PERIOSTIN: A MATRICELLULAR PROTEIN INVOLVED IN PERITONEAL INJURY DURING PERITONEAL DIALYSIS

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◆ **Background:** Periostin is a matricellular protein involved in tissue remodeling through the promotion of adhesion, cell survival, cellular dedifferentiation, and fibrogenesis. It can be induced by transforming growth factor beta and high glucose concentrations. We hypothesized that this protein might be expressed in the peritoneal cavity of patients on peritoneal dialysis (PD) and even more in patients with signs of encapsulating peritoneal sclerosis (EPS).

◆ **Method:** In this retrospective study, we included peritoneal biopsies from patients on PD with EPS ($n = 7$) and without signs of EPS ($n = 10$), and we compared them with biopsies taken during hernia repair from patients not on PD ($n = 11$) and during various procedures from uremic patients not on PD ($n = 6$). Periostin was localized by immunohistochemistry, scored semiquantitatively, and quantified by morphometry. Periostin protein concentrations were measured by ELISA in dialysates from 15 patients. Periostin messenger RNA was quantified *in vitro* in peritoneal fibroblasts.

◆ **Results:** In control biopsies, periostin was present in the walls of larger arteries and focally in extracellular matrix in the submesothelial zone. Patients on PD demonstrated interstitial periostin in variable amounts depending on the severity of submesothelial fibrosis. In EPS, periostin expression was very prominent in the sclerosis layer. The area of periostin was significantly larger in EPS biopsies than in control biopsies, and the percentage of periostin-positive area correlated with the thickness of the submesothelial fibrosis zone. Periostin concentrations in dialysate increased significantly with time on PD in patients without signs of EPS; in patients with EPS, periostin concentrations in dialysate were low and demonstrated the smallest increase with time. *In vitro*, periostin was found to be strongly expressed by peritoneal fibroblasts.

◆ **Conclusion:** Periostin is strongly expressed by fibroblasts and deposited in the peritoneal cavity of patients with EPS and with simple peritoneal fibrosis on PD. This protein might play a role in the progression of peritoneal injury, and low levels of periostin after prolonged time on PD might be a marker of EPS.

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During peritoneal dialysis (PD), exposure to dialysate (including high glucose, low pH, glucose degradation products) mediates peritoneal injury, with matrix expansion and angiopathy, resulting in so-called simple peritoneal fibrosis (1). This condition is an important cause of technique failure in patients on PD.

Encapsulating peritoneal sclerosis (EPS) is a rare but life-threatening complication of PD. The hallmark of EPS is thickening of the peritoneal membrane by layers of fibrin and fibrosis leading to encapsulation and bowel obstruction. Decreased appetite, weight loss, and signs of inflammation are early but nonspecific symptoms of EPS (2). Later, partial or complete bowel obstruction becomes clinically apparent. Computed tomographic

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imaging demonstrating peritoneal thickening, bowel tethering, peritoneal calcification, peritoneal enhancement, and loculated fluid collections is important for a diagnosis of EPS (3). The histologic picture of EPS is described as relatively nonspecific. Mesothelial denudation, hemorrhage, vasculopathy, calcification, peritoneal fibroblast swelling, interstitial fibrosis, angiogenesis with increased capillary numbers, and mononuclear cell infiltration have been described as typical (4,5). Fibrin deposits on the peritoneal membrane lead to adhesions and permanent scarring, which finally result in bowel obstruction (3).

Periostin was originally called osteoblast-specific factor 2 and was later renamed because of its intense expression in periosteum and periodontal ligament (6,7). It has a molecular weight of about 90 kDa (7) and mediates cell adhesion via the $\alpha_v\beta_3$ and $\alpha_v\beta_5$ integrins (8). Periostin is a profibrogenic molecule (9) that is involved in angiogenesis (10) and that has been shown to stiffen collagen matrices *in vitro* (11).

