomi H and Fujiyama Y. Expression of interleukin 1-like cytokine interleukin 33 and its receptor complex (ST2L and IL1RAcP) in human pancreatic myofibroblasts. 2010; 59:531-541.
 20. Marvie P, Lisbonne M, L'Helgoualc'h A, Rauch M, Turlin B, Preisser L, Bourd-Boittin K, Theret N, Gascan H, Piquet-Pellorce C and Samson M. Interleukin-33 overexpression is associated with liver fibrosis in mice and humans. *J Cell Mol Med*. 2010; 14:1726-1739.
 21. Kanda R, Hamada C, Kaneko K, Nakano T, Wakabayashi K, Hara K, Ito H, Horikoshi S and Tomino Y. Paracrine effects of transplanted mesothelial cells isolated from temperature-sensitive SV40 large T-antigen gene transgenic rats during peritoneal repair. *Nephrol Dial Transplant*. 2014; 29:289-300.
 22. Ma YL, Chen F, Yang SX, Chen BP and Shi J. MicroRNA-21 promotes the progression of peritoneal fibrosis through the activation of the TGF-beta/Smad signaling pathway: An in vitro and in vivo study. *Int J Mol Med*. 2018; 41:1030-1038.
 23. Sakairi T, Hiromura K, Yamashita S, Takeuchi S, Tomioka M, Ideura H, Maeshima A, Kaneko Y, Kuroiwa T, Nangaku M, Takeuchi T and Nojima Y. Nestin expression in the kidney with an obstructed ureter. *Kidney Int*. 2007; 72:307-318.
 24. Zhang H, Xiao B, Jiang L, Yao W, Shen H and Xiang X. Inhibition of mesenchymal stromal cells' chemotactic effect to ameliorate paraquat-induced pulmonary fibrosis. *Toxicol Lett*. 2019; 307:1-10.
 25. Gonzalez-Polo V, Pucci-Molineris M, Cervera V, Gambaro S, Yantorno SE, Descalzi V, Tiribelli C, Gondolesi GE and Meier D. Group 2 innate lymphoid cells exhibit progressively higher levels of activation during worsening of liver fibrosis. *Ann Hepatol*. 2019; 18:366-372.
 26. Yanaba K, Yoshizaki A, Asano Y, Kadono T and Sato S. Serum IL-33 levels are raised in patients with systemic sclerosis: association with extent of skin sclerosis and severity of pulmonary fibrosis. *Clin Rheumatol*. 2011; 30:825-830.
 27. McHedlidze T, Waldner M, Zopf S, Walker J, Rankin AL, Schuchmann M, Voehringer D, McKenzie AN, Neurath MF, Pflanz S and Wirtz S. Interleukin-33-dependent innate lymphoid cells mediate hepatic fibrosis. 2013; 39:357-371.
 28. Liang H, Xu F, Wen XJ, Liu HZ, Wang HB, Zhong JY, Yang CX and Zhang B. Interleukin-33 signaling contributes to renal fibrosis following ischemia reperfusion. *Eur J Pharmacol*. 2017; 812:18-27.
 29. Garbern JC, Williams J, Kristl AC, Malick A, Rachmin I, Gaeta B, Ahmed N, Vujic A, Libby P and Lee RT. Dysregulation of IL-33/ST2 signaling and myocardial periarteriolar fibrosis. *J Mol Cell Cardiol*. 2019; 128:179-186.

