

Matrix metalloproteinase-10 deficiency has protective effects against peritoneal inflammation and fibrosis via transcription factor NFκB pathway inhibition

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One of the most common causes of discontinued peritoneal dialysis is impaired peritoneal function. However, its molecular mechanisms remain unclear. Previously, by microarray analysis of mouse peritoneum, we showed that MMP (matrix metalloproteinase)-10 expression is significantly increased in mice with peritoneal fibrosis, but its function remains unknown. Chlorhexidine gluconate (CG) was intraperitoneally injected to wild-type and MMP-10 knockout mice to induce fibrosis to elucidate the role of MMP-10 on peritoneal injury. We also examined function of peritoneal macrophages and mesothelial cells obtained from wild-type and MMP-10 knockout mice, MMP-10-overexpressing macrophage-like RAW 264.7 cells and MeT-5A mesothelial cells, investigated MMP-10 expression on peritoneal biopsy specimens, and the association between serum proMMP-10 and peritoneal solute transfer rates determined by peritoneal equilibration test on patients. MMP-10 was expressed in cells positive for WT1, a mesothelial marker, and for MAC-2, a macrophage marker, in the thickened peritoneum of both mice and patients. Serum proMMP-10 levels were well correlated with peritoneal solute transfer rates. Peritoneal fibrosis, inflammation, and high peritoneal solute transfer rates induced by CG were all ameliorated by MMP-10 deletion, with reduction of CD31-positive vessels and VEGF-A-positive cells. Expression of inflammatory mediators and phosphorylation of NFκB subunit p65 at S536 were suppressed in both MMP-10 knockout macrophages and mesothelial cells in response to lipopolysaccharide stimulation. Overexpression of MMP-10 in RAW 264.7 and MeT-5A cells upregulated pro-inflammatory cytokines with phosphorylation of NFκB subunit p65. Thus, our results suggest that inflammatory responses induced by MMP-10

are mediated through the NFκB pathway, and that systemic deletion of MMP-10 ameliorates peritoneal inflammation and fibrosis caused by NFκB activation of peritoneal macrophages and mesothelial cells.

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Translational Statement

Impaired peritoneal function caused by peritoneal fibrosis is an important factor in peritoneal dialysis withdrawal. This study reveals that matrix metalloproteinase-10 (MMP-10) is upregulated in damaged peritoneum, that deletion of MMP-10 ameliorates peritoneal fibrosis, inflammation, and increased peritoneal solute transfer rates, and that MMP-10 is involved in inflammation induced by lipopolysaccharide through nuclear factor κB (NFκB) pathway activation in macrophages and mesothelial cells, suggesting that monitoring MMP-10 could be an indicator of peritoneal damage and that inhibition of MMP-10 could be a novel therapeutic target against peritoneal inflammation and fibrosis via NFκB pathway inhibition, leading to a longer period of peritoneal dialysis therapy.

Peritoneal dialysis (PD) is a well-established treatment modality for renal replacement with slower solute and fluid removal, resulting in higher quality of life more than hemodialysis.¹ Even though the prognosis of PD patients is comparable to that of hemodialysis,² PD may have to be discontinued because of loss of peritoneal function and PD-related infections. The main reasons of PD withdrawal have been reported to be peritonitis, cardiovascular diseases, and ultrafiltration failure.³ The peritoneal

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membrane dysfunction is caused when peritoneal inflammation is provoked because of various stimuli such as PD fluid or bacterial infection,^{4,5} and peritoneal inflammation induces structural abnormalities in the peritoneum, including peritoneal fibrosis and angiogenesis.⁶ Although inflammatory cytokines,⁷ high glucose concentrations of PD solution,⁸ and advanced glycation end products^{9,10} have been implicated in peritoneal deterioration process, the precise mechanism is still not clear.

Previously, our group performed microarray analysis of the peritoneum treated with chlorhexidine gluconate (CG) to identify novel genes that are involved in the pathogenesis of peritoneal fibrosis. In this microarray, it has been reported that pleiotrophin is markedly upregulated in CG-treated mice, and inflammation and increased peritoneal solute transfer rates are improved in its knockout mice.¹¹ In this study, the upregulated genes were examined in the same microarray and focused on matrix metalloproteinase (MMP) members. MMPs were originally discovered through their involvement in amphibian metamorphosis.¹² To date, 23 types of structurally related protease families have been discovered in humans (and 24 in mice), and these are collectively referred to as the MMP family.^{13,14} These MMPs not only degrade extracellular substrates but are now known to play important roles in many physiological activities such as inflammatory diseases and wound healing.^{15,16} Previous studies showed that overexpression of MMP-9 in the mouse peritoneum induces thickening of the submesothelial space and angiogenesis, and deletion of MMP-9-improved peritoneal solute transfer rates.¹⁷ MMP-2 levels in PD fluid are reported to reflect the solute transport rate of the peritoneal membrane.¹⁸ The correlation between MMPs and peritoneal function has attracted attention. However, the effects of MMPs other than MMP-2 and MMP-9 on peritoneal fibrosis are unknown, so we reanalyzed the gene array and investigated the significance of candidate MMPs associated with peritoneal deterioration.

METHODS

Human samples

Patients admitted to Kyoto University Hospital for the diagnosis and treatment of kidney or dialysis-related disease were enrolled in this study after obtaining informed consent. This study was approved by the Ethics Committee for Human Research of the Graduate School of Medicine, Kyoto University (approval no. R3781). The parietal peritoneal samples were taken from the near insertion site of the PD catheter placed in the lumbar region at the end of PD as biopsies and were embedded with paraffin or optimal cutting temperature. Sera were collected from 28 patients at the time of the peritoneal equilibration test from March 2010 to June 2014 and from October 2022 to March 2023. All primer sets of real-time quantitative polymerase chain reaction were listed in [Supplementary Table S1](#). The clinical features of the enrolled patients are listed in [Supplementary Tables S2 and S3](#).

Peritoneal injury model

The protocol for this study was approved by the Kyoto University Animal Experiment Ethics Review Committee (approval no. Med-Kyo22537). Peritoneal fibrosis was induced in 12-week-old wild-type

and MMP-10 knockout mice by i.p. injection of 0.1% CG (872619; Sumitomo Pharma Co., Ltd.) at 0.01 ml/g body weight dissolved in 15% ethanol and 85% phosphate-buffered saline (PBS) 3 times a week for 2 or 4 weeks, as previously described (8 mice in each group).^{11,19} Intraperitoneal injection of PBS was administered to control mice (5 mice in each PBS group). The experimental unit was a cage of animals and randomly assigned to each group. Mice were killed in a state of deep sedation at the day after the last i.p. injection, and the peritoneum was collected for mRNA and protein analyses, and histological examination.

Mouse primary cell culture

Mouse peritoneal mesothelial cells were obtained from the peritoneal wall of adult male wild-type and MMP-10 knockout mice using the trypsin digestion method as previously described^{11,20} and in [Supplementary Methods](#). After 24 hours of incubation of primary mesothelial cells with Dulbecco's modified Eagle's medium containing 0.3% fetal bovine serum in the presence of 100 ng/ml lipopolysaccharide (LPS; L3024; Sigma-Aldrich) or 10 ng/ml tumor necrosis factor- α (Proteintech Group), the cells were collected with appropriate buffers.

Mouse peritoneal macrophages were prepared as previously described^{21,22} and in [Supplementary Methods](#). Mouse primary peritoneal macrophages were stimulated with 100 ng/ml LPS or 10 ng/ml interleukin-6 (IL-6) (Proteintech Group) and collected after 2 or 24 hours.

Additional details for precise methods including reagents and antibodies used in this study are available in [Supplementary Methods](#).

RESULTS

MMP-10 is upregulated in the peritoneum of CG-treated mice and PD patients, mainly at mesothelial cells and macrophages

We searched upregulated genes in the peritoneum of CG-treated mice compared with PBS-treated mice from our previous GeneChip Microarray (GSE162029) and focused on the MMP family.¹¹ The expression levels of each MMP in the peritoneum are shown in [Table 1](#). Gene expression of many members of MMPs were upregulated by CG administration, among which MMP-10 was strongly upregulated. Real-time quantitative polymerase chain reaction showed that mRNA expression of MMP-10 was 30-fold higher in the peritoneum of CG-treated mice than that in the peritoneum of PBS-treated mice ([Figure 1a](#)). Zymographic analysis revealed upregulated proteolytic activity of MMP-10 in the CG-treated mice peritoneum ([Figure 1b and c](#)). Immunohistochemical and immunofluorescent staining were then performed to examine the localization of MMP-10 in the peritoneum. MMP-10 was positive mainly at the mesothelial layer in the peritoneum of PBS-treated mice, whereas the entire submesothelial layer was positive in the peritoneum of CG-treated mice ([Figure 1d](#)). Double immunofluorescent staining of WT1 and MMP-10 showed that MMP-10 was expressed by WT1-positive mesothelial cells in the peritoneum of PBS-treated mice, whereas WT1-positive cells with spindle shape were sporadically colocalized with MMP-10 in the submesothelial zone of CG-treated mice ([Figure 1e](#)).