During embryogenesis, periostin is involved in the normal development of teeth (12), bones, and the heart (13,14). Mice deficient in periostin demonstrate multiple defects such as incisor enamel defects, an early-onset periodontal-disease-like phenotype, and dwarfism (15). Periostin can be re-expressed in adults under pathologic conditions—for example, in heart failure (16), during myocardial infarction (17), in muscle injury, and in various forms of human cancers [reviewed in Ruan *et al.* (18)]. Our group was able to demonstrate that periostin, which is constitutively expressed in the human glomerular mesangium (19), was *de novo* expressed in tubulointerstitial renal fibrosis and correlated with loss of renal function (20). Recently, increased concentrations of periostin were reported in uremic hearts (21), in rat kidneys with 5/6 nephrectomy, and in urine from patients with chronic kidney disease (22).

High concentrations of glucose and transforming growth factor β (TGF- β) were found to induce periostin (7,23,24), and the presence of those two factors is well described during peritoneal injury related to PD and in patients with EPS (25–27). Periostin might also be involved in the propagation of cellular dedifferentiation, epithelial-to-mesenchymal transition, fibrosis, and angiogenesis in patients on PD. We therefore set out to localize periostin in peritoneal biopsies from patients on PD with EPS and without signs of EPS. We also quantified periostin protein levels in effluent from a second PD patient population. Finally, we quantified the expression of periostin *in vitro* in human peritoneal fibroblasts.

METHODS

STUDY POPULATION

Biopsies from parietal peritoneum were formalin-fixed and paraffin-embedded using routine protocols (28). The study included biopsies from patients on PD without signs of EPS ($n = 10$) and patients on PD with a clinical diagnosis of EPS ($n = 7$, Table 1). Biopsies from a second series of uremic patients, taken at the time of catheter implantation before the start of PD, were also studied ($n = 6$). Control biopsies were taken at the time of hernia repair from patients not on PD ($n = 11$).

Biopsies from patients on PD were obtained at the time of catheter implantation, removal, or correction of a dislocation, or at the time of any abdominal surgery (for example, hernia repair). Patients with an episode of peritonitis within the preceding 6 months were excluded. Biopsies from patients with EPS were taken at the time of peritonectomy. Clinically, all patients with EPS were at a very late stage of the disease, with recurrent abdominal pain caused by chronic bowel obstruction (Table 2). All patients gave informed consent regarding a scientific work-up of tissues taken during routine procedures.

IMMUNOHISTOCHEMISTRY

Immunohistochemistry was performed as previously described (29,30). In brief, paraffin-embedded tissue sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide (to block endogenous peroxidases). Antigen retrieval was performed in HCl (pH 0.9) heated by microwaving for 10 minutes (30). The primary antibody was applied for 1 hour. Incubation with biotinylated secondary reagents (Vector, Burlingame, CA, USA) for 30 minutes was followed by ABC reagent (Vector). As a detection system, we used 3,3'-diaminobenzidine (Sigma, Taufkirchen, Germany) with metal enhancement (resulting in a black-colored product). Nuclei were counterstained with methyl green.

A polyclonal anti-human periostin antibody was used (Biovendor, Heidelberg, Germany). As controls, we used sections from an allograft nephrectomy, including replacement of the antibody by diluent or by nonimmune serum [Figure 1(A,B)]. The latter controls did not demonstrate positive staining [Figure 1(B)]. Antigen retrieval at low pH reduces the nuclear counterstain and therefore nuclei appear “empty.” All sections (from controls, PD patients, and EPS patients illustrated in the quantitative figures) were evaluated by an observer blinded to the clinical diagnosis. Sites of positive staining were described, and a semiquantitative scoring system

TABLE 1
Clinical Data for the Biopsy Population

Variable	Normal peritoneum	Study group		
		Uremia	PD	EPS
Biopsies (n)	11	6	10	7
Mean age of subjects (years)	58.6±12.7	60.5±9.2	49.9±15.6	54.3±15.4
PD duration (months) ^a	—	—	21.3±26.1	70.6±34.1
Peritonitis (episodes/months at risk)	—	—	6/192	11/494
PD fluid				
Neutral	—	—	1	5
Acidic	—	—	1	1
Both or ND	—	—	5	4
Icodextrin (n)	—	—	1	5
Diabetes (n)	—	2	3	3
Smokers	—	1	1	3
PTH [pmol/L (normal: 1.1 – 7.3)]	ND	45.4±24.3	14.1±9.5	45.0±31.6
Urea nitrogen [mg/dL (normal: 10 – 25)]	ND	167.3±24.7	61.4±30.6	39.3±10.6
Creatinine [mg/dL (normal: 0.5 – 1.4)] ^a	0.92±0.31	7.3±1.67	7.69±3.77	6.99±2.17

PD = peritoneal dialysis; EPS = encapsulating peritoneal sclerosis; ND = not determined; PTH = parathyroid hormone.