Figures

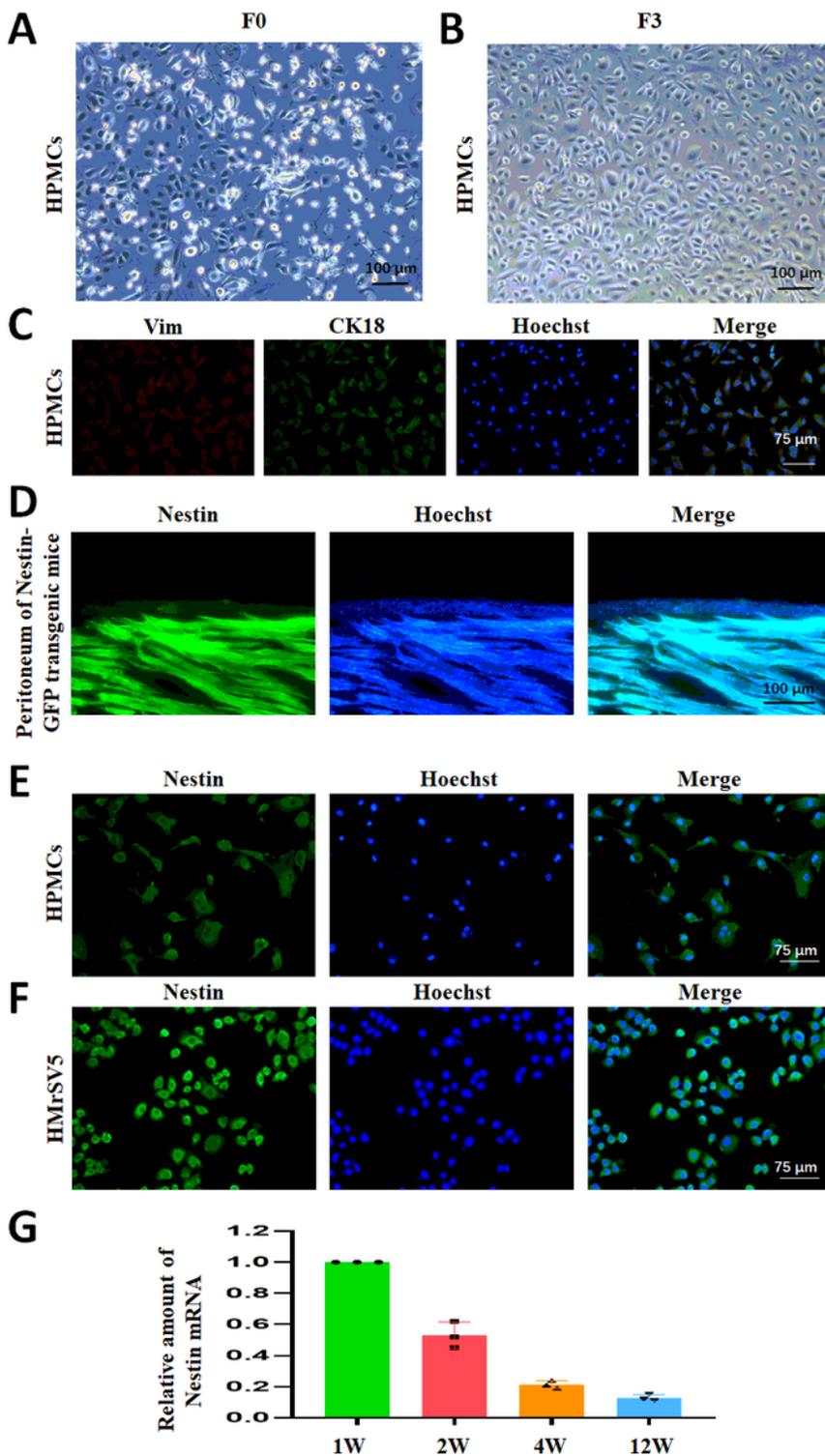


Figure 1

1A. Representative light microscopic features of cells isolated from PDE. Cells presented a stellate or fusiform appearance with good transparency at passage 0. Figure 1B. Cells isolated from PDE showed a typical cobblestone appearance at passage 3. Figure 1C. In PDE-derived culture at passage 3, the cells

were positively stained with anti-vimentin and anti-CK 18 antibodies. (1) Red indicates positive staining with the anti-vimentin antibody. (2) Green indicates positive staining with the CK 18 antibody. (3) Blue indicates positive Hoechst staining. (4) More than 90% of the cells were positively stained with the anti-vimentin and anti-CK 18 antibodies. Figure 1D. Nestin was expressed in the mouse peritoneum. (1) Green indicates positive expression of GFP (Nestin). (2) Blue indicates positive Hoechst staining. Figure 1E. Nestin was expressed in HPMCs derived from PDE. (1) Green indicates positive staining with the Nestin antibody. (2) Blue indicates positive Hoechst staining. Figure 1F. Nestin was expressed in HMrSV5. (1) Green indicates positive staining with the Nestin antibody. (2) Blue indicates positive Hoechst staining. Figure 1G. Nestin was expressed continuously in the neonatal and adult peritoneum of the mice. The mRNA levels of Nestin were observed in the peritoneum of the mice at 1, 2, 4, and 12 weeks, as shown by RT-PCR. Abbreviations: HPMCs human peritoneal mesothelial cells; PDE peritoneal dialysis effluent; CK 18 cytokeratin 18.