Table 1 | MMP changed in parietal peritoneum in CG-treated mice compared with PBS-treated mice after 3 weeks of CG treatment

Gene title	ID	Fold change of gene upregulated or downregulated in CG-treated mice compared with PBS-treated mice	Gene symbol	Common name
Matrix metalloproteinase 14	NM_008608	24.2515	<i>Mmp14</i>	MT1-MMP
Matrix metalloproteinase 10	NM_019471	8.5742	<i>Mmp10</i>	Stromelysin-2
Matrix metalloproteinase 8	NM_008611	8.0000	<i>Mmp8</i>	Collagenase-2
Matrix metalloproteinase 13	NM_008607	6.4980	<i>Mmp13</i>	Collagenase-3
Matrix metalloproteinase 3	NM_010809	6.0629	<i>Mmp3</i>	Stromelysin-1
Matrix metalloproteinase 2	NM_008610	4.0000	<i>Mmp2</i>	Gelatinase A
Matrix metalloproteinase 11	NM_008606	3.7321	<i>Mmp11</i>	Stromelysin-3
Matrix metalloproteinase 20	NM_013903	3.7321	<i>Mmp20</i>	Enamelysin
Matrix metalloproteinase 23	NM_011985	3.4822	<i>Mmp23</i>	CA-MMP
Matrix metalloproteinase 19	NM_021412	3.2490	<i>Mmp19</i>	Stromelysin-4
Matrix metalloproteinase 16	NM_019724	2.1435	<i>Mmp16</i>	MT3-MMP
Matrix metalloproteinase 24	NM_010808	0.8706	<i>Mmp24</i>	MT5-MMP
Matrix metalloproteinase 15	NM_008609	0.6598	<i>Mmp15</i>	MT2-MMP
Matrix metalloproteinase 17	NM_011846	0.6156	<i>Mmp17</i>	MT4-MMP
Matrix metalloproteinase 9	NM_013599	0.3078	<i>Mmp9</i>	Gelatinase B
Matrix metalloproteinase 7	NM_010810	0.2500	<i>Mmp7</i>	Matrilysin
Matrix metalloproteinase 12	NM_008605	0.2176	<i>Mmp12</i>	Metalloelastase

CG, chlorhexidine gluconate; MMP, matrix metalloproteinase; PBS, phosphate-buffered saline.

A bold gene indicates a gene of interest.

Macrophage antigen-2 (MAC-2)–positive macrophages were scarcely observed in the peritoneum of PBS-treated mice but were abundant in the peritoneum of CG-treated mice and colocalized with MMP-10 (Figure 1f). Immunofluorescence staining of peritoneal biopsy specimens from patients with PD withdrawal was performed to investigate MMP-10 expression in the human peritoneum, and MMP-10 was expressed in both infiltrating macrophages and mesothelial cells (Figure 1g and h). Hematoxylin and eosin staining of each biopsy specimen is shown in Supplementary Figure S1A, and clinical features of the presented cases are listed in Supplementary Table S2. These results are consistent with the findings in mice, indicating that MMP-10 expression is upregulated in the injured peritoneum in both humans and mice, especially in macrophages and mesothelial cells. Furthermore, we examined serum proMMP-10 levels by enzyme-linked immunosorbent assay in PD patients to assess the relevance between MMP-10 and peritoneal transport. Patients' background is shown in Supplementary Table S3. Serum proMMP-10 levels showed positive correlation with the dialysate/plasma ratio of creatinine (D/P Cr) and negative correlation with the dialysate/initial dialysate ratio of glucose (D/D₀ Glu; Figure 1i and j), suggesting the positive correlation of serum proMMP-10 and peritoneal solute transfer rates. Serum proMMP-10 levels also showed positive correlation with PD effluent Interleukin-6 (IL-6) levels (Figure 1k), and PD effluent IL-6 levels showed positive correlation with D/P Cr (Figure 1l). Residual kidney function examined by 24-hour urine collection tended to show a weak negative correlation with serum proMMP-10 levels, even though the correlation was not significant (Supplementary Figure S1B). Residual kidney function in PD patients was not correlated with D/P Cr and D/D₀ Glu (Supplementary Figure S1C and D).

These results indicate that MMP-10 is increased in the damaged peritoneal membrane, mainly in macrophages and mesothelial cells, and is associated with peritoneal solute transfer rates.

Peritoneal thickening, fibrosis, and inflammation are ameliorated in CG-treated MMP-10 knockout mice

To further investigate the role of MMP-10 in peritoneal thickening, we intraperitoneally administered CG or PBS to MMP-10 knockout or wild-type mice and analyzed their peritoneal membranes. Deletion of MMP-10 was confirmed by Western blot analysis and real-time polymerase chain reaction of ovary lysate, because it is known to highly express MMP-10 (Supplementary Figure S2A–C). The peritoneal expression of MMP-10 in MMP-10 knockout mice was undetectable by real-time polymerase chain reaction (Figure 2a) and immunohistochemical staining (Figure 2b). MMP-10 activity was not observed in the MMP-10 knockout mouse peritoneum by zymography (Figure 2c). There was no difference in body weight between each group before i.p. injection, but PBS-treated mice gained weight after 4 weeks of i.p. injection, whereas CG-treated mice did not (Supplementary Figure S2D–F). There was no difference in peritoneal thickening between MMP-10 knockout and wild-type mice in the PBS-treated control group. In contrast, wild-type mice showed clear and marked peritoneal thickening, which was suppressed in MMP-10 knockout mice in the CG-treated group (Figure 2d). Quantitative analysis showed that peritoneal thickening was reduced by approximately 50% in MMP-10 knockout mice (282.7 μ m in wild-type mice vs. 143.1 μ m in MMP-10 knockout mice, Figure 2f). Moreover, the fibrosis area in the submesothelial zone was increased in CG-treated wild-type mice, and its increase was reduced in MMP-10 knockout mice in Sirius Red

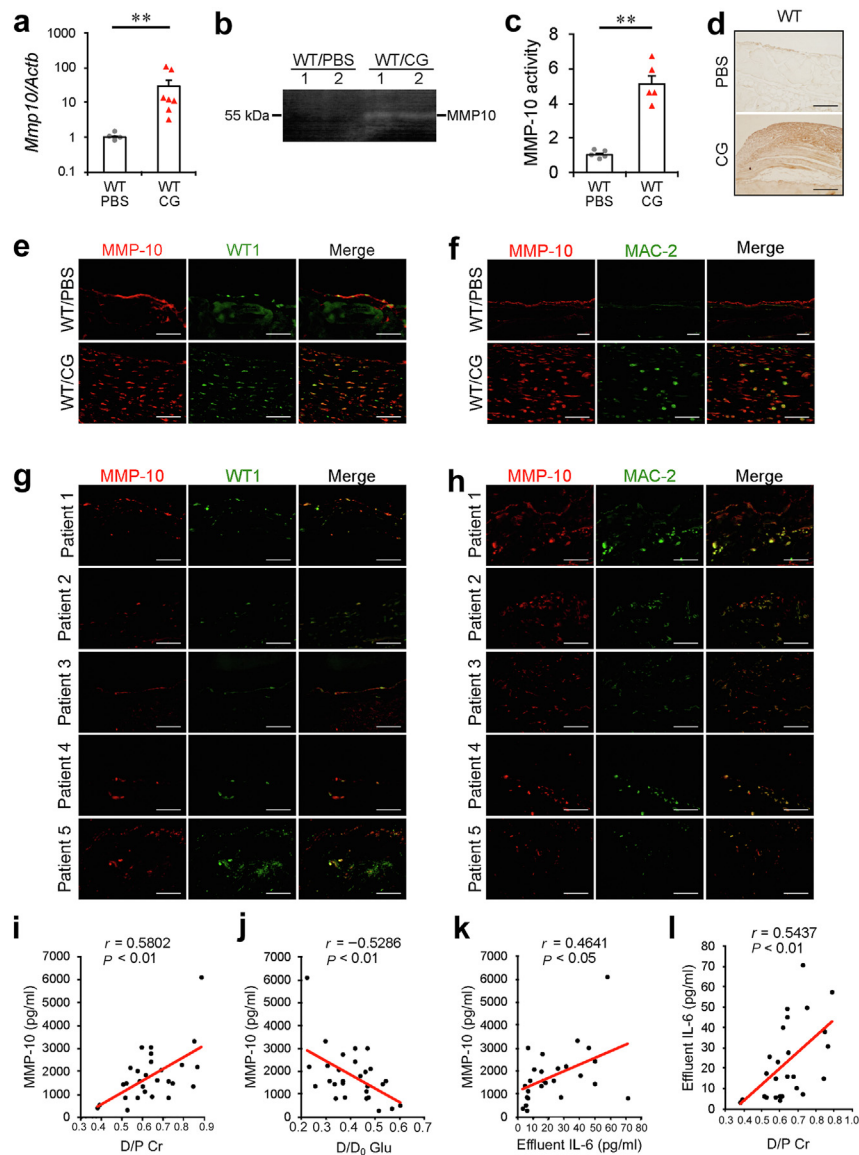


Figure 1 | Peritoneal matrix metalloproteinase-10 (MMP-10) expression and localization in wild-type mice (WT). (a) Expression of MMP-10 mRNA in the mouse peritoneum treated with phosphate-buffered saline (PBS) or chlorhexidine gluconate (CG). WT/PBS, n = 5; WT/CG, n = 8. (b) Gelatin zymography of the WT peritoneum treated with PBS or CG. (c) Zymographic activity of MMP-10 in the peritoneum treated with PBS or CG (n = 5, each). (d) Immunohistochemical staining for MMP-10 in the WT peritoneum treated with PBS or CG. (e) Double immunofluorescence staining for MMP-10 and WT1 in PBS- or CG-treated mouse peritoneum. (f) Double immunofluorescence staining for MMP-10 and macrophage antigen-2 (MAC-2) in PBS- or CG-treated mouse peritoneum. (g) Double immunofluorescence staining for MMP-10 and WT1 in the human peritoneum after long-term treatment of peritoneal dialysis (PD). Patients' data are shown in [Supplementary Table S2](#). (h) Double immunofluorescence staining for MMP-10 and MAC-2 in the human peritoneum after long-term treatment of PD. (i) Correlation between serum proMMP-10 and the dialysate/plasma ratio of creatinine (D/P Cr) in PD patients. (j) Correlation between serum proMMP-10 and the dialysate/initial dialysate ratio of glucose (D/D₀ Glu) in PD patients. (k) Correlation between serum proMMP-10 and PD effluent interleukin-6 (IL-6). (l) Correlation between PD effluent IL-6 and D/P Cr. Values are expressed as means $\pm$ SEM. Bar = 50 μ m. ** $P < 0.01$. r, Pearson's rank correlation coefficient. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

