J Nephrol (2014) 27:507–514
DOI 10.1007/s40620-014-0133-5

ORIGINAL ARTICLE

Adipose-derived mesenchymal stem cells transplantation facilitate experimental peritoneal fibrosis repair by suppressing epithelial–mesenchymal transition

Keiichi Wakabayashi · Chieko Hamada · Reo Kanda · Takanori Nakano · Hiroaki Io · Satoshi Horikoshi · Yasuhiko Tomino

Received: 23 May 2014 / Accepted: 12 August 2014 / Published online: 22 August 2014

Abstract
Background Prevention or reversal of peritoneal damage is critical in peritoneal dialysis. Although autologous cell transplantation has beneficial effects on tissue repair in various organs, few studies have investigated the effects of transplantation of adipose-derived mesenchymal stem cells (ASCs) on peritoneal fibrosis (PF). Thus, we examined the mechanism of facilitated peritoneal reconstruction induced by ASC transplantation on chlorhexidine gluconate (CG)-induced PF in rats.
Methods To induce PF in rats, continuous-infusion pumps containing 8 % CG were placed in the abdominal cavity for 21 days. The pumps were removed on day 22 and ASCs were immediately injected into the peritoneal cavity. Morphological alterations and mRNA expression levels of fibrosis-related factors were examined on days 29 and 35.
Results ASC transplantation significantly facilitated peritoneal repair. mRNA expression of tumor necrosis factor-α, interleukin-1β, monocyte chemotactic protein-1, and epithelial–mesenchymal transition (EMT) markers such as Snail and α-smooth muscle actin were suppressed, whereas that of vascular endothelial growth factor (VEGF) and platelet-derived growth factor-BB (PDGF-BB) were overexpressed after ASC transplantation. Immunofluorescence indicated that some transplanted ASCs expressed VEGF and PDGF-BB and differentiated into vascular cells.
Conclusions ASC transplantation facilitates peritoneal repair by suppressing EMT and modulating inflammation and angiogenesis during the early phase of tissue repair in experimental PF.

Keywords Peritoneal fibrosis · Adipose-derived mesenchymal stem cells · Epithelial–mesenchymal transition · Angiogenesis

K. Wakabayashi · C. Hamada · R. Kanda · T. Nakano · H. Io · S. Horikoshi · Y. Tomino (✉)
Division of Nephrology, Department of Internal Medicine, Juntendo University Faculty of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-8421, Japan
e-mail: yasu@juntendo.ac.jp

Introduction

Peritoneal fibrosis (PF) remains a life-threatening complication in peritoneal dialysis [1]. Bio-incompatible dialysates and infectious peritonitis are known to upregulate the production of fibrosis-related factors in the peritoneum, resulting in mesothelial cell loss, submesothelial compact (SMC) zone thickening, and abnormal angiogenesis [2].

Excessive production of vascular endothelial growth factor (VEGF) and transforming growth factor-β (TGF-β) induces both abnormal angiogenesis and an epithelial–mesenchymal transition (EMT), resulting in the development of PF [2, 3]. Several studies have shown that TGF-β as well as tumor necrosis factor-α (TNF-α) and hypoxia inducible factor-1α (HIF-1α) are capable of inducing EMT through Snail activation [4].

There are considerable ongoing efforts to examine the therapeutic potential of mesenchymal stem cells (MSCs) in various organs [5, 6]. Since MSCs possess many characteristics, including a multi-lineage potential [7], immunomodulatory properties [8], and self-renewal capability in vitro [9], they have been found to decrease fibrosis in the heart [10], lungs [11] and kidneys [12]. Moreover, injected bone marrow-derived MSCs (BMSCs) have been shown to differentiate into peritoneal mesothelial cells [13] and

repair PF morphologically and functionally in animal models through anti-inflammatory and anti-EMT effects [14–16].

The immunomodulatory properties of adipose-derived mesenchymal stem cells (ASCs) may be more potent than those of BMSCs [17]. In addition, the use of ASCs is more advantageous than that of BMSCs because harvesting ASCs is minimally invasive and in vitro culturing yields an unlimited supply [18]. A previous report demonstrated that treatment with ASCs decreased kidney damage caused by ischemia–reperfusion injury through suppressing oxidative stress and inflammation [19]. Takahashi et al. [20] also demonstrated that ASCs appeared to have the capacity to differentiate into endothelial cells (ECs) as well as secrete paracrine factors that stimulated EC repair in a rat femoral artery wire injury model.

To determine the effects of intraperitoneal ASC transplantation, we injected ASCs into rats with chlorhexidine gluconate (CG)-induced PF.

Subjects and methods

Animals

Thirty-five male Sprague–Dawley rats (body weight 200–250 g; Sankyo Labo Service Corporation Inc, Tokyo, Japan) were housed in standard rodent cages under conventional laboratory conditions of 22 °C and a 12-h light/dark cycle with free access to laboratory chow and tap water. Our experimental protocol was approved by the Ethics Review Committee of Animal Experimentation at Juntendo University Faculty of Medicine, Tokyo, Japan.