^a $p < 0.01$.

TABLE 2
Clinical Findings, Laboratory Values, and Peritoneal
Dialysis (PD) Characteristics in Seven Patients
with Encapsulating Peritoneal Sclerosis

Variable	Incidence [n (%)]
Clinical findings	
Pain	7 (100)
Diarrhea	1 (14)
Malnutrition	4 (57)
Ascites/hemoperitoneum	4 (57)
Bowel obstruction	7 (100)
On tamoxifen or immunosuppressants	0 (0)
Laboratory values	
C-Reactive protein > 0.5 mg/dL	5 (71)
PD characteristics	
Transport status	2 High (29) 4 High-average (57) 1 ND (14)

ND = not determined or peritoneal equilibration test more than 6 months in the past.

was used (0 = no or faint focal staining of extracellular matrix; 1 = prominent but focal staining of extracellular matrix; 2 = diffuse staining of extracellular matrix).

The slides stained for periostin by immunohistochemistry were scanned using the Mirax Midi digital slide scanner (Carl Zeiss MicroImaging, Jena, Germany), and

the area of periostin positivity was quantified using the HistoQuant module of the Mirax Viewer software (release 1.12: Carl Zeiss MicroImaging). The positive area was expressed as a percentage of the evaluated area.

The submesothelial fibrotic zone was measured using the Leica Application Suite (version 3.4.0: Leica Microsystems, Heerbrugg, Switzerland) in combination with a Leica Microscope (Leica DMRXA2). To illustrate the association with extracellular matrix, consecutive sections were stained with Masson–Goldner trichrome, or sirius red, or both.

DOUBLE IMMUNOFLUORESCENCE

Double immunofluorescence was performed for periostin and alpha-smooth muscle actin (α -SMA). The monoclonal antibody for α -SMA (M0851: Dako, Glostrup, Denmark) was visualized with a Cy3-labeled secondary antibody (Jackson ImmunoResearch, Suffolk, UK). Periostin was visualized with a biotinylated secondary antibody and labeled with FITC bound to streptavidin (Vector). Negative controls consisted of isotype immunoglobulins or nonimmune serum on renal allografts (not illustrated).

STUDIES ON PERITONEAL FIBROBLASTS *IN VITRO*

The isolation and characterization of peritoneal fibroblasts has previously been described in detail (31). Briefly, human peritoneal fibroblasts were isolated

Figure 1 — Periostin in control tissue. Immunohistochemistry (A,C,E) was performed using a polyclonal antiserum against periostin on tissue sections (A,B) from an allograft nephrectomy and (C–F) from a peritoneal biopsy obtained at the time of hernia repair (original magnification 200 $\times$). (A) Note the prominent presence of periostin in areas of interstitial fibrosis (arrowhead); (B) no black color product was present when the primary antibody was replaced with non-immune rabbit serum (negative control). (C) In the biopsies, the thin layer of submesothelial fibrotic tissue was commonly negative for periostin (arrowhead), but focal staining for periostin was present within the interstitial tissue (arrow). (E,F) Smooth-muscle cells of arteries in the biopsies were positive for periostin [representative view of same specimen as (C,D)]. (D,F) Consecutive sections of the peritoneal biopsy stained with sirius red.