staining (Figure 2e and g). In addition, we investigated the peritoneal mRNA expression changes of *Fn1* and *Ctgf* by real-time polymerase chain reaction, which was upregulated in CG-treated wild-type mice and markedly suppressed in CG-treated MMP-10 knockout mice (Figure 2h and i).

Immunohistochemical staining for alpha smooth muscle actin (α SMA), F4/80, and MAC-2 was performed to investigate the effects of MMP-10 on inflammation. The

α SMA-positive cells were significantly increased in the peritoneum of CG-treated wild-type mice, whereas they were reduced in MMP-10 knockout mice (Figure 3a and b). MAC-2- and F4/80-positive cells were markedly increased in wild-type peritoneum but were significantly reduced in MMP-10 knockout mice (Figure 3c–f). These results suggest that deletion of MMP-10 reduced fibrosis and inflammation in the peritoneum. In addition, the

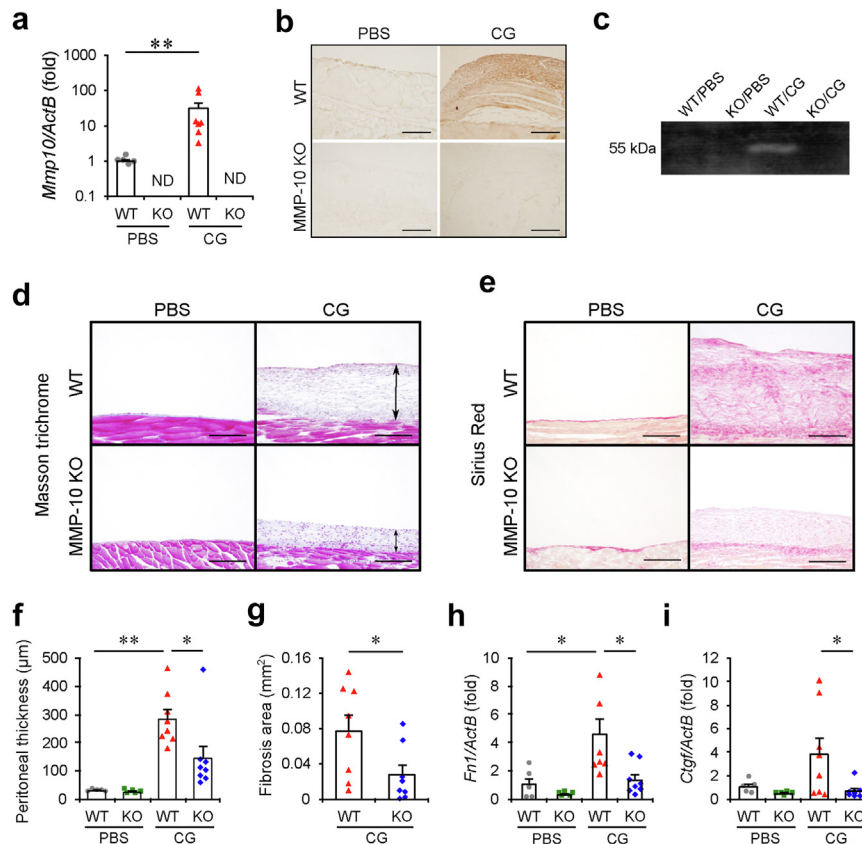


Figure 2 | Deletion of matrix metalloproteinase-10 (MMP-10) ameliorated peritoneal thickening and fibrosis. (a) Expression of MMP-10 mRNA in wild-type (WT) and MMP-10 knockout mice (KO) peritoneum treated with phosphate-buffered saline (PBS) or chlorhexidine gluconate (CG). (b) Immunohistochemical staining for MMP-10 in WT and MMP-10 KO mice peritoneum treated with PBS or CG. The images of the WT peritoneum are same as Figure 1b. (c) Gelatin zymography of WT and MMP-10 KO mice peritoneum treated with PBS or CG for detecting MMP-10 activity. (d,e) Representative images of Masson trichrome and Sirius Red staining of the peritoneum after 4 weeks of CG injection in WT and MMP-10 KO mice. The double-headed arrows represent the submesothelial zone. (f,g) Peritoneal membrane thickness and fibrosis area of the submesothelial zone. (h,i) Peritoneal mRNA expression of *Fn1* (h) and *Ctgf* (i) mRNA in WT and MMP-10 KO mice treated with CG or PBS. β -Actin was used as control. WT/PBS, $n = 5$; KO/PBS, $n = 5$; WT/CG, $n = 8$; KO/CG, $n = 8$. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$. Bar = 200 μ m. ND, not detected. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

proinflammatory genes such as *Ccl2* and *Emr1* were markedly suppressed in MMP-10 knockout mice (Figure 4a and b). We also examined *Mmp2* and *Mmp9* as major MMPs, which were found to be significantly suppressed in CG-treated MMP-10 knockout mice (Figure 4c and d). However, mRNA expression of *Mmp3* and *Mmp14* was not significantly changed in CG-treated MMP-10 knockout mice compared with CG-treated wild-type mice (Supplementary Figure S3A and B). Phosphorylation of nuclear factor κ B (NF κ B) subunit p65 at S536, known as a major regulator of inflammatory responses, was induced by CG administration and suppressed in the MMP-10 knockout peritoneum (Figure 4e and f). Furthermore, we investigate phosphorylation of mitogen-activated protein kinases (MAPKs) including p38 MAPK, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase. All MAPK phosphorylation was upregulated in CG-treated wild-type mice, but only phospho-p38 MAPK and phospho-ERK were suppressed in CG-treated MMP-10

knockout mice (Figure 4g–j). These results suggest that deletion of MMP-10 ameliorates peritoneal fibrosis and inflammation.

Peritoneal solute transfer rates and angiogenesis were improved in MMP-10 knockout mice

Subsequently, we performed a peritoneal equilibration test in wild-type and MMP-10 knockout mice treated with PBS or CG for 2 weeks to confirm changes in the peritoneal solute transfer rates. There was no difference in peritoneal solute transfer rates between wild-type and MMP-10 knockout mice in the PBS-treated control group. Meanwhile, CG-treated wild-type mice showed an increased ratio of dialysate to plasma concentration of urea nitrogen (D/P UN) and that of D/P Cr, and decreased volume of the effluent and D/D₀ Glu. In CG-treated MMP-10 knockout mice, D/P UN was significantly improved compared with wild-type mice (Figure 5a), but D/P Cr, D/D₀ Glu, and volume of effluent were not significant (Figure 5b–d).