Animal models of peritoneal fibrosis

Rats were continuously infused with 8 % CG using infusion pumps as previously described [21, 22]. In brief, infusion pumps were placed in the abdominal cavity for 21 days and the anterior abdominal peritoneum was harvested on days 0, 22, 29 and 35 (CG group).

ASC transplantation

After removing the infusion pumps, ASCs harvested from subcutaneous adipose tissue of green fluorescent protein (GFP)-transgenic rats and cultured as previously described [23] were injected (3×10^7 cells/rat) into the peritoneal cavity on day 22 and sections were harvested on days 29 and 35 (Tx group). Peritoneal samples were immediately fixed in 20 % formalin solution, embedded in paraffin, and stained with Masson's trichrome.

Histological analysis

As previously described [24], the maximum SMC thickness (μm) was measured in each section. Five sections were randomly selected using a KS400 Imaging System (Kortron Elektronik GmbH, Eching, Germany) [25]. The average peritoneal thickness was determined for each specimen.

Immunohistochemical analysis

Immunohistochemical analysis was performed using formalin fixed tissue sections after microwave antigen retrieval as previously described [21]. The mouse anti-rat alpha-smooth muscle actin (α-SMA) antibody (Abcam, Cambridge, UK) was used as primary antibody. The negative control was confirmed by incubation without primary antibodies and exhibited no positive cells. The number of α-SMA positive cells in the peritoneum were counted in ten random regions in each tissue. The average number was then calculated (the number of positive cells/SMC mm^2) using the KS400 Imaging System.

Double-immunofluorescence

Immunofluorescence was performed using formalin fixed tissue sections after microwave antigen retrieval as previously described [21]. Sections were incubated overnight with the following antibodies: fluorescein isothiocyanate (FITC)-conjugated goat anti-GFP antibody (Abcam, Tokyo, Japan), mouse anti-VEGF antibody (Abcam), mouse anti-CD31 antibody (Abcam), rabbit anti-platelet-derived growth factor-BB (PDGF-BB) antibody (Abcam), rabbit anti-PDGF-Rβ antibody (Abcam), and rabbit anti-α-SMA antibody (Abcam). After incubation, sections were mounted in diluted Alexa 555 (Molecular Probes, Inc., Eugene, OR, USA). Negative controls omitted primary antibodies. In all fluorescent images, cell nuclei were stained using 4′,6-diamidino-2-phenylindole (DAPI).

Real-time polymerase chain reaction (PCR)

RNA extraction and real-time PCR analysis were performed as previously described [21]. For quantification, samples were normalized against glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. PCR primers are shown in Table 1.

Statistical analysis

Results are expressed as mean ± standard deviation (SD). Group results were compared using one-way analysis of variance (ANOVA). Statistical analysis was performed

Table 1 The PCR primers were designed as follows

Gene	Forward primer	Reverse primer
GAPDH	5′-ACGTAAACGGCCACAAGTTC-3′	5′-AAGTCGTGCTGCTTCATGTG-3′
Type 3 collagen	5′-CCACCCTGAACTCAAGAGCGGAGAA-3′	5′-GGGACAGTCATGGGACTGGCATTT-3′
TNF-α	5′-TCGGTCCCAACAAGGAGGAGAAGT-3′	5′-TGATCTGAGTGTGAGGGTCTGGGC-3′
IL-1β	5′-TGGAGAAGCTGTGGCAGCTACCTAT-3′	5′-CACAGAGGACGGGCTCTTCTTCAA-3′
MCP-1	5′-AGGTCTCTGTCACGCTTCTGGG-3′	5′-TAGCAGCAGGTGAGTGGGGCAT-3′
Snail	5′-ACAGCGAACTGGACACACACACA-3′	5′-AGGGGAGTGGAATGGAACTGCTGA-3′
TGF-β	5′-ATGGTGGACCGCAACAACGCAAT-3′	5′-CAGCTCTGCACGGGACAGCAAT-3′
HIF-1α	5′-ACACACAGAAATGGCCCAGTGAGAA-3′	5′-GCACCTTCCACGTTGCTGACTTGAT-3′
α-SMA	5′-AGTCGCCATCAGGAACCTCGAGAA-3′	5′-ACCAGCAAAGCCCGCCTTACA-3′
VEGF	5′-ACTGGACCCTGGCTTTACTGCTG-3′	5′-TTCACCACTTCATGGGCTTTCTGCT-3′
PDGF-BB	5′-AGCGAGAGTGTGGGCAGGGTTA-3′	5′-GCACCATTGGCCCGTCCGAATCA-3′

PCR polymerase chain reaction, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase, *TNF* tumor necrosis factor, *IL* interleukin, *MCP* monocyte chemoattractant protein, *TGF* transforming growth factor, *HIF* hypoxia inducible factor, *SMA* smooth muscle actin, *VEGF* vascular endothelial growth factor, *PDGF-BB* platelet-derived growth factor-BB, *ASC* adipose-derived mesenchymal stem cells, *SMC* submesothelial compact, *DAPI* 4′,6-diamidino-2-phenylindole

using Graph Pad Prism version 5.0 software (Graph Pad Software, Inc., San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

Results

Effects of ASC transplantation on PF and collagen mRNA expression

Serial morphological changes are shown in Fig. 1a. SMC thickness on day 35 in the Tx group significantly decreased (Fig. 1b). Type 3 collagen mRNA levels on day 29 in the Tx group were lower than those in the CG group (Fig. 1c); however those on day 35 were not significantly different between groups (data not shown). ASCs were widely distributed in the peritoneum on day 25, and decreased on days 27 and 29 and disappeared on day 35 (Fig. 1d).