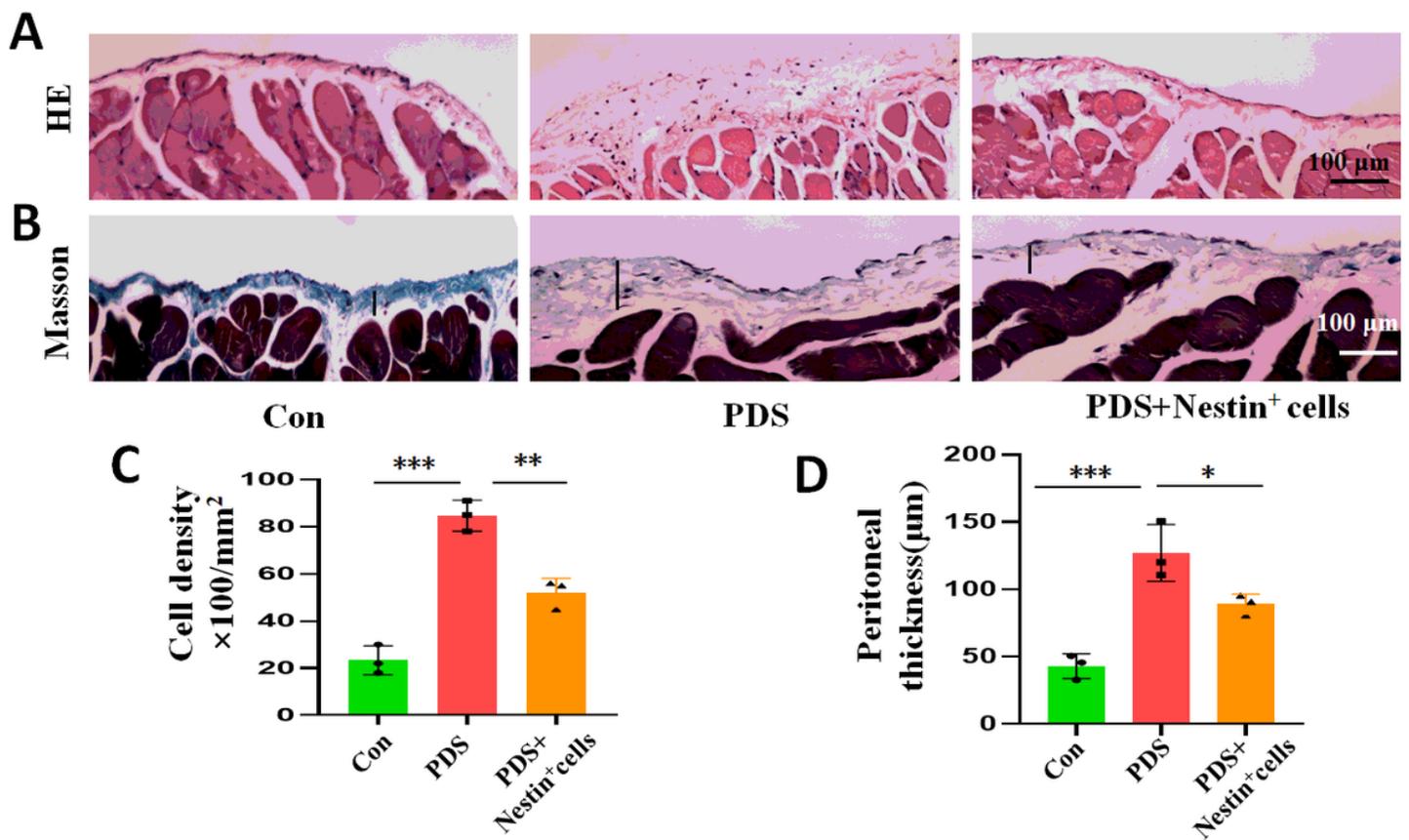


Figure 2

Nestin⁺ cells isolated from the peritoneum attenuate peritoneal cell density and thickening in PDS-injected mice. Figure 2A, B. Representative light microscopic features of peritoneal tissues on day 28 (A: hematoxylin-eosin staining; B: Masson's staining) in control mice, PF mice with an intraperitoneal administration of 4.25% PDS only and PDS-injected mice treated with Nestin⁺ cells. Figure 2A, B. The thickness of the submesothelial compact zone increased along with its cellularity until day 28 in PF mice injected with PDS. Cell density and thickening of the zone were suppressed in mice treated with Nestin⁺