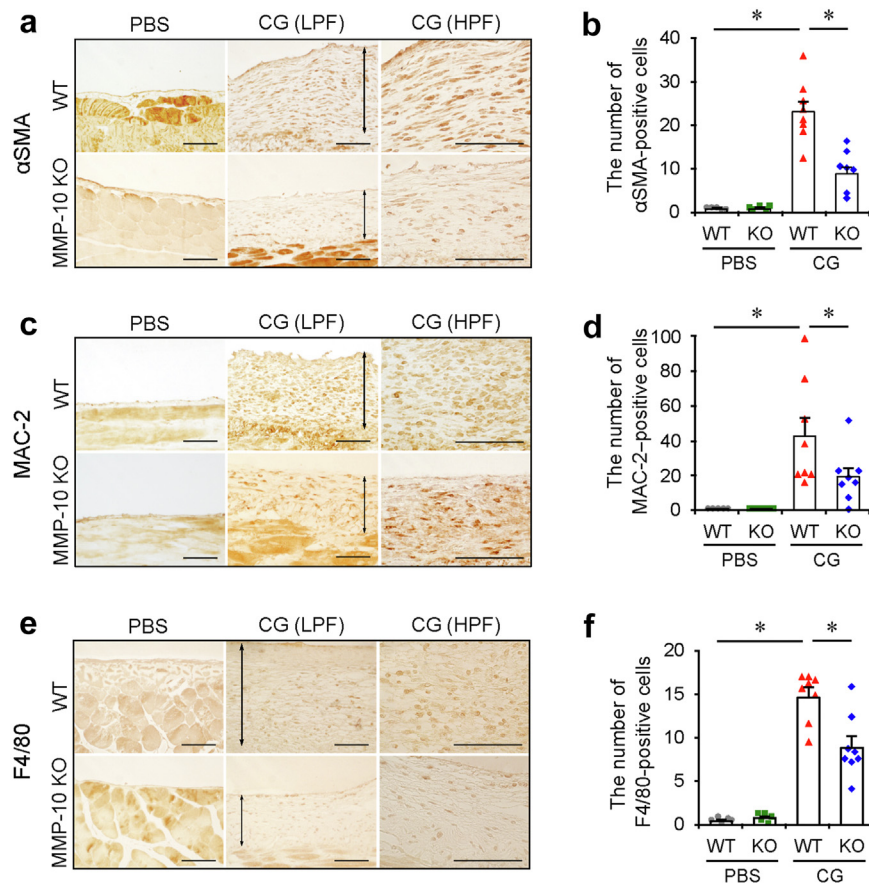


Figure 3 | Immunohistochemical staining for alpha smooth muscle actin (α SMA), F4/80, and macrophage antigen-2 (MAC-2) in matrix metalloproteinase-10 (MMP-10) knockout mice (KO). (a) Immunohistochemical staining for α SMA in the peritoneum at 4 weeks after the induction of peritoneal fibrosis. Photomicrographs at low and high magnification. (b) The number of α SMA-positive cells in the peritoneum. (c) Immunohistochemical staining for F4/80 in the peritoneum. Photomicrographs at low and high magnification. (d) The number of F4/80-positive cells in the peritoneum. (e) Immunohistochemical staining for MAC-2 in the peritoneum. Photomicrographs at low and high magnification. (f) The number of MAC-2-positive cells in the submesothelial layer of the peritoneum. The double-headed arrows represent the submesothelial zone. Bar = 100 μ m. WT/PBS, n = 5; KO/PBS, n = 5; WT/CG, n = 8; KO/CG, n = 8. Values are expressed as means $\pm$ SEM. * P < 0.05. CG, chlorhexidine gluconate; HPF, high-power field; LPF, low-power field; PBS, phosphate-buffered saline; WT, wild-type mice. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Immunohistochemical staining of CD31 and vascular endothelial growth factor A (VEGF-A) was also performed as there was a correlation between peritoneal solute transfer rates and angiogenesis.²³ Increased CD31-positive vessels and VEGF-A-positive cells observed in CG-treated wild-type mice were ameliorated in the peritoneum of CG-treated MMP-10 knockout mice (Figure 5e–h).

Proinflammatory changes in response to LPS stimulation are suppressed in MMP-10 knockout macrophages and mesothelial cells

To further evaluate the findings of *in vivo* experiments, we focused on the function of MMP-10 in mesothelial cells and macrophages. Because various types of cells are involved in the process of peritoneal thickening, we aimed to determine what kinds of changes occur in the function of each cell by MMP-10 deletion. We first collected thioglycollate-inducible peritoneal macrophages from wild-type and MMP-10 knockout mice and then stimulated them with LPS (100 ng/ml; Figure 6a).

Unstimulated macrophages showed no difference in *Ccl2*, *Il6*, and *Mmp9* mRNA expression between wild-type and MMP-10 knockout mice (Figure 6c). LPS stimulation induced upregulation of *Mmp10* and inflammation-related genes in wild-type macrophages. In contrast, MMP-10 knockout mice-derived macrophages showed reduced expression of these genes. Furthermore, phosphorylation of NF κ B subunit p65 at S536 induced by LPS stimulation was significantly suppressed in MMP-10 knockout macrophages (Figure 6e). IL-6-stimulated MMP-10 knockout macrophages showed reduced inflammatory gene-related mRNA expression compared with wild-type macrophages (Supplementary Figure S4). Next, we collected peritoneal mesothelial cells using the trypsin–ethylenediamine tetraacetic acid methods (Figure 6b). Cytokeratin staining for primary mesothelial cells is shown in Supplementary Figure S5. LPS-stimulated peritoneal mesothelial cells lacking MMP-10 showed suppressed inflammatory cytokine responses (Figure 6d) and phosphorylation of NF κ B subunit p65 at S536 (Figure 6f). Tumor necrosis factor- α -stimulated MMP-10

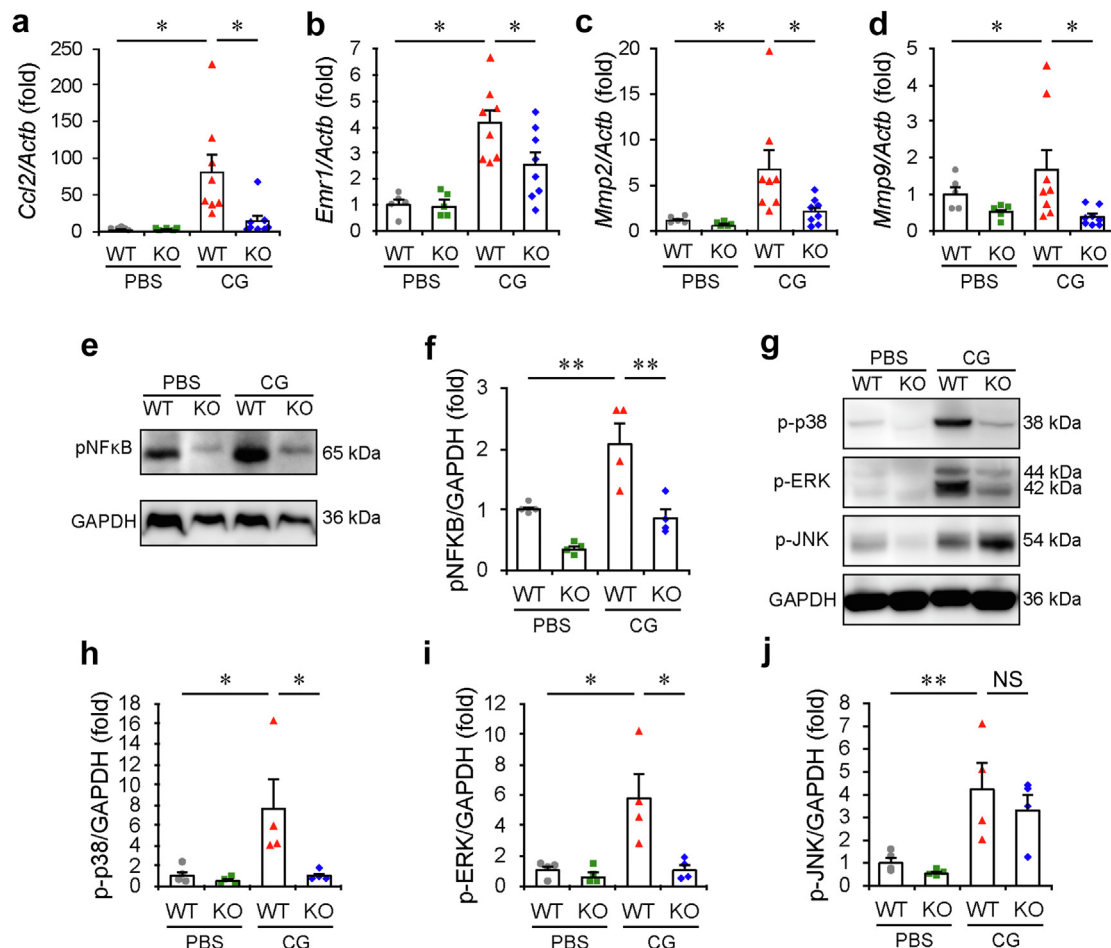


Figure 4 | Inflammatory changes in peritoneum. (a–d) Inflammatory mRNA expression of peritoneum in wild-type (WT) and matrix metalloproteinase-10 (MMP-10) knockout mice (KO) treated with chlorhexidine gluconate (CG) or phosphate-buffered saline (PBS) 3 times a week for 4 weeks. Expression of *Ccl2* (a), *Emr1* (b), *Mmp2* (c), and *Mmp9* (d) mRNA. β -Actin was used as control. (e,f) Western blot analyses of phosphorylated nuclear factor κ B (NF κ B) subunit p65 at S536, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in peritoneum treated with PBS or CG from WT and MMP-10 KO. (g) Western blot analyses of phosphorylated mitogen-activated protein kinases (MAPKs) and GAPDH in peritoneum treated with PBS or CG from WT and MMP-10 KO. (h–j) Quantitative analyses of (h) phosphorylated p38 MAPK, (i) phosphorylated extracellular signal-regulated kinase (p-ERK), (j) phosphorylated c-Jun N-terminal kinase (p-JNK). GAPDH was used as control. Values are expressed as means $\pm$ SEM. * P < 0.05, ** P < 0.01. D/D₀ Glu, dialysate/initial dialysate ratio of glucose; D/P Cr, dialysate/plasma ratio of creatinine; D/P UN, dialysate to plasma concentration of urea nitrogen; NS, not significant; VEGF-A, vascular endothelial growth factor A.

knockout mesothelial cells also showed reduced inflammatory gene-related mRNA expression compared with wild-type mesothelial cells (Supplementary Figure S6). In addition, fibroblasts were collected from mouse ear and then were stimulated with LPS (Supplementary Figure S7A). However, LPS stimulation did not upregulate MMP-10 expression and induced no obvious changes in mRNA expression of IL-6 between MMP-10 knockout and wild-type fibroblasts (Supplementary Figure S7B).

