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Decorin inhibits Epithelial-to-mesenchymal Transition and Fibronectin Synthesis in Peritoneal Mesothelial Cells

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INTRODUCTION

Peritonitis is an important cause of peritoneal membrane failure in patients on peritoneal dialysis (PD) (1). Decorin is a dermatan sulfate proteoglycan that possesses anti-fibrotic properties (2). Its role in peritoneal fibrosis remains to be fully defined. This study examined the relationship between dialysate levels of decorin and pro-inflammatory and fibrotic mediators in patients with PD associated peritonitis, and investigated the role of decorin in mesothelial cell fibrogenesis.

METHODS

- Serial aliquots (50ml) of PD fluid were collected from 43 patients with PD-related peritonitis. Dialysate samples from 20 PD patients who were peritonitis-free in the past 12 months were included as controls.
- Dialysate concentrations of decorin, TGF-β1, IL-1β, IL-6 and IL-8 were measured using commercially available ELISAs.
- Mesothelial cells were obtained from omental specimens by enzymatic digestion as previously described (3) and cultured in Medium-199 supplemented with 10% FCS.
- All experiments were conducted in mesothelial cells of the second passage that had been growth arrested for 72h. Cultured mesothelial cells were incubated with spent PD fluid in the presence or absence of exogenous decorin (1μg/ml), and the expression of SNAIL and fibronectin determined by Western blot analysis.

RESULTS

- Dialysate decorin level was significantly higher at the onset of peritonitis compared to non-peritonitis dialysate ($P<0.05$) (Figure 1).
- Decorin level peaked 3 days after the onset of peritonitis and its level at 3 months remained significantly higher than that in non-peritonitis patients ($P<0.05$) (Figure 1).

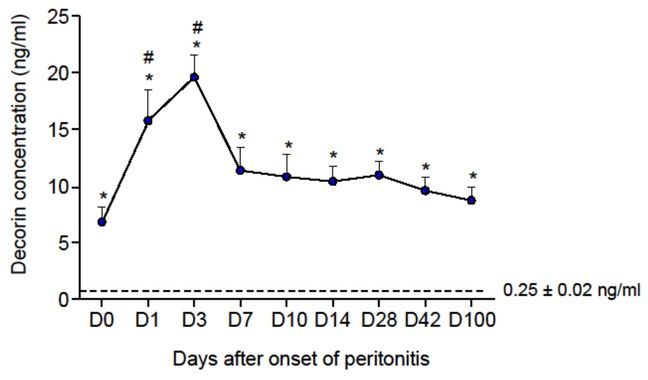

Figure 1. Decorin levels in spent dialysate

*Decorin concentrations were measured in longitudinal spent dialysate from 43 PD patients with bacterial-complicating peritonitis. Dash line represents mean decorin concentration in PD patients who were peritonitis-free for at least 12 months. Data presented as mean ± SEM. *P<0.001, compared to peritonitis-free PD fluid, #P<0.01, compared to onset of peritonitis (D0).*

- Dialysate decorin level showed a positive correlation with TGF-β1 (Figure 2A), and an inverse correlation with dialysate IL-1β, IL-6 and IL-8 levels (Figure 2B-D).

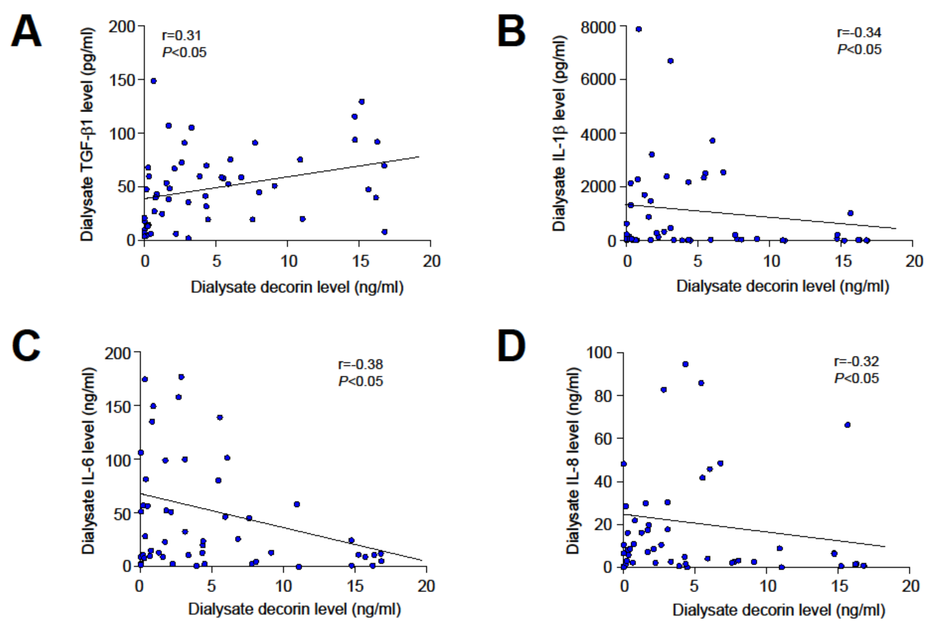

Figure 2. Correlation of dialysate decorin levels with TGF-β1, IL-1β, IL-6 and IL-8 levels at the onset of peritonitis (D0)

- Spent PD fluid at the onset of peritonitis induced phenotypic changes in mesothelial cells with a loss of their epithelial morphology and the acquisition of fibroblastic features (Figure 3).
- Phenotypic changes in mesothelial cells were accompanied by increased fibronectin and SNAIL synthesis (Figure 4).
- Exogenous decorin improved mesothelial cell morphology (Figure 3) and decreased fibronectin and SNAIL synthesis (Figure 4), mediated in part though reduced p38 MAPK activation (Figure 5).