Effects of ASC transplantation on EMT and inflammation

Since ASCs had almost disappeared on day 35 as above, we compared the manners of ASCs on peritoneal repair between days 22 and 29. Snail mRNA levels on day 29 in the Tx group were significantly lower than those in the CG group; however those of TGF-β were comparable to those in the CG group (Fig. 2a). α-SMA positive cells were widely distributed in the peritoneum on day 22—the distribution of α-SMA positive cells in both CG and Tx groups is shown in Fig. 2b. ASCs suppressed α-SMA mRNA expression and the number of α-SMA-positive cells in the Tx group on day 29 (Fig. 2c). The FITC-positive transplanted cells (in green), which migrated into the peritoneal interstitium, did not express α-SMA (in red) (Fig. 2d). IL-1β, TNF-α, and monocyte chemoattractant protein-1 (MCP-1) mRNA levels on day 29 in the Tx group were significantly lower than those in the CG group (Fig. 2e).

Effects of ASC transplantation on angiogenesis

VEGF and PDGF-BB mRNA levels on day 29 in the Tx group were significantly higher than those in the CG group (Fig. 3a, b), however that of HIF-1α was significantly decreased (Fig. 3c). The average vascular density on day 29 in the Tx group tended to decrease compared to that in the CG group, but the differences were not statistically significant (Fig. 3d). Immunofluorescence showed that a part of the ASCs expressed VEGF on day 29 (Fig. 4e). In addition, a part of the transplanted ASCs that formed lumens also expressed PDGF-BB and PDGF-Rβ (Fig. 4f, g). Transplanted ASCs on day 29 also expressed CD31, which is a marker of ECs (Fig. 4h). Furthermore, to exclude the non-specific staining, we show the figures in which the sections were incubated with FITC-conjugated anti-GFP antibody on day 0 (Fig. 4a) and day 22 (Fig. 4b). We also show the negative controls for Fig. 4e, f (Fig. 4c) and Fig. 4g, h (Fig. 4d), in which the sections were stained with only secondary antibodies.

Discussion

To the best of our knowledge, this is the first study to show that intraperitoneal ASC transplantation facilitated peritoneal repair in rats with CG-induced PF. SMC thickness on day 35 was significantly reduced after