cells. *P < 0.05, **P < 0.01, ***P < 0.001 versus PF mice injected with PDS. Abbreviations: HE hematoxylin-eosin staining; Con control; PDS peritoneal dialysis solution; CK 18 cytokeratin 18; PF peritoneal fibrosis; PMC peritoneal mesothelial cells.

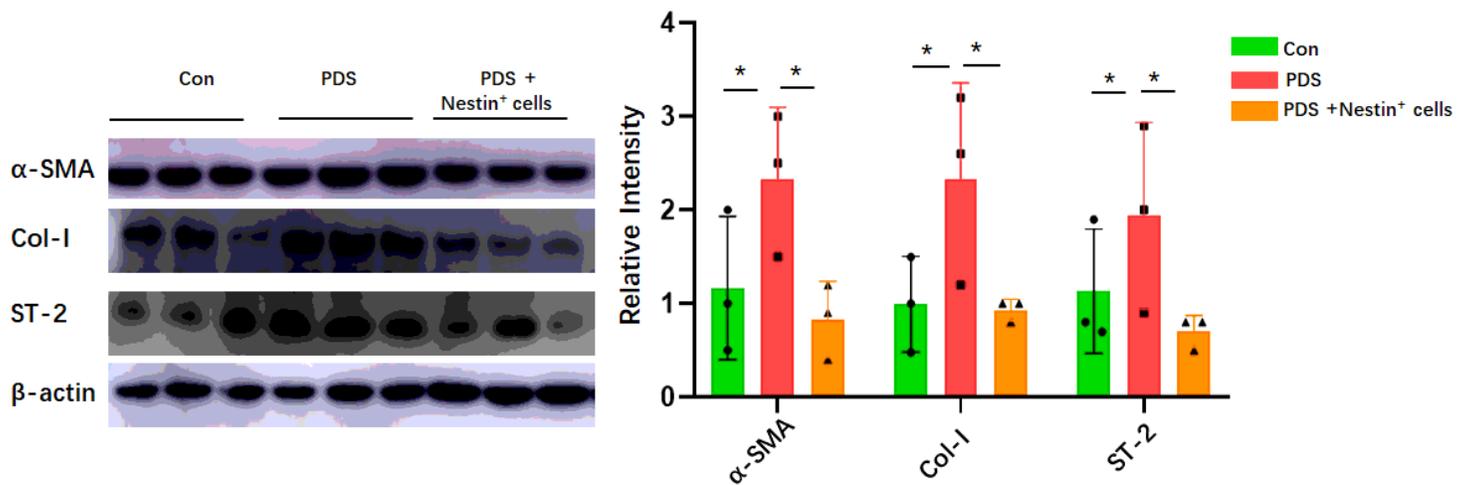


Figure 3

Nestin⁺ cells isolated from the peritoneum suppressed the expression of Col I, α -SMA and ST-2 in mice with PF. Compared to those of the control group, the levels of Col I, α -SMA and ST-2 increased significantly in PF mice with PDS injection, as shown by western blots. However, Nestin⁺ cells isolated from the peritoneum reduced these expression levels. *P < 0.05 versus PF mice injected with PDS. Abbreviations: Col I collagen I, α -SMA, α -smooth muscle actin, Con control; PDS peritoneal dialysis solution; PF peritoneal fibrosis