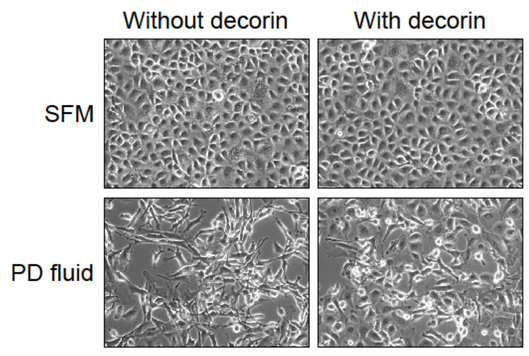

Figure 3. Peritonitis PD fluid induced EMT in mesothelial cells

Mesothelial cells were incubated with serum free medium (SFM) or spent peritonitis PD fluid (D0) for 24h, and cell morphology assessed by phase contrast microscopy. Representative images are shown. Original magnification x200.

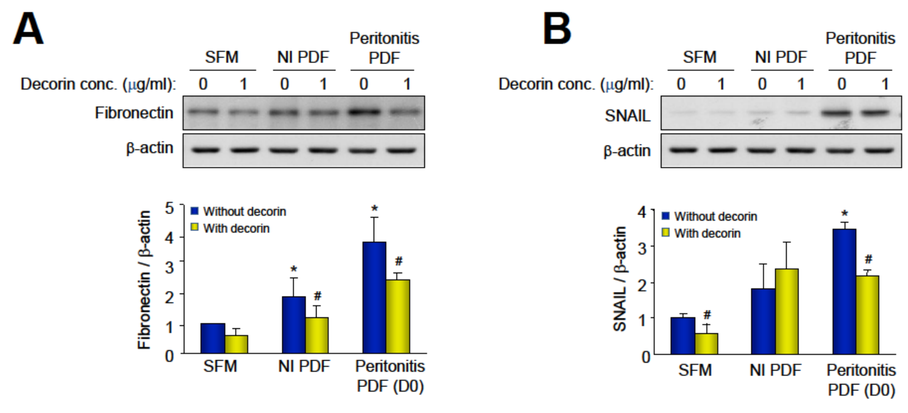

Figure 4. Peritonitis PD fluid induced fibronectin and SNAIL synthesis

*Representative Western blots showing the effect of serum free medium (SFM), non-infected PD fluid (NI PDF) and peritonitis PD fluid with or without decorin on the synthesis of (A) fibronectin or (B) SNAIL (upper panels). The intensity of the bands were normalized to β-actin (bottom panels). *P<0.05, compared to SFM, #P<0.05, with vs without decorin for the same stimulation.*

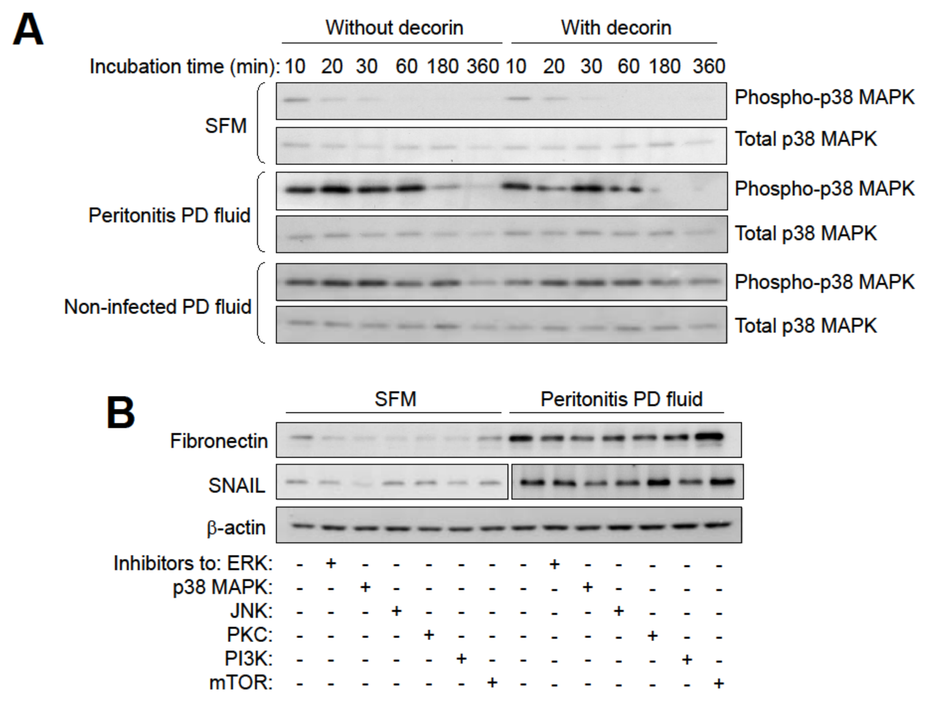

Figure 5. The effect of PD fluid and decorin on p38 MAPK activation, and signaling pathways involved in fibronectin and SNAIL synthesis

Representative Western blots showing (A) the effect of SFM or PD fluid in the presence or absence of decorin on p38 MAPK activation, and (B) the effect of specific inhibitors to MAPK, PKC, PI3K and mTOR on fibronectin and SNAIL synthesis. Results show that p38 MAPK mediates at least in part, synthesis of both fibrotic markers.

CONCLUSIONS

Our data suggest a beneficial role for decorin in ameliorating peritonitis-mediated fibrotic processes in peritoneal mesothelial cells.

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