from specimens of omentum by enzymatic digestion. Cells were grown to confluence and rendered quiescent in serum-free medium for 48 hours. Cells were then exposed to glucose (75 mmol/L) or mannitol (75 mmol/L) for 12 hours, 1 day, 2 days, or 4 days. In a second experiment, the cells were stimulated with tumor necrosis factor α (TNF- α) 1 ng/mL or interleukin 1 β (IL-1 β) 1 ng/mL for at least 6 hours and up to 3 days. At designated time intervals, total RNA was isolated using RNeasy. Reverse transcription was performed using 1 μ g total RNA in a reaction volume of 20 μ L. Expression of periostin messenger RNA (mRNA) was

quantified by real-time reverse-transcription polymerase chain reaction as previously described (29,32). Pre-developed TaqMan reagents were used for human periostin and the housekeeping genes GAPDH and 18S rRNA (Applied Biosystems, Darmstadt, Germany). Expression of mRNA was analyzed using the delta delta Ct method (25).

QUANTIFICATION OF PERIOSTIN IN DIALYSATE

An ELISA was established to quantify periostin in dialysate, as previously described in Ben *et al.* (33). A

96-well plate was coated overnight at 4°C with 1 µg/mL (0.1 µg per well) monoclonal anti-periostin antibody (MAB2955: R&D Systems, Minneapolis, USA) diluted in 0.05 mol/L carbonate-bicarbonate (C3041: Sigma). After a blocking step with solution containing 3% phosphate-buffered saline with bovine serum albumin for 1 hour at room temperature, undiluted samples were added to the wells for 2 hours at room temperature. A rabbit polyclonal antibody to periostin (Ab14041: Abcam, Cambridge, UK) diluted 1:500 in phosphate-buffered saline was added to the plate for 1 hour at room temperature, followed by a horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin [1:500 dilution in phosphate-buffered saline (BD Biosciences, Allschwil, Switzerland)]. As a detection substrate, we used 3,3',5,5'-tetramethylbenzidine (555214: BD Biosciences), and the reaction was terminated by the addition of 1 N sulfuric acid. Absorbance was measured at 450 nm with an ELISA reader (Infinite 200 Pro: Tecan Schweiz, Männedorf, Switzerland). For determination of periostin levels, a standard curve with diluted recombinant periostin (3548-F2: R&D Systems) at concentrations ranging from 3.125 ng/mL to 50 ng/mL was used. Negative controls included incubation of wells with phosphate-buffered saline or solution containing 3% phosphate-buffered saline with bovine serum albumin.

Samples from 15 patients were available for the study. Patients with fast transport status ($n = 5$) and with low or low-average transport status ($n = 5$) were included. Transport status was assessed during a modified peritoneal equilibration test, using a 3.86% glucose-based dialysate for a 4-hour dwell 4–6 weeks after PD start. Five patients who developed EPS were included. For each patient, the first sample after the start of PD, and the last sample before the patient stopped PD were evaluated. In 5 patients (2 with fast transport status and 3 with EPS), intermediate samples during the time course were available.

STATISTICAL ANALYSIS

An observer blinded to the diagnosis completed the semiquantitative scoring of the periostin staining. For comparisons of means, the nonparametric Kruskal-Wallis and Dunn multiple comparison tests were used. Spearman rank correlations were performed for correlations with the thickness of the submesothelial area (InStat software, version 3.05: Intuitive Software for Science, San Diego, CA, USA). A value of $p < 0.05$ was considered significant. Error bars demonstrate the standard error of the mean.

RESULTS

Table 1 summarizes the clinical data for the patient cohort.

PERIOSTIN EXPRESSION

Control Tissue: A total of 11 control biopsies taken during hernia repair in patients not on PD were included [Figure 1(C–F)]. These tissue samples consisted of a thin submesothelial layer covering the fat tissue, with very little interstitial tissue and larger vessels [Figure 1(C,D)]. Periostin was commonly absent from the thin submesothelial area [Figure 1(C,D)]. Faint staining was focally present in some biopsies [Figure 1(C)]. In contrast, constitutive expression of periostin was present in the walls of arteries in the interstitial tissue [Figure 1(E,F)]. In the group of control biopsies, the blinded scoring resulted in a mean score of 1.1 [range: 0–2; Figure 2(A)]. The percentage periostin-positive area (including positive vessel walls) was 15% [range: 7%–33%; Figure 2(B)].