Overexpression of MMP-10 exacerbates inflammation via the NF κ B pathway

Deletion of MMP-10 ameliorated peritoneal inflammation *in vivo* and *ex vivo*, and we investigated the inflammatory changes by overexpressing mouse MMP-10 in RAW 264.7, a mouse macrophage cell line, and MeT-5A, immortalized human mesothelial cells isolated from noncancerous

pleural fluids (Figure 7a). Expression of mouse MMP-10 was confirmed by immunofluorescent study for MMP-10 (Figure 7b) and Western blot analysis for FLAG (DYKDDDDK)-tagged protein (Figure 7c). The expression of inflammatory cytokines such as IL-1 β and IL-6 was significantly upregulated in both macrophages and mesothelial cells with MMP-10 overexpression, but the changes were more pronounced in macrophages. Furthermore, *Ccl2* was significantly upregulated in MMP-10-overexpressing RAW 264.7 cells, and *CTGF* was upregulated in MMP-10-overexpressing MeT-5A cells (Figure 7d and e). In addition, phosphorylation of NF κ B subunit p65 at S536 was also facilitated, which suggested that MMP-10 enhanced inflammation via NF κ B pathways (Figure 7f and g). All changes observed in mouse MMP-10 overexpression cells were observed in human MMP-10 overexpression cells (Supplementary Figures S8 and S9).

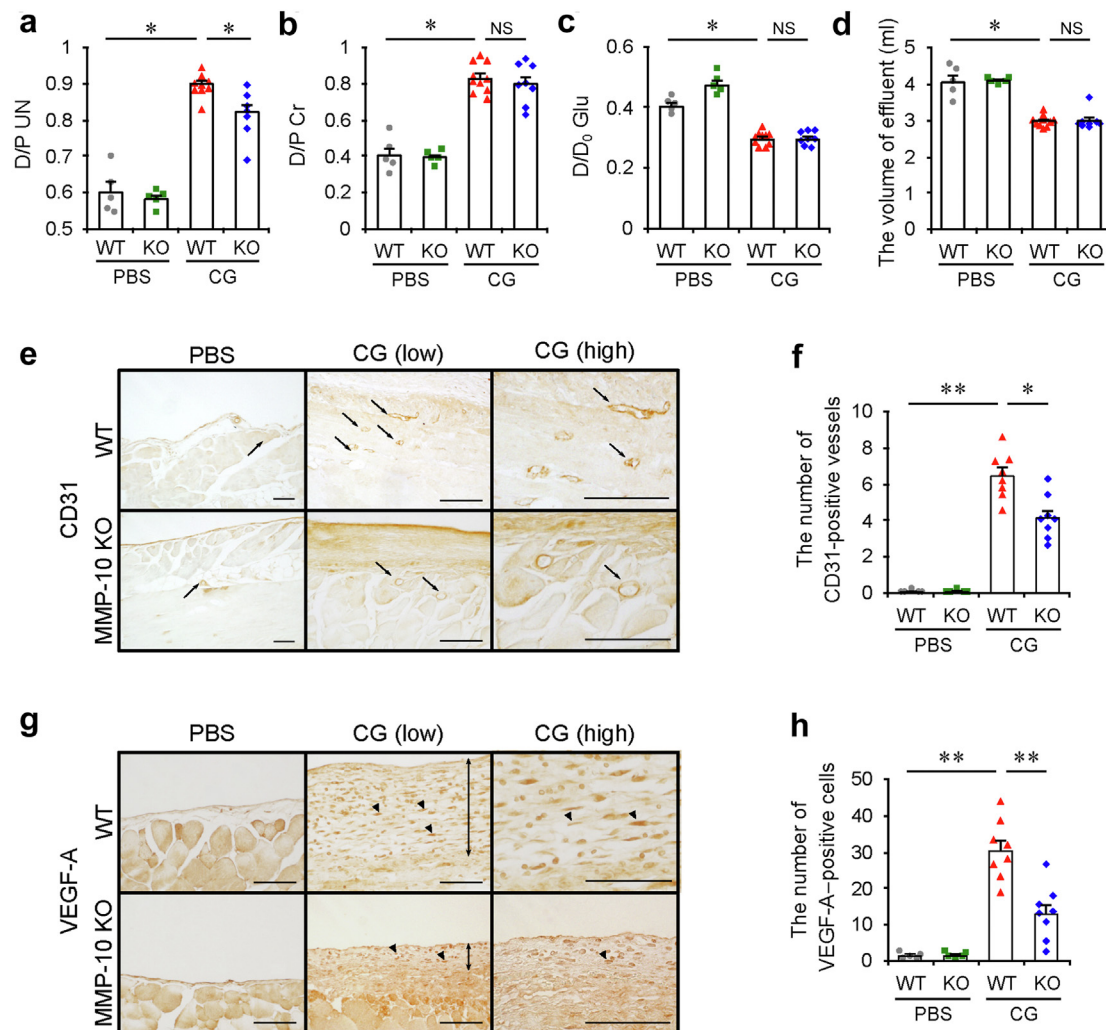


Figure 5 | Modified peritoneal equilibration test (PET) and immunohistochemical staining of CD31 and vascular endothelial growth factor A (VEGF-A). (a) D/P UN: the urea concentration in dialysate divided by the urea concentration in plasma. (b) D/P Cr: the creatinine concentration in dialysate divided by the creatinine concentration in plasma. (c) D/D₀ Glu: the glucose concentration in dialysate at 2 hours divided by the glucose concentration in dialysate at 0 hour. (d) The volume of effluent after 2-hour duration of i.p. storage of 3 ml of 4.25% glucose solution. PET was performed after 2 weeks of i.p. injection of chlorhexidine gluconate (CG) or phosphate-buffered saline (PBS). Wild type (WT)/PBS, n = 5; knockout (KO)/PBS, n = 5; WT/CG, n = 10; KO/CG, n = 8. (e) Immunohistochemical staining of CD31 in the peritoneum after 4 weeks of i.p. injection of CG or PBS. The arrows indicate CD31-positive vessels. (f) The area of CD31-positive vessels in the submesothelial zone per distance. (g) Immunohistochemical staining of VEGF-A in the peritoneum after 4 weeks of i.p. injection of CG or PBS. The arrowheads indicate VEGF-A-positive cells. The double-headed arrows represent the submesothelial zone. (h) The number of VEGF-A-positive cells in the submesothelial zone. Bar = 100 μ m. Values are expressed as means $\pm$ SEM. * P < 0.05, ** P < 0.01. LPS, lipopolysaccharide; MMP-10, matrix metalloproteinase-10; NS, not significant. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

DISCUSSION

In this study, we focused on the role of MMP-10 in peritoneal deterioration by activation of the NF κ B pathway in a CG model mouse, and deletion of MMP-10 led to preservation of peritoneal function (Figure 8). MMP-10 is also known as stromelysin-2 and classified into the simple hemopexin domain structural class without fibronectin-like domain found in gelatinases.²⁴ It has been reported to be involved in wound healing^{25,26} and malignant tumor invasion,²⁷ as well as correlating with the degree of atherosclerosis and aortic valve calcification^{28,29} and progression of diabetic nephropathy.³⁰ Although there is a controversy regarding the efficacy of

MMP-10 inhibition, a recent study has revealed that MMP-10 deletion has a protective effect against Adriamycin nephropathy.³¹ However, the function of MMP-10 in the peritoneal deterioration is still unclear. In the present study, we showed that MMP-10 mRNA expression was strongly upregulated in the peritoneum injured by CG administration by nearly 30-fold. In general, mRNA expression of MMP-10 is reported to be at a very low level in normal tissues³² but is readily induced in response to injury or inflammatory stimuli. MMP-10 knockout mice significantly improved peritoneal inflammation, fibrosis, and hyperperitoneal solute transfer rates that were typical phenotypes of the CG-treated peritoneum.¹⁹

