Inhibiting core fucosylation attenuates glucose-induced peritoneal fibrosis in rats

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Ultrafiltration failure is a major complication of long-term peritoneal dialysis, resulting in dialysis failure. Peritoneal fibrosis induced by continuous exposure to high glucose dialysate is the major contributor of ultrafiltration failure, for which there is no effective treatment. Overactivation of several signaling pathways, including transforming growth factor-β1 (TGF-β1) and platelet-derived growth factor (PDGF) pathways, contribute to the development of peritoneal fibrosis. Therefore, simultaneously blocking multiple signaling pathways might be a potential novel method of treating peritoneal fibrosis. Previously, we showed that core fucosylation, an important posttranslational modification of the TGF-β1 receptors, can regulate the activation of TGF-β1 signaling in renal interstitial fibrosis. However, it remains unclear whether core fucosylation affects the progression of peritoneal fibrosis. Herein, we show that core fucosylation was enriched in the peritoneal membrane of rats accompanied by peritoneal fibrosis induced by a high glucose dialysate. Blocking core fucosylation dramatically attenuated peritoneal fibrosis in the rat model achieved by simultaneously inactivating the TGF-β1 and PDGF signaling pathways. Next the protective effects of blocking core fucosylation and imatinib (a selective PDGF receptor inhibitor) on peritoneal fibrosis were compared and found to exhibit a greater inhibitory effect over imatinib alone, suggesting that blocking activation of multiple signaling pathways may have superior inhibitory effects on the development of peritoneal fibrosis. Thus, core fucosylation is essential for the development of peritoneal fibrosis by regulating the activation of multiple signaling pathways. This may be a potential novel target for drug development to treat peritoneal fibrosis.

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Peritoneal dialysis (PD) is an effective and convenient renal replacement treatment for patients with end-stage renal disease.[1–3] However, ultrafiltration failure due to peritoneal fibrosis is a major cause of PD failure.[4–8] Although glucose dialysate is the most widely used solution around the world, it is the main contributing factor in the induction of peritoneal fibrosis in patients undergoing long-term PD treatment.[9–12] The underlying pathophysiological mechanisms of glucose dialysate–mediated peritoneal fibrosis are not fully elucidated and remain unresolved,[13,14] and there are few effective methods of treating peritoneal fibrosis once it develops. Recent studies have shown that alanyl-glutamine can ameliorate PD-induced peritoneal damage partially by modulating interleukin 17 expression in animal models.[4] Moreover, gefitinib was also found to attenuate high glucose-induced peritoneal fibrosis in rats by inhibiting epidermal growth factor receptors.[15] However, new targets for protecting the peritoneal membrane against fibrosis are urgently required.

The overactivation of several key cell signaling pathways have been shown to promote the progression of peritoneal fibrosis, particularly transforming growth factor-β1 (TGF-β1)[16–18] and platelet-derived growth factor (PDGF) pathways.[19–21] Blocking TGF-β1 signaling was also found to protect the peritoneal membrane from glucose dialysate–induced peritoneal damage,[17] and blocking PDGF signaling also attenuated peritoneal fibrosis.[15] However, because multiple signaling pathways are involved in peritoneal fibrosis, blocking 1 signaling pathway may not be sufficient for preventing peritoneal fibrosis. Thus, we hypothesized that simultaneously inactivating multiple signaling pathways might provide more effective protection against peritoneal fibrosis. We sought methods by which we could block multiple cell signaling pathways by inhibiting 1 target. Emerging studies have suggested that the posttranslational modification of

proteins can alter their function and affect the activation of multiple signaling pathways. Core fucosylation is an important posttranslational modification of proteins,[22–24] and it has been established that some important proteins involved in the epithelial-mesenchymal transition and inflammation are modified by core fucosylation (e.g., TGF-β1 and PDGF receptors).[25–29] Our previous work demonstrated that reducing core fucosylation ameliorated the progression of both renal and pulmonary fibrosis.[28,29] Because the TGF-β1 and PDGF signaling pathways are 2 major pathways involved in promoting peritoneal fibrosis, we hypothesized that blocking core fucosylation may inactivate these signaling pathways and ameliorate peritoneal fibrosis more efficiently. Our recent study has shown that blocking core fucosylation could ameliorate the rat peritoneal mesothelial cell epithelial-mesenchymal transition induced by a high glucose solution *in vitro*.[30] Thus, in this study, we further detected the expression of core fucosylation in the peritoneal membrane of rats with peritoneal fibrosis. We then investigated the effects of inhibiting core fucosylation on the development and progression of peritoneal fibrosis, and finally compared the protective effects of core fucosylation inhibition with imatinib (an available selective PDGF receptor inhibitor). Our data indicate that inhibiting core fucosylation can significantly attenuate the progression of peritoneal fibrosis with a superior effect compared with the effects of imatinib and might be a potential novel therapeutic target against glucose-induced peritoneal fibrosis, especially for long-term PD patients.