Tissue from Patients on PD Without Signs of EPS: Ten biopsies from patients on PD were included. The severity of fibrosis varied between the biopsies, with a mean submesothelial fibrotic thickness of 188 µm (range: 59–599 µm). Periostin was found to be present in the extracellular matrix and the arterial walls. Figure 3 illustrates biopsies with increasing severity of peritoneal fibrosis, from early lesions [Figure 3(A–D)], to lesions of moderate severity [Figure 3(E,F)], to a severe form [Figure 3(G,H)]. Thickness of the periostin-positive layer and percentage of the periostin-positive area increased with the severity of fibrosis (Figure 3).

The presence of periostin ranged from no or minimal staining of extracellular matrix (score 0), to focal staining (score 1), to diffuse presence of periostin [Figure 3(G,H)]. The mean score was 1.3 [range: 0–2; Figure 2(A)]. The percentage of periostin-positive area (including positive vessel walls) was 19% [range: 5%–46%; Figure 2(B)]. There was also a morphologic correlation, though nonsignificant, between the severity of submesothelial thickness and periostin staining. The mean percentage of periostin-positive area in peritoneal biopsies from uremic patients who had not started dialysis was 17% (range: 6%–27%). That value was not significantly different from those of the other groups studied.

Tissue from PD Patients With EPS: Figure 4 illustrates the 7 biopsies from patients on PD with clinical signs of EPS. The mean thickness of the sclerotic area was 760 µm (range: 418–1250 µm; $p = 0.001$ vs controls, $p = 0.05$).

Figure 2 — Quantification of periostin staining. (A) Periostin was scored semi-quantitatively in a blinded fashion as described in the Methods section. Mean scores for the study groups are shown (error bars illustrate the standard error of the mean; n.s. = nonsignificant; PD = peritoneal dialysis; EPS = encapsulating peritoneal sclerosis). (B) Percentage of periostin staining in the submesothelial area for the study groups quantified by morphometry. (C) Percentage periostin-positive area by tertile of submesothelial thickness. (D) Correlation between submesothelial thickness (in micrometers) and the percentage periostin-positive area for the study groups.

vs PD patients). All biopsies demonstrated strong and diffuse staining of the sclerotic zone [Figure 4(A,C)]. The periostin staining appeared in layers of the sclerotic zone [Figure 4(A,B)]. The periostin-positive sclerotic zone was commonly covered by a periostin-negative layer [Figure 4(C,D)]. Entire cross-sections through the thick sclerotic membranes were impressively positive for periostin. By immunohistochemistry, periostin-negative fibroblasts were seen as a negative contrast [Figure 4(E)]. All 7 biopsies were scored as 2 (diffuse periostin staining), which was significantly different compared with the scoring for the controls [Figure 2(A)]. The mean percentage of periostin-positive area in peritoneal biopsies from patients with EPS was 30% [range: 15% – 41%; Figure 2(B)]. The morphometric quantification of periostin demonstrated a significant increase in the percentage of periostin-positive area compared with that in control biopsies [Figure 2(B)]. Using double immunofluorescence (Figure 5), the α -SMA-positive fibroblasts were embedded in the periostin-positive extracellular matrix [Figure 5(G–I)].

ASSOCIATION BETWEEN PERIOSTIN AND SUBMESOTHELIAL FIBROSIS

Figure 3 describes the morphologic association between submesothelial thickness and periostin staining

for patients on PD. The mean percentage of periostin-positive staining increased with the severity of submesothelial fibrosis, illustrated in Figure 2(C) for the tertiles of submesothelial thickness (the figure includes all biopsies). Although the number of biopsies included in the present study was low, a significant correlation was observed between the thickness of the submesothelial fibrotic zone and periostin staining as reflected by the semiquantitative score or the percentage of positive area [Figure 2(D)]. A strong overlap was observed between the controls and the PD group [Figure 2(D), left side]. The EPS group had little overlap in thickness [Figure 2(D), right side], but there was some overlap in the percentage of periostin positivity.