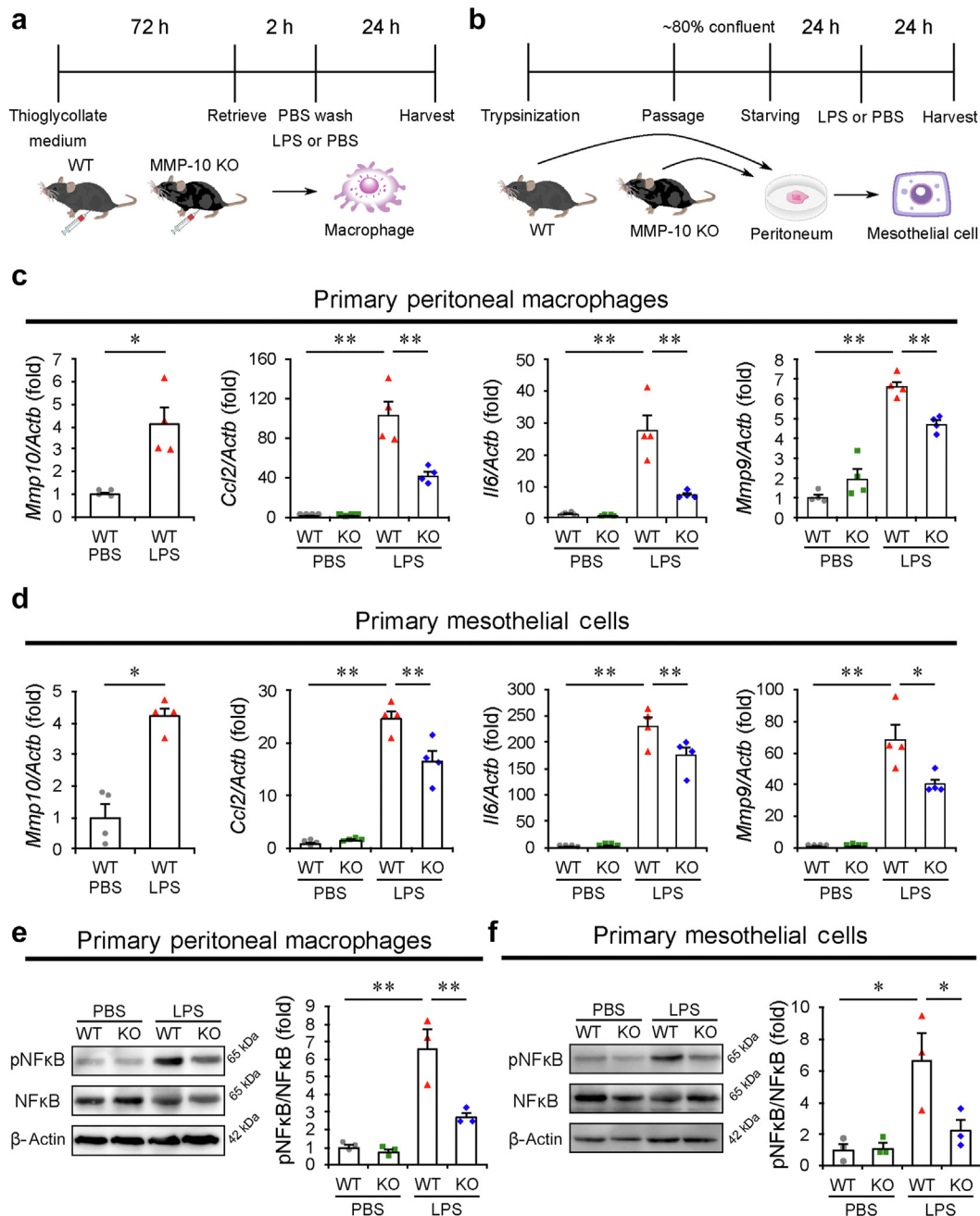


Figure 6 | Gene and protein expression of stimulated primary macrophages and mesothelial cells. (a) The cell culture protocol of primary macrophages from wild-type (WT) and matrix metalloproteinase-10 (MMP-10) knockout mice. (b) The cell culture protocol of primary mesothelial cells from WT and MMP-10 knockout mice (KO). (c) Expression of *Mmp10*, *Ccl2*, *Il6*, and *Mmp9* in primary macrophages after 24-hour stimulation of lipopolysaccharide (LPS, 100 ng/ml) or phosphate-buffered saline (PBS). (d) Expression of *Mmp10*, *Ccl2*, *Il6*, and *Mmp9* in mesothelial cells after 24-hour stimulation of LPS or PBS. (e) Western blot analyses of phosphorylated nuclear factor κ B (NF κ B) subunit p65 (S536), NF κ B subunit p65, and β -actin in peritoneal macrophages from WT and MMP-10 KO. (f) Western blot analyses of phosphorylated NF κ B (pNF κ B) subunit p65 (S536), NF κ B subunit p65, and β -actin in mesothelial cells from WT and MMP-10 KO. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$.

This is the first report that demonstrated the role of MMP-10 in peritoneal fibrosis. MMP-2 activity has been shown to be increased in the peritoneum of rats treated with CG,³³ and thickening of the submesothelial layer is observed in the peritoneum of MMP-9-overexpressing mice.¹⁷ In addition, MMP-2 in the PD fluid is elevated even before the onset of

encapsulating peritoneal sclerosis and expected to be a biomarker.³⁴ Therefore, MMPs are closely associated with peritoneal fibrosis and are well known as important therapeutic targets.

This study revealed that MMP-10 was expressed in mesothelial cells and macrophages in the mouse and human

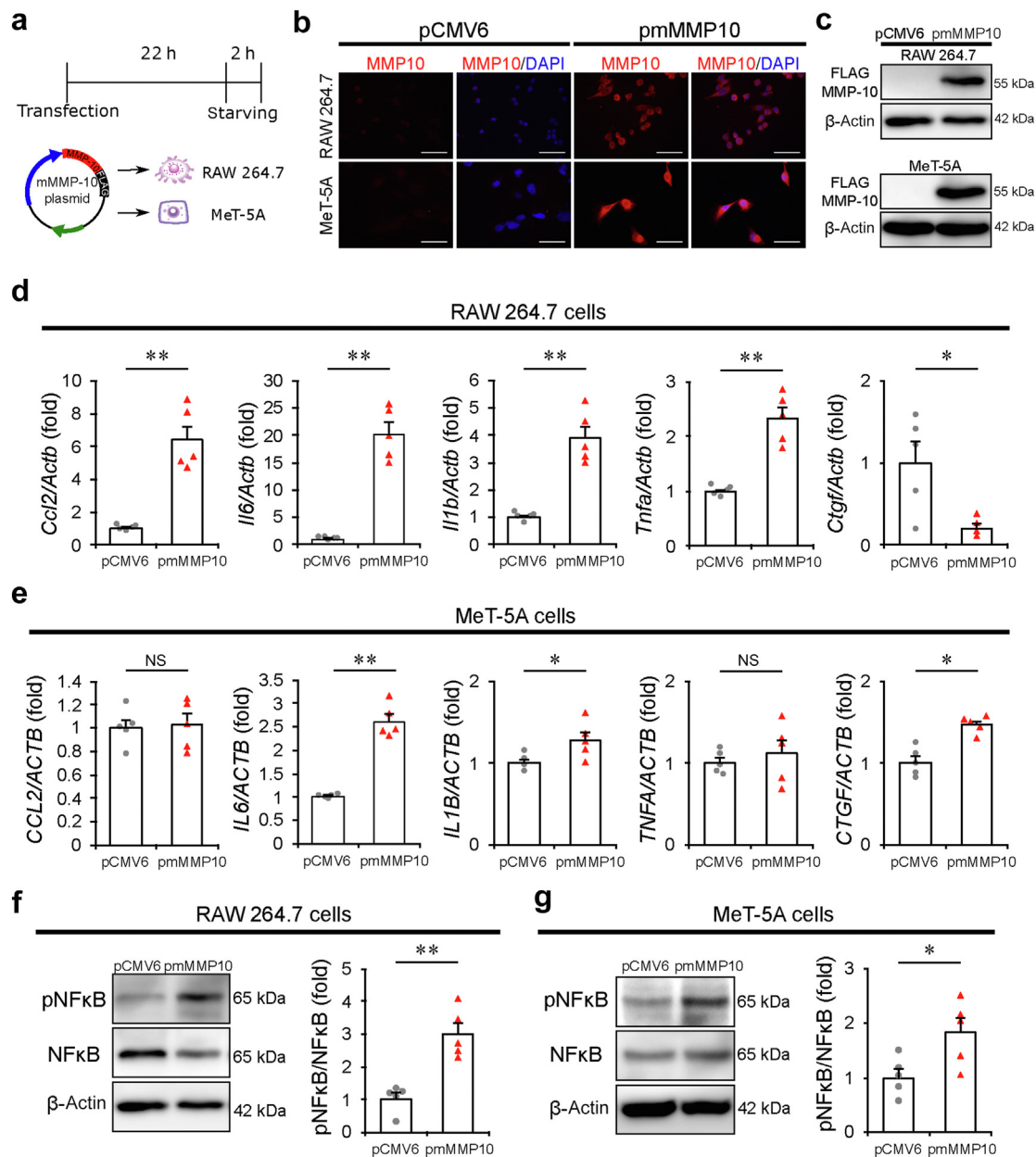


Figure 7 | Gene and protein expression of mouse matrix metalloproteinase-10 (MMP-10) overexpression RAW 264.7 cells and MeT-5A cells. (a) The cell culture protocol for the transfection of mouse MMP-10 (mMMP-10) expression plasmid to RAW 264.7 and MeT-5A cells. **(b)** Representative images of mMMP-10-overexpressing cells. Red color indicates MMP-10, and blue indicates 4',6-diamidino-2-phenylindole (DAPI). **(c)** Western blot analyses of RAW 264.7 cells and MeT-5A cells for FLAG (DYKDDDDK) and β -actin with or without mMMP-10 overexpression. **(d)** Expression of *Ccl2*, *Il6*, *Il1b*, *Tnfa*, and *Ctgf* mRNA in RAW 264.7 cells with or without mMMP-10 overexpression. **(e)** Expression of *CCL2*, *IL6*, *IL1B*, *TNFA*, and *CTGF* mRNA in MeT-5A cells with or without mMMP-10 overexpression. **(f)** Western blot analyses of phosphorylated nuclear factor κ B (NF κ B) subunit p65 (S536), NF κ B subunit p65, and β -actin in RAW 264.7 cells with or without mMMP-10 overexpression. **(g)** Western blot analyses of MeT-5A cells with or without mMMP-10 overexpression. Bar = 50 μ m. Values are expressed as means $\pm$ SEM. * P < 0.05, ** P < 0.01. NS, not significant; pCMV6; empty plasmid, pmMMP10; plasmid expressing mouse MMP-10; pNFkB, phosphorylated NF κ B. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

peritoneum. MMP-10 is reported to be expressed in keratinocytes,³⁵ podocytes,³¹ and macrophages,³⁶ and this study confirmed that it was also expressed in mesothelial cells. MMP-10 is demonstrated to be expressed in a similar pattern in humans for the first time. Furthermore, it has already been known that serum levels of MMP-10 were correlated with serum creatinine, albuminuria,³¹ and severity of

atherosclerosis,³⁷ and this study revealed that serum levels of MMP-10 were correlated with increased peritoneal solute transfer rates in PD patients. This result supported the importance of MMP-10 in maintenance of human peritoneal function. Serum MMP-10 was also correlated with IL-6 levels in PD effluent. Because PD effluent IL-6 levels were reported to have a strong correlation with peritoneal solute transfer