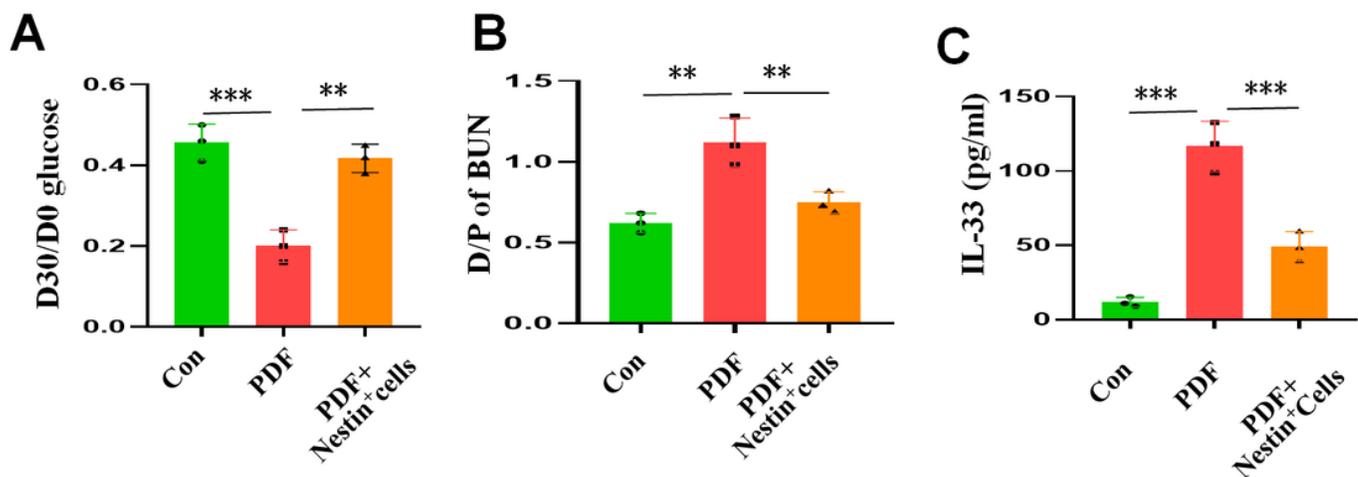


Figure 4

Nestin⁺ cells reduced the functional impairments of the peritoneal membrane and IL-33 secretion in mice with PF. The peritoneal absorption of glucose from the dialysate (D30/D0) (A) and the dialysate-to-plasma (D/P) ratio of urea (B) were assessed in control mice, PF mice with an intraperitoneal administration of 4.25% PDS only and PDS-injected mice treated with Nestin⁺ cells. The absorption rate

of glucose from the dialysate and the transport rate of blood urea from the plasma were significantly higher in mice given the PDS only than in control mice, whereas these parameters were significantly improved in PDS-injected mice treated with Nestin+ cells. The cytokine IL-33 was significantly increased in the PDE of mice with PDS-induced PF (C). Treatment with Nestin+ cells significantly suppressed the secretion of IL-33 compared with treatment with PDS only. **P < 0.01, ***P < 0.001 versus PF mice injected with PDS. Abbreviations: PMC peritoneal mesothelial cells; Con control; PDS peritoneal dialysis solution; PF peritoneal fibrosis.