PERITONEAL FIBROBLASTS

To further study the expression of periostin by peritoneal fibroblasts, *in vitro* studies were performed using primary peritoneal fibroblasts. Expression of periostin was quantified by real-time reverse-transcription polymerase chain reaction. Prominent expression of periostin mRNA could be documented with threshold cycles (Ct) between 23 and 26 (GAPDH: 19 – 23 Ct). High osmolarity [Figure 6(A)] and exposure to cytokines [Figure 6(B)] did not result in significant regulation of periostin.

Figure 3 — Periostin in peritoneal dialysis (PD) patients. Peritoneal biopsies from PD patients were stained with (A,B,C,E,G) a polyclonal antibody against periostin, (D,H) Sirius red, and (F) Masson trichrome. Original magnification (A,C–H) 200 $\times$ and (B) 630 $\times$. (A–D) These two biopsies come from the first tertile of submesothelial thickness [described in Figure 2(C)] and demonstrate early peritoneal changes, with periostin staining of the submesothelial sclerotic zone. (E) A moderate form of peritoneal fibrosis [second tertile of submesothelial thickness, indicated by the arrowheads in (A,C,E)]. (G) A severe form of peritoneal fibrosis (third tertile of submesothelial thickness). Note that the basal end of the sclerotic zone is no longer visible at this magnification. Note the prominent increase in the periostin-positive area with the progression of peritoneal fibrosis.

PERIOSTIN QUANTIFICATION IN EFFLUENT

Effluent samples from 15 patients were available for quantification of periostin [Table 3, Figure 6(C)]. For each patient, the first sample available after the start

of PD and the last sample before PD withdrawal were included. In patients without EPS, a significantly higher concentration of periostin was present in the last PD effluent than in the initial effluent. Figure 6(C) illustrates periostin protein concentration normalized for its

Figure 4 — Periostin in peritoneal dialysis (PD) patients with encapsulating peritoneal sclerosis (EPS). (A,C,E) Immunohistochemistry was performed using a polyclonal antiserum against periostin. Original magnification (A,C) 200 $\times$ and (E) 630 $\times$. Consecutive sections were stained with (B) sirius red or (D) Masson trichrome. Original magnification (D) 200 $\times$ and (F) 630 $\times$. (A,C,E) In all biopsies from patients with EPS, extracellular matrix demonstrated positive severe diffuse staining for periostin. The mesothelial cell layer was absent [arrowhead in (A)]. (A,B) Areas of fibrosis correlated with periostin-positive areas. (E) Fibroblasts and vessels were embedded in a periostin-positive extracellular matrix (arrowhead), therefore appearing in negative contrast (arrow).

concentration in the first effluent in patients without EPS ($p < 0.01$). Low concentrations of periostin were found in the last available effluent from EPS patients [Table 3, Figure 6(C)]. Intermediate samples from 5 patients (2 with fast transport status, and 3 with EPS) were available. The 3 patients with EPS demonstrated only a small nonsignificant rise in effluent periostin concentration (without a significant peak during the time course).

DISCUSSION

The mechanisms and factors underlying so-called simple peritoneal fibrosis and EPS are under intense investigation, but are still incompletely understood (27). Here, we describe a relatively new matricellular protein that is prominently expressed in both lesions. The localization to sites of fibrosis results in a positive association with the

Figure 5 — Double immunofluorescence for periostin and smooth muscle actin (SMA). Double immunofluorescence was performed using (A,D,G) a polyclonal antiserum against periostin and (B,E,H) a monoclonal antibody against SMA on tissue sections from peritoneal biopsies taken (A–C) during hernia repair, (D–F) from patients on PD, and (G–I) from PD patients with encapsulating peritoneal sclerosis (EPS). (C,F,I) Immunofluorescence overlay. All images, original magnification 400 $\times$. (A–C) In the control tissue, no positive staining for periostin was evident (arrowhead localizes the tissue border). Prominent staining for periostin (arrow) was present (D) in a patient on PD and (G) in a PD patient with EPS. (E,H) Fibroblasts positive for SMA (red) are embedded in the periostin-positive matrix (arrowheads).

thickness of the submesothelial fibrotic area. Periostin protein localized by immunohistochemistry does not discriminate between EPS and simple fibrosis, but it might be involved in the pathogenesis of both lesions. Only in EPS biopsies was the area of periostin staining significantly increased compared with staining in controls. Biopsies with increased submesothelial fibrosis demonstrated

larger areas of periostin staining in patients on PD. On the other hand, periostin in effluent increased with time on PD, but patients with EPS demonstrated low effluent periostin concentrations at the time of EPS diagnosis. Based on our data and the current literature, several hypotheses concerning the role of periostin in peritoneal tissue remodeling can be advanced.