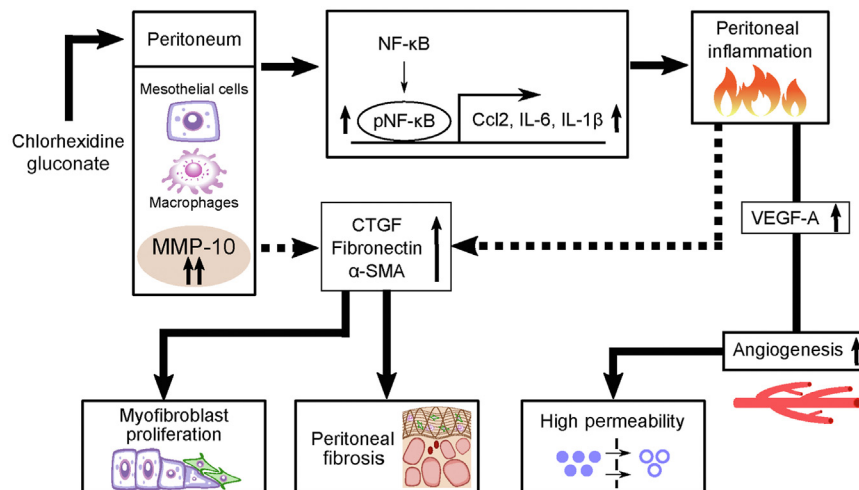


Figure 8 | Schematic images of matrix metalloproteinase-10 (MMP-10) in inflammation. I.p. administration of chlorhexidine gluconate elevates MMP-10 expression in the peritoneum. The upregulation of MMP-10 results in phosphorylation of nuclear factor κ B (NF κ B) subunit p65 at S536, the key modulator of inflammatory signaling, and increases the expression of its downstream chemokines and cytokines. The intensity of peritoneal fibrosis and permeability are associated with the severity of peritoneal inflammation in MMP-10 knockout mice. The dashed arrow represents the estimated or already known pathway. α -SMA, alpha smooth muscle actin; CTGF, connective tissue growth factor; pNF κ B, phosphorylated NF κ B; VEGF-A, vascular endothelial growth factor A.

rates³⁸ and represent inflammation in peritoneal cavity, it was suggested that peritoneal inflammation played an important role in elevated serum proMMP-10 and high peritoneal solute transfer rates. Expressions of MMP-2 and MMP-9 were also suppressed in the CG-treated MMP-10 knockout peritoneum, which is consistent with a previous report that MMP-9 is directly cleaved and activated by MMP-10.³⁹ MMP-9 knockout mice have shown suppression of myofibroblasts and angiogenesis, potentially involved in maintaining peritoneal function, but not suppression of peritoneal fibrosis and inflammation, indicating that MMP-10 is closely associated with other MMPs.

It has been reported that the number of α SMA-positive cells and macrophages is not suppressed in MMP-10 knockout mice during the skin healing process,⁴⁰ but, on the contrary, the present research shows the suppression of α SMA-positive cells and macrophages. This could be a reason of organ differences or the difference between multiple administration of CG and a single skin puncture. Zuo *et al.*³¹ reported that expressions of fibronectin and α SMA were suppressed in MMP-10 knockout mice podocytes treated with Adriamycin, which is consistent with the present study. Severe inflammation is the common feature between the 2 experiments, which may have a different mechanism than simple fibrosis in a relatively mild inflammatory state.

In the peritoneum degraded by CG administration, water permeability was reduced because of decreased aquaporin-1 expression and solute permeability was increased because of the widening of the endothelial intercellular gap,^{11,41} whereas small molecule permeability was improved in MMP-10 knockout mice. This result is supported by a lower number of CD31-positive vessels in the submesothelial region and a lower number of VEGF-A-positive cells. A previous study

about the relationship between VEGF-A and MMP-10 in vascular endothelium reported that VEGF stimulation increases MMP-10 expression and suppression of MMP-10 inhibits VEGF-A-induced angiogenesis.²³ IL-6 signaling plays an important role in the activation of VEGF-A,⁴² and this study demonstrated that deletion of MMP-10 leads to the reduction of IL-6 in macrophages and mesothelial cells. Taken together, deletion of MMP-10 leads to improvement of hyperperitoneal solute transfer rates partly through suppressing IL-6. It has also been reported that zonula occludens-1 was cleaved by MMP-10,³¹ and zonula occludens-1 expression of human peritoneal mesothelial cells was down-regulated by glucose degradation products through the generation of VEGF.⁴³ Zonula occludens-1 was reported to be related to solute transport in the mesothelial monolayer.⁴⁴ Therefore, zonula occludens-1 can be involved in the reduced permeability in MMP-10 knockout mice.

Peritoneal fibrosis is caused by infection, mechanical stress, and long-term exposure to osmotic pressure gradients and develops through the interaction of various cells.⁴⁵ Each cell plays an important role, but the mesothelial cells, myofibroblasts, and macrophages are thought to be the main players.⁴⁶ For *ex vivo* studies, we performed primary cultures of murine peritoneal macrophages and mesothelial cells, showing that the LPS-induced upregulation of proinflammatory gene expression was suppressed in MMP-10 knockout mice. There are some discrepancies with previous reports on macrophage results in this study. McMahan *et al.*⁴⁷ reported that MMP-10 knockout mice infected with *Pseudomonas aeruginosa* showed increased mortality compared with wild-type mice and increased *Ccl2* expression in bone marrow-derived macrophages and alveolar macrophages, which is the opposite of the results reported in this study. The use of thioglycolic acid-

induced peritoneal macrophages in this report may have led to different results. It was reported that the number of F4/80 positive cells tends to be suppressed in the skin of MMP-10 knockout mice 10 days after wound⁴⁰ and that the number of F4/80 positive cells in glomeruli is reduced in streptozotocin-treated MMP-10 knockout mice compared with streptozotocin-treated wild-type mice,⁴⁸ which is consistent with the present study. MMP-10 knockout mice have been reported to have improved podocyte damage in Adriamycin nephropathy.³¹ We have previously shown that *Ccl2* mRNA expression is upregulated in Adriamycin-treated podocytes,⁴⁹ suggesting that the CCL2 pathway was responsible for the reduced damage in MMP-10 knockout mice. NFκB is the main regulator of inflammatory response including the control of proinflammatory cytokines such as macrophage chemoattractant protein-1 (protein of *Ccl2* mRNA), and its phosphorylation is a critical factor in the activation of the NFκB pathway.⁵⁰ Phosphorylation of NFκB subunit p65 at S536, p38 MAPK, and ERK was enhanced in CG-treated wild-type mice and suppressed in MMP-10 knockout mice. These results indicate that p38 MAPK/ERK/NFκB pathways might be one of the main regulators of MMP-10-dependent peritoneal injuries. It was reported that the activator protein-1 binding factor (c-Fos, downstream of p38 MAPK, or ERK pathway) was the major regulator of MMP-10 gene expression.⁵¹ Although the MAPK/c-Fos pathway is speculated to be important in peritoneal inflammation and fibrosis in MMP-10 knockout mice, precise interaction between MAPK and MMP-10 was unclear. Primary mesothelial cells also showed the similar results concerning *Ccl2* and NFκB pathway in this study.

Recently, MMP-10 knockout mice have been known to aggravate fibrosis and increased expression of macrophage chemoattractant protein-1 in the liver after bile duct ligation.⁵² On the other hand, it has been reported that MMP-10 expression is elevated in blood vessels and macrophages of patients with pulmonary hypertension and that MMP-10-overexpressing pulmonary artery smooth muscle cells show increased proliferation and migration involved in vascular remodeling. The increased expression of MMP-10 in blood vessels was thought to be caused by infiltrating macrophages. We further analyzed the function of MMP-10 on peritoneal inflammation using primary macrophages from MMP-10 knockout mice and MMP-10-overexpressing macrophages. This study results also showed that cytokine and chemokine mRNA expressions such as *Ccl2* and *Il6* were increased in MMP-10-overexpressing RAW 264.7 cells. The study findings can suggest that the function of MMP-10 in macrophages may play an important role in inflammatory responses and organ remodeling.