RESULTS

Core fucosylation is upregulated in the peritoneal membrane of rats with peritoneal fibrosis induced by glucose dialysate

An i.p. injection with standard glucose PD fluids for a period of 6 weeks resulted in a typical fibrotic lesion (Figure 1a), and the peritoneal ultrafiltration rate dramatically decreased in rats with peritoneal fibrosis (Figure 1b). Increased peritoneal thickness and impaired peritoneal ultrafiltration function confirmed the successful establishment of the peritoneal fibrosis model in our study. We then detected the expression of core fucosylation in the rat peritoneal membrane by fluorescent *Lens culinaris* agglutinin-fluorescein isothiocyanate (LCA-FITC). Our data revealed a significant enrichment of LCA-FITC in the peritoneal membrane of rats with peritoneal fibrosis compared with the control rats (Figure 1c and d). These findings suggest that core fucosylation is upregulated in the peritoneal membrane of rats with peritoneal fibrosis.

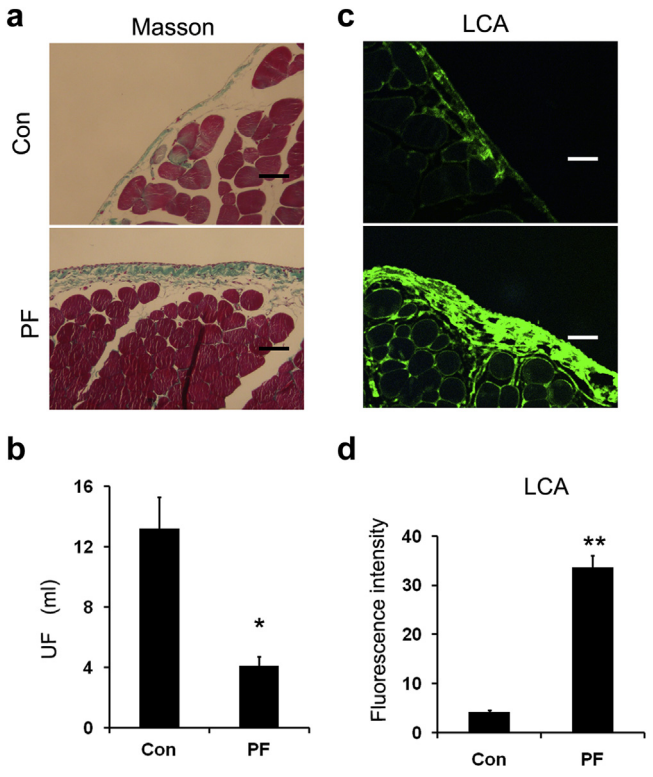

Figure 1 | Core fucosylation is upregulated in the peritoneal membrane of rats with peritoneal fibrosis (PF). The parietal peritoneal membrane was obtained from control (Con; $n = 6$) and peritoneal fibrosis rats ($n = 6$). (**a**) Representative photographs of Masson staining show that the peritoneal thickness was dramatically increased in the rats with peritoneal fibrosis compared with that in the control rats. Bar = 200 μm. (**b**) Ultrafiltration (UF) rate dramatically decreased in the rats with peritoneal fibrosis compared with that in the control rats. (**c**) Representative photographs of *Lens culinaris* agglutinin (LCA)-fluorescein complex in the control and peritoneal fibrosis rats. Bar = 200 μm. (**d**) Analysis of immunofluorescence intensity in the control and peritoneal fibrosis rats. Results are expressed as the mean ± SEM of 6 rats per group. $^{*}P < 0.05$; $^{**}P < 0.01$ versus control group. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

Inhibition of core fucosylation ameliorates glucose dialysate–induced peritoneal lesions

To investigate the effect of core fucosylation inhibition on peritoneal fibrosis *in vivo*, we knocked down endogenous α-1,6 fucosyltransferase (Fut8) in the rat peritoneal membrane, the unique fucosyltransferase responsible for core fucosylation,[31,32] via an i.p. injection of a Fut8 short hairpin RNA (shRNA) recombinant adenovirus vector constructed in our previous study.[28] Our results showed that LCA levels were increased in the peritoneal membrane of both the peritoneal fibrosis and adenovirus-control (Ad-con) rats. However, these levels were significantly down-regulated in adenovirus-Fut8–treated (Ad-Fut8) rats at weeks 3 and 6 (Figure 2). These data indicated the successful generation of a Fut8-knockdown in a rat model of peritoneal fibrosis. Peritoneal pathology was investigated by Masson staining at weeks 3 and 6 (Figure 3a) and revealed an increase in the peritoneal thickness in both the peritoneal fibrosis and Ad-con rats. Fut8shRNA treatment significantly decreased the peritoneal thickness ($P < 0.05$) compared with the peritoneal fibrosis and Ad-con rats (Figure 3a and b). The peritoneal equilibrium test results demonstrated that the absorption rate of glucose from the