Figure 6 — Quantification of periostin *in vitro* and *in vivo* in effluent. Periostin messenger RNA (mRNA) expression in peritoneal fibroblasts *in vitro* after exposure (A) to glucose or (B) to cytokines as described in the Methods section. Although periostin was prominently expressed by peritoneal fibroblasts, no regulation was detectable under the conditions tested. (C) Periostin was quantified by ELISA in the first and last effluent available from 15 patients. The graph illustrates the factor by which periostin was induced in effluent, normalized to the concentration in the first effluent from patients without encapsulating peritoneal sclerosis (EPS). Significant induction was found in the last available effluent sample from patients without EPS ($p < 0.01$). Patients with EPS demonstrated a low level of periostin in the last effluent sample available (n.s. = nonsignificant).

Simple peritoneal fibrosis and EPS have, in part, overlapping morphology, because EPS develops in long-term PD patients with simple fibrosis. In both processes, the

typical features in which periostin might be involved are fibroblast activation with extracellular matrix formation, angiogenesis, and epithelial-to-mesenchymal

TABLE 3
Clinical Characteristics of the Study Population for Periostin in Effluent

Variable	Pts (n)	Sex (female)	Age (years)	Time on PD (months)	Protein	Periostin Induction factor
High transport	5	1	66 (51–80)	31.9 (23–44)	1±0.26	4.9±3.6
Low transport	5	4	54 (28–81)	41.3 (11–80)	1±0.1	7±2.7 ^b
EPS	5	1	51 (39–62)	61.5 (34–101)	1±0.15	1.6±0.19
All	15	6	57 (28–81)	44.9 (11–101)	1±0.1	4.5±1.7 ^c

PD = peritoneal dialysis; EPS = encapsulating peritoneal sclerosis.

^a Values expressed as mean and range for age and time on peritoneal dialysis; periostin protein and factor for induction of the protein (± standard error of the mean) in effluent normalized to first effluent for the corresponding group; the “low transport” group combines low and low-average transporters.

^b $p < 0.05$ compared with first effluent.

^c $p < 0.001$.

differentiation (27). Periostin staining did not help to distinguish the lesions, but all patients with EPS demonstrated severe diffuse deposition of periostin. Because time on PD was longer in the EPS group, we cannot definitively determine whether the increased periostin expression was a result of the pathogenesis of EPS or simply a reflection of longer time on PD with more prominent lesions of simple fibrosis. We have to acknowledge here the main limitation of our study, which is its small sample size and an absence of significance for both scored and measured periostin protein in biopsies from PD patients.

Transforming growth factor β has been described to be induced during PD and is a key factor in peritoneal fibrosis and fibroblast activation (27). This growth factor directly induces formation of extracellular matrix, but it also induces expression of periostin (7). The prominent expression of periostin in biopsies from patients on PD and with EPS is therefore consistent with data describing induction of TGF- β in PD and EPS. On the other hand, periostin can, *per se*, induce TGF- β expression (11). A vicious cycle can easily be envisioned in which induction of periostin might promote ongoing formation of active TGF- β . Additionally, periostin promotes collagen fibrillogenesis and crosslinking and leads to stiffening of the matrix (11). The expression of matrix metalloproteinase 2 (MMP2), a tissue remodeler, was found to be increased by periostin (34). The presence of MMP2 in PD effluents (35,36) and in experimental peritoneal fibrosis (37) has been described. Expression of MMP2 might therefore be a downstream event. These complex interactions can now be evaluated in future studies.