This investigation has some limitation. The method of primary mesothelial cell extraction has risk of fibroblast contamination. The purity of mesothelial cells was approximately 80%–90%, but LPS stimulation did not upregulate *Mmp10* mRNA expression in primary fibroblasts. The number of PD patients included in this examination was limited. As the

CG administration peritoneal injury model is a model of inducing severe inflammation, it should be noted that it might be different from the clinical course of PD patients. The precise mechanism activating the NFκB pathway by MMP-10 is still unclear, but several hypotheses can be formulated. Several MMPs like MMP-2 and MMP-9 are known to cleave precursors of tumor necrosis factor-α⁵³ and IL-1β⁵⁴ into active forms, and suppression of MMPs may have inhibited such cytokines activity. As upregulation of MMP-10 has been reported to induce activation of epidermal growth factor receptor via degradation of heparin-binding epidermal growth factor-like growth factor,⁵⁵ it is possible that the epidermal growth factor receptor–NFκB pathway may have an important role in peritoneal inflammation. These findings suggest the multiple mechanisms of NFκB pathway activation by MMP-10. MMPs play a wide variety of roles, including invasion and metastasis of malignant tumors and activation of latent signaling molecules.¹³ However, further studies are needed to clarify how MMP-10 functions in various diseases and organs.

In conclusion, deletion of MMP-10 protects the peritoneum and maintains peritoneal solute transfer rates by suppressing fibrosis, inflammation, and angiogenesis via NFκB pathway inhibition. MMP-10 is expressed on mesothelial cells and macrophages. These findings indicate the importance of MMP-10 in peritoneal inflammation and fibrosis and may contribute to future investigations of the peritoneal deterioration.

DISCLOSURE

HYo has received research grants from Mitsubishi Tanabe Pharmaceutical Corporation and speakers bureau from AstraZeneca. MY has received research grants from Mitsubishi Tanabe Pharmaceutical Corporation and Boehringer Ingelheim. All the other authors declared no competing interests.

DATA STATEMENT

The data sets analyzed in this study are available from the Gene Expression Omnibus repository under the following accession numbers: GSE162029.

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AUTHOR CONTRIBUTIONS

TI and HYo conceived and designed the study, wrote the manuscript, and are the guarantors of this work, had full access to all the data in

the study, and take responsibility for the integrity of the data and the accuracy of the data analysis. TI conducted experiments. Als, HYA, KO, KPM, NT, SO, YK, TH, SS, Alk, and HN interpreted the results and contributed to discussion. HYO and MY supervised this study. All authors approved the final version of the manuscript.

SUPPLEMENTARY MATERIAL

[Supplementary File \(PDF\)](#)

Supplementary Methods.

Supplementary Table S1. Primer and dual-labelled probe sequences for real-time polymerase chain reaction (PCR).

Supplementary Table S2. Clinical characteristic of patients presented in [Figure 1g](#) and [h](#).

Supplementary Table S3. Clinical characteristic of patients receiving peritoneal dialysis.

Supplementary Figure S1. Hematoxylin and eosin (HE) staining of human peritoneum and relationship between residual kidney function and serum pro-matrix metalloproteinase-10 (proMMP-10) or peritoneal solute transfer rate. **(A)** HE staining of human peritoneum from peritoneal dialysis (PD) patients. Patient number is the same as in [Figure 1g](#) and [h](#), and clinical features of these patients are listed in [Supplementary Table S2](#). The double-headed arrows represent the submesothelial area. Bar = 100 μ m. **(B)** Correlation between serum proMMP-10 levels and creatinine clearance (CCr) in PD patients. **(C)** Correlation between the dialysate/plasma ratio of creatinine (D/P Cr) and CCr in PD patients. **(D)** Correlation between the dialysate/initial dialysate ratio of glucose (D/D0 Glu) and CCr in PD patients. HPF, high power field; LPF, low power field; r, Pearson's rank correlation coefficient.

Supplementary Figure S2. Matrix metalloproteinase-10 (MMP-10) expression in wild-type and MMP-10 knockout mice, and body weight in each group. **(A,B)** Western blot analyses of ovary lysates from wild-type and MMP-10 knockout mice for MMP-10 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). **(C)** Expression of *Mmp10* in wild-type and MMP-10 knockout mouse peritoneum and ovaries. **(D)** Mouse body weight (BW) before i.p. injection. **(E)** Mouse BW after 4 weeks of i.p. injection. **(F)** Mouse BW change. # $P < 0.05$ vs. BW before i.p. injection. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$. CG, chlorhexidine gluconate; KO, knockout mice; ND, not detected; PBS, phosphate-buffered saline; WT, wild-type mice.

Supplementary Figure S3. mRNA expression of *Mmp3* and *Mmp14* in the mouse peritoneum. **(A)** mRNA expression of *Mmp3* in wild-type and matrix metalloproteinase-10 (MMP-10) knockout mice peritoneum treated with chlorhexidine gluconate (CG) or phosphate-buffered saline (PBS). **(B)** mRNA expression of *Mmp14* in wild-type and MMP-10 knockout mice peritoneum treated with CG or PBS. WT/PBS, $n = 5$; KO/PBS, $n = 5$; WT/CG, $n = 8$; KO/CG, $n = 8$. Values are expressed as means $\pm$ SEM. * $P < 0.05$. KO, knockout mice; n.s., not significant; WT, wild-type mice.

Supplementary Figure S4. Expression of inflammatory cytokines in primary peritoneal macrophages with interleukin-6 (IL-6) stimulation. **(A)** The cell culture protocol of primary mesothelial cells from wild-type and matrix metalloproteinase-10 (MMP-10) knockout mice. **(B)** Expression of *Il1b* and *Tnfa* in primary peritoneal macrophages after 24-hour stimulation of IL-6 (10 ng/ml) or vehicle (0.1% bovine serum albumin [BSA]). Values are expressed as means $\pm$ SEM. ** $P < 0.01$. KO, knockout mice; n.s., not significant; WT, wild-type mice.

Supplementary Figure S5. Light microscopic, immunohistochemical and immunofluorescence images of mouse primary mesothelial cells. **(A)** Light microscopic images of mouse primary mesothelial cells showing cobblestone appearance. **(B)** Immunohistochemical staining of pan-cytokeratin for mouse primary mesothelial cells. **(C)** Immunofluorescent staining of pan-cytokeratin for mouse primary

mesothelial cells. Bar = 100 μ m. HPF, high-power field; LPF, low-power field.

Supplementary Figure S6. Expression of inflammatory cytokines in primary mesothelial cells with tumor necrosis factor- α (TNF- α) stimulation. **(A)** The cell culture protocol of primary mesothelial cells from wild-type and matrix metalloproteinase-10 (MMP-10) knockout mice. **(B)** Expression of *Mmp10*, *Ccl2*, *Il6*, and *Tnfa* in primary mesothelial cells after 2-hour stimulation of TNF- α (10 ng/ml) or vehicle. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$. KO, knockout mice; WT, wild-type mice.

Supplementary Figure S7. Expression of inflammatory cytokines in primary fibroblasts with lipopolysaccharide (LPS) stimulation. **(A)** The cell culture protocol of primary fibroblast from wild-type and matrix metalloproteinase-10 (MMP-10) KO mice. **(B)** Expression of *Mmp10*, *Ccl2*, *Il6*, and *Mmp9* in primary fibroblasts after 24-hour stimulation of LPS or phosphate-buffered saline (PBS). Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$. KO, knockout mice; n.s., not significant; WT, wild-type mice.

Supplementary Figure S8. Expression of FLAG-tagged matrix metalloproteinase-10 (MMP-10) in COS-7 transfected with human MMP-10-expressing vector. **(A)** The cell culture protocol of COS-7 cells transfected with human MMP-10-expression plasmid. **(B,C)** Western blot analysis of COS-7 cells after transfection of human MMP-10-expression plasmid or pCMV6 for FLAG and β -actin. **(D,E)** Western blot analysis of COS-7 cells after transfection of mouse MMP-10-expression plasmid or pCMV6 for FLAG and β -actin. Values are expressed as means $\pm$ SEM. * $P < 0.05$. pCMV6, empty plasmid; pHMMP10, plasmid expressing human MMP-10; pmMMP10, plasmid expressing mouse MMP-10.

Supplementary Figure S9. Gene and protein expression of human matrix metalloproteinase-10 (MMP-10) overexpression RAW 264.7 cells and MeT-5A cells. **(A)** The cell culture protocol for transfection of human MMP-10 (hMMP-10) expression plasmid to RAW 264.7 and MeT-5A cells. **(B)** Representative images of hMMP-10-overexpressing cells. Red color indicates MMP-10, and blue indicates 4',6-diamidino-2-phenylindole (DAPI). **(C)** Expression of *Ccl2*, *Il6*, *Il1b*, *Tnfa*, and *Ctgf* mRNA in RAW 264.7 cells with or without hMMP-10 overexpression. **(D)** Expression of *CCL2*, *IL6*, *IL1B*, *TNFA*, and *CTGF* mRNA in MeT-5A cells with or without hMMP-10 overexpression. **(E)** Western blot analyses of phosphorylated nuclear factor κ B (NF κ B) subunit p65 (S536), NF κ B subunit p65, and β -actin in RAW 264.7 cells with or without hMMP-10 overexpression. **(F)** Western blot analyses of MeT-5A cells with or without hMMP-10 overexpression. Bar = 50 μ m. Values are expressed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$. pCMV6, empty plasmid; pHMMP10, plasmid expressing human MMP-10.

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