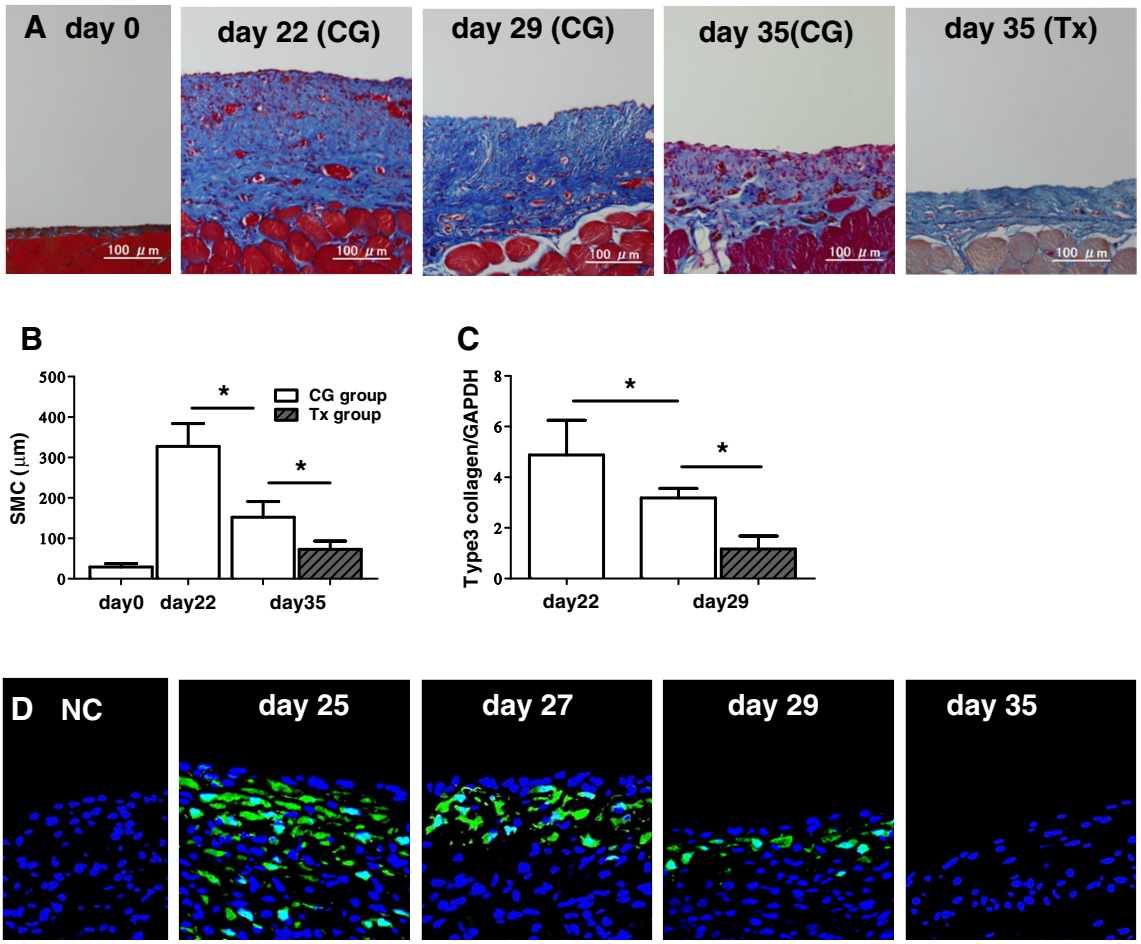


Fig. 1 Effects of ASC transplantation on morphological change and collagen mRNA expression. **a** Serial morphological changes in the CG group and Tx group. Masson's trichrome stain; magnification: ×200. **b** SMC thickness in the Tx group on day 35 was significantly decreased compared to that in CG group. **c** Type 3 collagen mRNA levels in the Tx group significantly decreased compared to the CG group. D: ASCs- (*green*) and DAPI-stained (*blue*) nuclei were visualized in the peritoneum. ASCs were widely distributed on day 25. ASC numbers decreased on days 27 and 29 and disappeared on day 35; magnification: ×60. *Error bars* represent standard deviations (SDs). *Significantly different ($p < 0.05$). *ns* no significance, *NC* negative control (sections incubated with secondary antibody), *ASC* adipose-derived mesenchymal stem cells, *SMC* submesothelial compact, *DAPI* 4′,6-diamidino-2-phenylindole

injection of ASCs. Transplanted ASCs appeared in the peritoneum until day 29. Since the transplanted ASCs may conduct to facilitate tissue repair transiently, we focused the manners at early stage of transplantation. ASCs modified mRNA expression of fibrosis-related factors on day 29. And ASCs suppressed Snail and α-SMA mRNA levels, but transplanted ASCs did not indicate α-SMA. Since TGF-β levels in the Tx group were comparable to those in the CG group, EMT may be regulated by TNF-α and HIF-1α in our study. When we examined the effects of ASCs on inflammation and angiogenesis, ASCs remarkably suppressed TNF-α levels. Moreover, VEGF and PDGF-BB levels significantly increased, and some ASCs secreted VEGF and PDGF-BB and differentiated into vascular cells, resulting in decreased HIF-1α levels and vascular density.

Recent studies suggested that one of the functions of MSCs is to act as guardians against excessive inflammatory responses. Prockop et al. [8] demonstrated that activated MSCs induced by pro-inflammatory factors modulated a negative feedback loop of a generic pathway of TNF-α-regulated inflammation. In our study, ASCs significantly suppressed IL-1β, TNF-α, and MCP-1 mRNA levels during the early repair phase.

Ueno et al. [14] reported that BMSCs ameliorated PF by suppressing inflammation and inhibiting TGF-β1 signaling. In our study, mRNA levels of Snail, which is a transcriptional repressor of E-cadherin and an inducer of EMT, were significantly inhibited independently of TGF-β levels. Regulation of several EMT-related factors was altered by activation of the TGF-β pathway, such as glycogen synthase kinase-3β, mitogen-activated protein