Angiogenesis and vasculopathy are prominent findings in peritoneal fibrosis associated with PD and might be the driving force in technique failure (38). Vascular

endothelial growth factor A (VEGFA) has been shown to be important in that process (38,39). Tumors promote angiogenesis through the release of periostin (40). Periostin mediates angiogenesis through an integrin-dependent induction of vascular endothelial growth factor receptor 2 expression (40). Periostin in the peritoneum might therefore modulate the response of endothelial cells to VEGFA.

The transformation of epithelial cells to cells with fibroblastic appearance during PD has recently attracted much attention (38,41). This transformation might be a source for fibroblasts, the major contributors to extracellular matrix production. Periostin has been shown to promote cellular dedifferentiation and transformation [reviewed in Ruan *et al.* (18)]. Those three processes (dedifferentiation, extracellular matrix deposition, and angiogenesis) show complex interaction because TGF- β is a major mediator of cellular transformation, and mesothelial cells undergoing dedifferentiation demonstrate increased expression of growth factors such as VEGFA (38). Further studies in human and animal models, including knockout mice, are necessary to illuminate the exact role of this protein in the pathogenesis of peritoneal fibrosis during PD.

With time on PD, the amount of periostin in dialysate increased significantly in patients with simple fibrosis. Consistently, the area of periostin staining was associated with submesothelial thickness (also, the mean percentage of periostin in PD patients was not significantly different from that in control subjects). Our first hypothesis, that periostin might be regulated by osmolarity or cytokines (or both) was not confirmed by our *in vitro* experiments. Rather, we found prominent expression, an association with tissue fibrosis, and the presence of myofibroblasts. The increased number of

Figure 7 — Hypothetical schema for periostin capture in the peritoneal membrane in encapsulating peritoneal sclerosis (EPS). (A) The normal mesothelial cell layer with some periostin positivity in the submesothelial tissue and the vessel wall. (B) The situation of peritoneal fibrosis during peritoneal dialysis (PD), with loss of mesothelial cells, thickening of the submesothelial zone, increasing deposition of periostin in the extracellular matrix, and a significant amount of periostin in the peritoneal fluid. (C) The situation in EPS, with complete loss of mesothelial cells and formation of a fibrin layer on the mesothelial surface (shown in gray). Deposition of periostin is prominent in the sclerotic zone and sclerotic vessels, but the peritoneal fluid contains only a little periostin, which might be a result of either periostin trapping by the submesothelial extracellular matrix or capture of periostin by the fibrin-positive layer, or both.

fibroblasts might therefore be the main cause of the increased deposition of periostin, rather than increased expression per fibroblast.

An unexpected finding was the low level of periostin in effluent from patients with EPS [Figure 6(C)]. Morphologically, periostin is prominently present in the thick areas of sclerosis found in EPS [Figure 7(C)]. Morphologic features that might help to differentiate EPS from simple fibrosis are deposition of fibrin and increased thickness of the degenerative compact zone (42). Periostin might find its way into effluent in patients on PD without EPS, but not in patients with signs of EPS [Figure 7(B,C)]. Comparing the first and last effluents during the time course of dialysis, patients with EPS demonstrated the smallest increase in periostin. That finding might be related either to the thick deposition of fibrin present on the peritoneal surface or to periostin being “trapped” by the matrix in the compact zone. To determine whether periostin could be a biomarker for EPS development, future experiments should include more longitudinal measurements of periostin in effluent from long-term PD patients at risk for EPS. We had multiple effluent samples only from 3 patients with EPS, and periostin levels were low throughout the time course in those patients.

CONCLUSIONS

Here, we present the first data that localize periostin in the lesions of simple peritoneal fibrosis and EPS in patients on PD. Our hypothesis is that, during peritoneal exposure to PD solutions, induction of TGF- β stimulates fibroblast proliferation and the production

and deposition of periostin. The periostin might promote ongoing matrix expansion (fibrosis), angiogenesis, and cellular transformation, resulting in technique failure. That sequence of events is supported by a higher periostin concentration with prolonged time on PD. This hypothesis can now be addressed with *in vitro* and animal models. Finally, periostin might be a suitable therapeutic target to prevent peritoneal remodeling in PD patients.

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DISCLOSURES

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