A

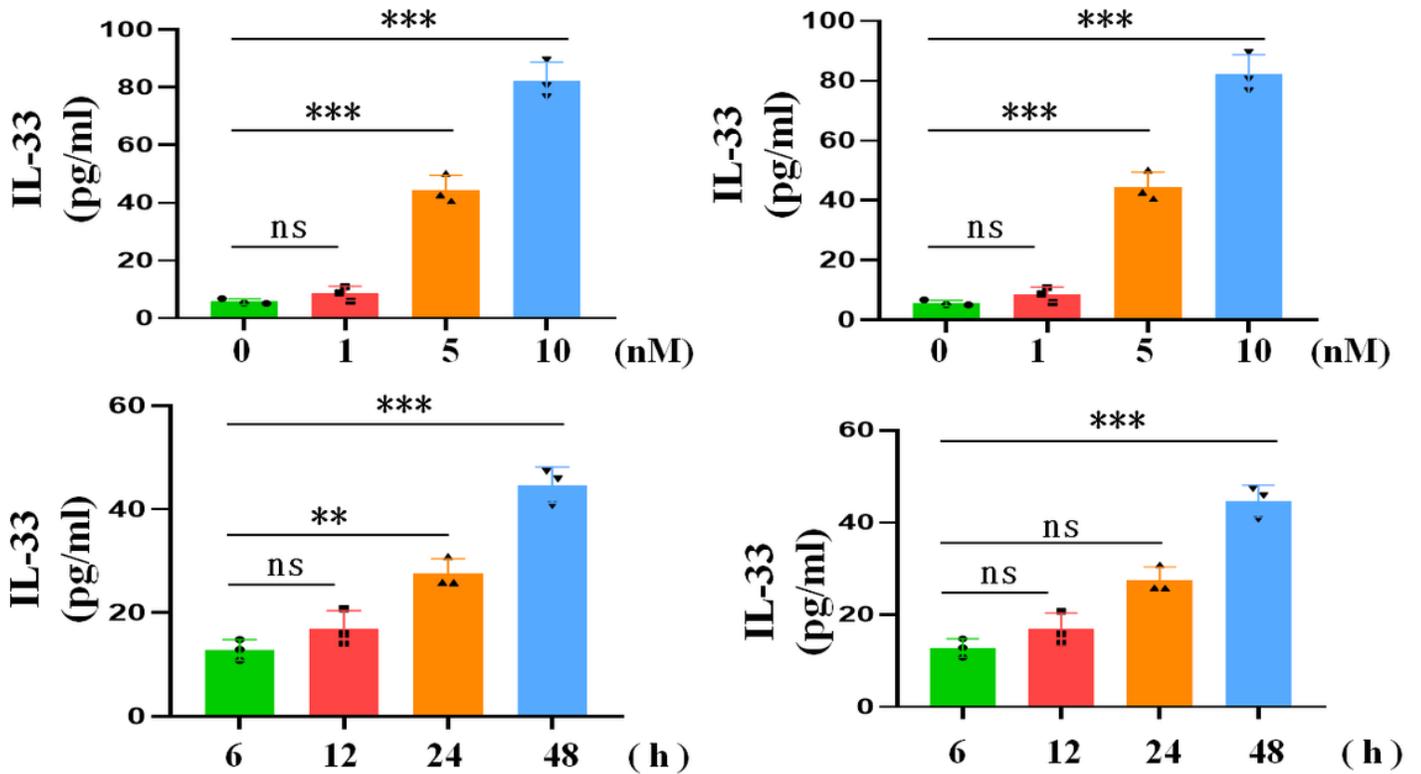


Figure 5

Effects of different TGF-β1 concentrations and durations on IL-33 secretion in HPMCs HPMCs were stimulated with different concentrations of TGF-β1 (0, 1, 5, and 10 nM) for 24 h (Figure 6A, B). Then, HPMCs were stimulated with TGF-β1 at a concentration of 5 nM for different durations (6, 12, 24 and 48 h) (Figure 6C, D), and the levels of IL-33 were detected by ELISAs. **P < 0.01, ***P < 0.001 versus the control group. Abbreviations: IL-33, interleukin-33; HPMCs human peritoneal mesothelial cells; TGF-β1 transforming growth factor β1.