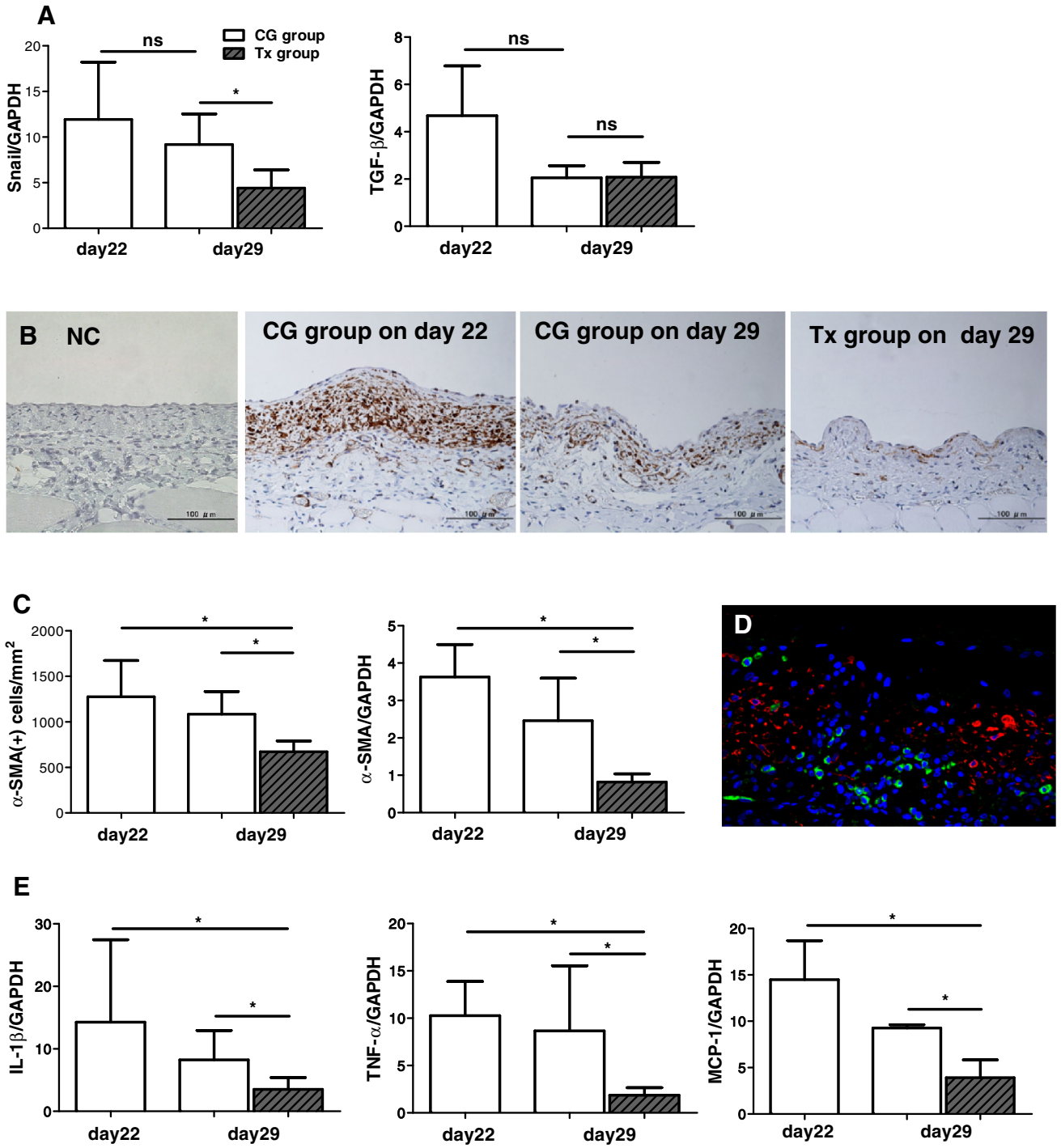


Fig. 2 Effects of ASC transplantation on EMT and inflammation. **a** Snail mRNA levels in the Tx group were significantly decreased on day 29, although TGF-β levels were not significantly different. **b**, **c** α-SMA-positive cells were widely distributed in the peritoneum on day 22. α-SMA mRNA levels and the number of α-SMA-positive cells in the Tx group were significantly decreased on day 29; magnification: ×200. **d** We detected transplanted cells that migrated into the peritoneal interstitium; however, they were not double-stained by either GFP (*green*) or α-SMA antibody (*red*); magnification: ×40. **e** IL-1β, TNF-α, and MCP-1 mRNA levels on day 29 in the Tx group were significantly lower than those in the CG group. *Error bars* represent SDs. *Significantly different ($p < 0.05$). *ns* no significance, *NC* negative control (sections incubated with secondary antibody), *SMA* smooth muscle actin, *GFP* green fluorescent protein, *IL* interleukin, *TNF* tumor necrosis factor, *MCP* monocyte chemoattractant protein. For other abbreviations, see Fig. 1

kinase, and Smad-related proteins, which were also examined in the present study, but we found no significant difference in expression levels between the CG and Tx groups (data not shown). Previous reports have demonstrated that several signals emerging from the extracellular microenvironment are necessary for EMT induction [4, 26]. TGF-β as well as TNF-α and hypoxia converge on nuclear factor kappa-B activation, resulting in Snail upregulation. According to our results, Snail mRNA suppression was probably due to the inhibition of HIF-1α and TNF-α expression. Actually, EMT suppression in the present study was demonstrated by confirming the reduction not only in Snail expression but also in the α-SMA expressions. Also, our results suggest that the transplanted ASCs, which migrated into peritoneal interstitium, did not transform into fibroblasts.

It has been established that angiogenesis is induced by tissue injury and hypoxia. Pinelopi et al. [27] demonstrated that pharmacological inhibition of HIFs before acute kidney injury induced by occlusion of renal arteries ameliorated renal fibrosis. In our study, VEGF and PDGF-BB levels were significantly higher, whereas HIF-1α levels were lower in the Tx group. PDGF-BB/PDGF-Rβ signaling plays an important role in vascular maturation and has been well documented by genetic studies [28, 29]. We observed a belt-like distribution of PDGF-BB on day 29 in the Tx group. Because PDGF stimulates its own expression by resident cells through a paracrine positive feedback effect and released PDGF binds to surrounding extracellular matrix, PDGF expression appeared band-like. Thus, amplification of both VEGF and PDGF signaling at the early repair stage improved tissue ischemia through a substantial microvessel network.

Moreover, a previous report suggested that ASCs have the potential to differentiate into ECs and pericytes [30]. Gehmert et al. [31] reported that PDGF-BB exert potent biological effects on mural cells and that ASCs express PDGF-Rβ as a receptor for PDGF-BB. Immunofluorescence showed that some PDGF-BB- and PDGF-Rβ-positive ASCs formed lumens. Therefore, the facilitative effect of ASC transplantation was not only the stimulation of resident cells to produce angiogenesis-related factors, but also the induction of ASCs themselves to differentiate into vascular cells that, in turn, secrete PDGF-BB and express PDGF-Rβ. However, it seems that the main inducer of the facilitative effects of ASCs on PF are paracrine effects of humoral factors rather than differentiation capacity because the cells that differentiated into CD31- and PDGF-Rβ-positive cells were only a small part of engrafted cells in the present study.

The present study has several limitations. First, we could not determine peritoneal function. Second, although several factors contributing to activation of the TGF-β pathway were measured, we found no significant differences on day 29. Therefore, serial examinations to assess the effects of ASCs in earlier periods are needed. Third, ASCs' differentiation capacity into vascular cells is a novel finding in a field of peritoneal dialysis, but the number of differentiated cells which we could detect were only a small part of the engrafted cells. So further investigation, e.g. transplanting with a far higher number of cells or transplanting them in masses or sheets, is required.

In conclusion, we propose that ASC transplantation may be an effective method for treating PF by contributing to anti-EMT and anti-inflammation effects, and improving hypoxia at an early phase of repair.

Fig. 4 Immunofluorescence analysis on angiogenesis. Sections incubated with FITC-conjugated anti-GFP antibody on day 0 (**a**) and day 22 (**b**). No GFP-positive cells were detected in either **a** or **b**. **c**, **d** Negative controls (NC; sections stained with secondary antibodies) for **e**, **f** (**c**) and **g**, **h** (**d**). **e** Transplanted ASCs (*green*) expressed VEGF (*red*) on day 29 in the Tx group; magnification: ×40. **f**–**h** A part of the transplanted ASCs (*green*) that formed lumens also expressed PDGF-BB (**f** *red*; ×40), PDGF-Rβ (**g** *red*; ×40) and CD31 (**h** *red*; ×60). *FITC* fluorescein isothiocyanate. For other abbreviations, see previous figures

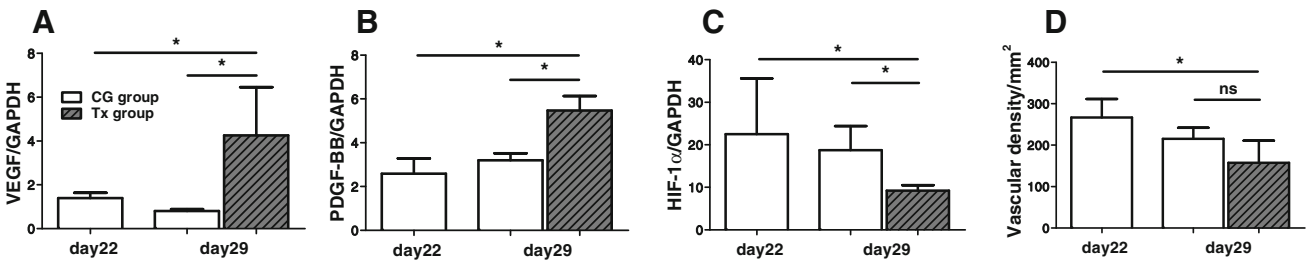
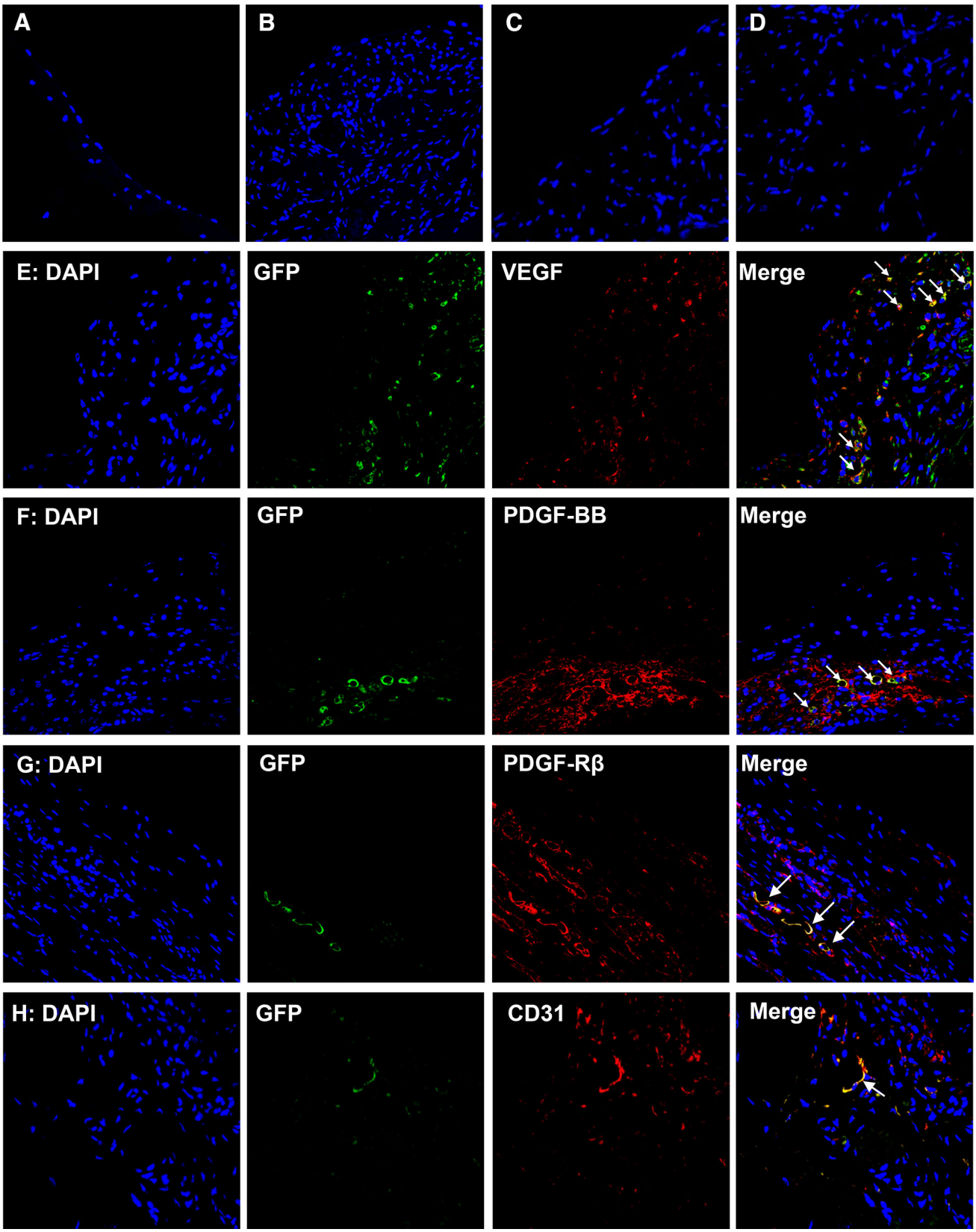