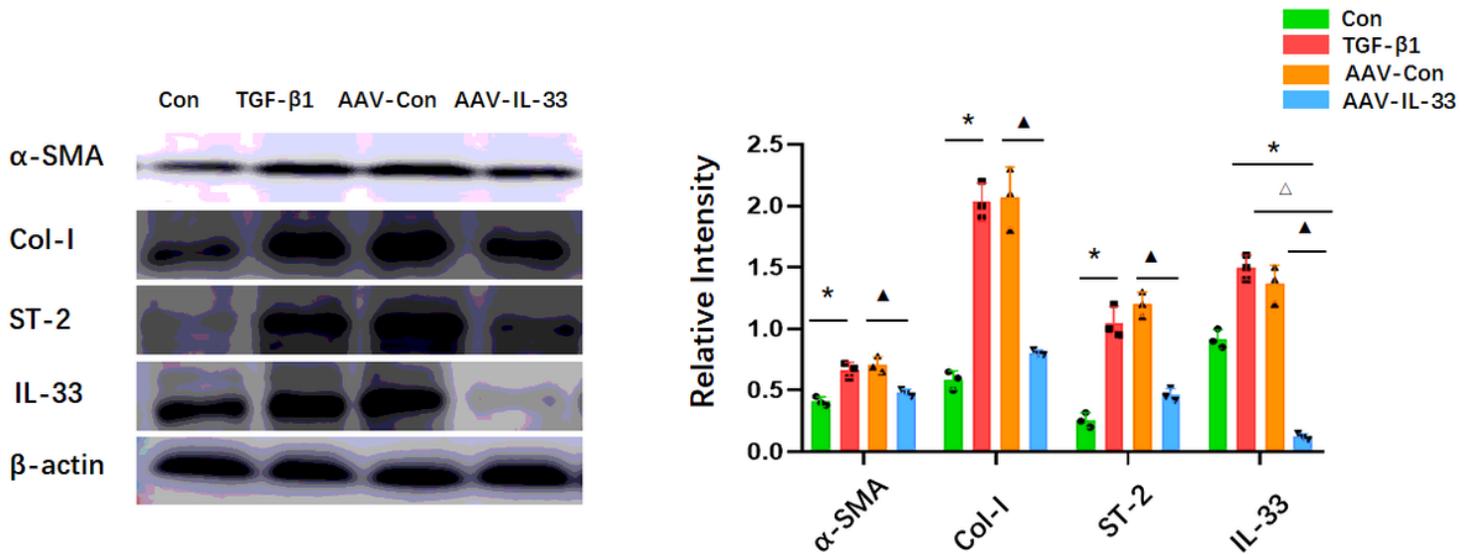


Figure 6

Downregulation of IL-33 expression decreased the TGF- β 1-induced EMT of HPMCs, as shown by western blots. The levels of IL-33 decreased significantly after AAV-RNAi-IL-33 interference, as shown by western blots. The levels of α -SMA, Col-I and ST-2 were significantly increased following TGF- β 1 stimulation compared with those of the control group. Furthermore, in the AAV-RNAi-IL-33 group, these proteins were decreased significantly compared with those of the AAV-RNAi-vector group. *P < 0.05 versus the control group, \blacktriangle P < 0.05 versus the AAV-RNAi-control group, \triangle P < 0.05 versus the TGF- β 1 group. Abbreviations: HPMCs human peritoneal mesothelial cells; TGF- β 1, transforming growth factor β 1; Col I, collagen I; α -SMA, α -smooth muscle actin; Con, control; AAV, adeno-associated virus.

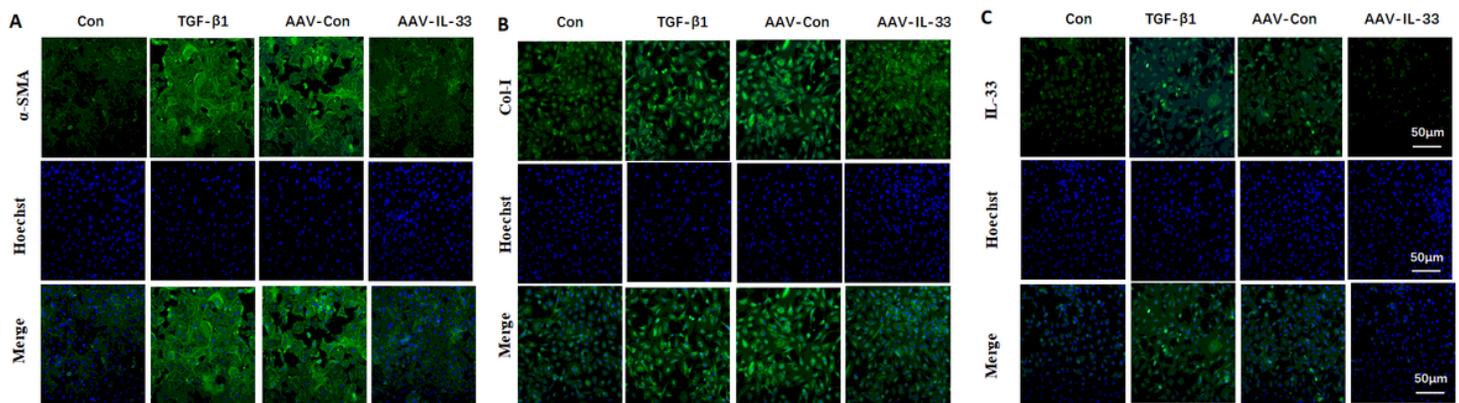


Figure 7

Downregulation of IL-33 expression increased the levels of Col I and α -SMA and IL-33 secretion in HPMCs. The levels of α -SMA and Col-I were significantly increased following TGF- β 1 stimulation compared with those of the control group. Furthermore, in the AAV-RNAi-IL-33 group, these proteins were decreased significantly compared with those of the AAV-RNAi-vector group. Abbreviations: TGF- β 1,

transforming growth factor β 1; Col I, collagen I; α -SMA, α -smooth muscle actin; Con, control; AAV, adeno-associated virus; HPMCs human peritoneal mesothelial cells.