Fig. 3 Effects of ASC transplantation on angiogenesis. **a**, **b** VEGF and PDGF-BB mRNA levels on day 29 in the Tx group were significantly increased. **c** HIF-1α levels on day 29 in the Tx group were significantly decreased. **d** Average density on day 29 tended to decrease in the Tx group compared to that in the CG group. *Error bars* represent SDs. *Significantly different ($p < 0.05$). *VEGF* vascular endothelial growth factor, *PDGF-BB* platelet-derived growth factor-BB, *HIF* hypoxia inducible factor, *SD* standard deviation. For other abbreviations, see previous figures


A
B
C
D
E: DAPI
GFP
VEGF
Merge
F: DAPI
GFP
PDGF-BB
Merge
G: DAPI
GFP
PDGF-Rβ
Merge
H: DAPI
GFP
CD31
Merge

Acknowledgments This study was supported by Grants-in-Aid for science from the Ministry of Education, Culture, Sport, Science, and Technology of Japan. We thank Terumi Shibata for her excellent technical assistance. We also thank Takako Ikegami and Tomomi Ikeda (Division of Molecular and Biochemical Research, Juntendo University Graduate School of Medicine) for their excellent technical assistance.

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Kawaguchi Y, Saito A, Kawanishi H et al (2005) Recommendations on the management of encapsulating peritoneal sclerosis in Japan, 2005: diagnosis, predictive markers, treatment, and preventive measures. Perit Dial Int 25(Suppl 4):S83–S95
2. Schilte MN, Celie JW, Wee PM et al (2009) Factors contributing to peritoneal tissue remodeling in peritoneal dialysis. Perit Dial Int 29(6):605–617
3. Io H, Hamada C, Ro Y et al (2004) Morphologic changes of peritoneum and expression of VEGF in encapsulated peritoneal sclerosis rat models. Kidney Int 65(5):1927–1936
4. Giannoni E, Parri M, Chiarugi P (2012) EMT and oxidative stress: a bidirectional interplay affecting tumor malignancy. Antioxid Redox Signal 16:1248–1263
5. Prockop DJ (2009) Repair of tissues by adult stem/progenitor cells (MSCs): controversies, myths, and changing paradigms. Mol Ther 17(6):939–946
6. Prockop DJ, Kota DJ, Bazhanov N et al (2010) Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med 14(9):2190–2199
7. Pittenger MF, Mackay AM, Beck SC et al (1999) Multilineage potential of adult human mesenchymal stem cells. Science 284(5411):143–147
8. Prockop DJ, Oh JY (2012) Mesenchymal stem/stromal cells (MSCs): role as guardians of inflammation. Mol Ther 20(1): 14–20
9. Parfenova H, Leffler CW, Tcheranova D et al (2010) Epileptic seizures increase circulating endothelial cells in peripheral blood as early indicators of cerebral vascular damage. Am J Physiol Heart Circ Physiol 298(6):H1687–H1698
10. Nagaya N, Kangawa K, Itoh T et al (2005) Transplantation of mesenchymal stem cells improves cardiac function in a rat model of dilated cardiomyopathy. Circulation 112(8):1128–1135
11. Ortiz LA, Gambelli F, McBride C et al (2003) Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA 100(14):8407–8411
12. Ninichuk V, Gross O, Segerer S et al (2006) Multipotent mesenchymal stem cells reduce interstitial fibrosis but do not delay progression of chronic kidney disease in collagen4A3-deficient mice. Kidney Int 70(1):121–129
13. Sekiguchi Y, Hamada C, Ro Y et al (2012) Differentiation of bone marrow-derived cells into regenerated mesothelial cells in peritoneal remodeling using a peritoneal fibrosis mouse model. J Artif Organs 15(3):272–282
14. Ueno T, Nakashima A, Doi S et al (2013) Mesenchymal stem cells ameliorate experimental peritoneal fibrosis by suppressing inflammation and inhibiting TGF-beta1 signaling. Kidney Int 84(2):297–307
15. Wang N, Li Q, Zhang L et al (2012) Mesenchymal stem cells attenuate peritoneal injury through secretion of TSG-6. PLoS ONE 7(8):e43768
16. Bastug F, Gunduz Z, Tulpar S et al (2013) Mesenchymal stem cell transplantation may provide a new therapy for ultrafiltration failure in chronic peritoneal dialysis. Nephrol Dial Transplant 28(10):2493–2501
17. Banas A, Teratani T, Yamamoto Y et al (2008) IFATS collection: in vivo therapeutic potential of human adipose tissue mesenchymal stem cells after transplantation into mice with liver injury. Stem Cells 26(10):2705–2712
18. Leu S, Lin YC, Yuen CM et al (2010) Adipose-derived mesenchymal stem cells markedly attenuate brain infarct size and improve neurological function in rats. J Transl Med 8:63
19. Chen YT, Sun CK, Lin YC et al (2011) Adipose-derived mesenchymal stem cell protects kidneys against ischemia–reperfusion injury through suppressing oxidative stress and inflammatory reaction. J Transl Med 9:51
20. Takahashi M, Suzuki E, Oba S et al (2010) Adipose tissue-derived stem cells inhibit neointimal formation in a paracrine fashion in rat femoral artery. Am J Physiol Heart Circ Physiol 298(2):H415–H423
21. Kanda R, Hamada C, Kaneko K et al (2014) Paracrine effects of transplanted mesothelial cells isolated from temperature-sensitive SV40 large T antigen gene transgenic rats during peritoneal repair. Nephrol Dial Transplant 29(2):289–300
22. Komatsu H, Uchiyama K, Tsuchida M et al (2008) Development of a peritoneal sclerosis rat model using a continuous-infusion pump. Perit Dial Int 28(6):641–647
23. Lopez MJ, Spencer ND (2011) In vitro adult rat adipose tissue-derived stromal cell isolation and differentiation. Methods Mol Biol 702:37–46
24. Honda K, Hamada C, Nakayama M et al (2008) Impact of uremia, diabetes, and peritoneal dialysis itself on the pathogenesis of peritoneal sclerosis: a quantitative study of peritoneal membrane morphology. Clin J Am Soc Nephrol 3(3):720–728
25. Shimaoka T, Hamada C, Kaneko K et al (2010) Quantitative evaluation and assessment of peritoneal morphologic changes in peritoneal dialysis patients. Nephrol Dial Transplant 25(10): 3379–3385
26. Selgas R, Bajo A, Jimenez-Heffernan JA et al (2006) Epithelial-to-mesenchymal transition of the mesothelial cell—its role in the response of the peritoneum to dialysis. Nephrol Dial Transplant. 21 Suppl 2:ii2–ii7
27. Kapitsinou PP, Jaffe J, Michael M et al (2012) Preischemic targeting of HIF prolyl hydroxylation inhibits fibrosis associated with acute kidney injury. Am J Physiol Renal Physiol 302(9):F1172–F1179
28. Andrae J, Gallini R, Betsholtz C (2008) Role of platelet-derived growth factors in physiology and medicine. Genes Dev 22(10):1276–1312
29. Pierce GF, Tarpley JE, Allman RM et al (1994) Tissue repair processes in healing chronic pressure ulcers treated with recombinant platelet-derived growth factor BB. Am J Pathol 145(6):1399–1410
30. Szoke K, Brinchmann JE (2012) Concise review: therapeutic potential of adipose tissue-derived angiogenic cells. Stem Cells Transl Med 1(9):658–667
31. Gehmert S, Gehmert S, Hidayat M et al (2011) Angiogenesis: the role of PDGF-BB on adipose-tissue derived stem cells (ASCs). Clin Hemorheol Microcirc 48(1):5–13