

## Mechanisms of Epithelial-Mesenchymal Transition of Peritoneal Mesothelial Cells During Peritoneal Dialysis

A growing body of evidence indicates that epithelial-mesenchymal transition (EMT) of human peritoneal mesothelial cells (HPMC) may play an important role in the development and progression of peritoneal fibrosis during long-term peritoneal dialysis (PD) leading to failure of peritoneal membrane function. Here, we review our own observations and those of others on the mechanisms of EMT of HPMC and suggest potential therapeutic strategies to prevent EMT and peritoneal fibrosis during long-term PD. We found that high glucose and H<sub>2</sub>O<sub>2</sub> as well as transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) induced EMT in HPMC and that high glucose-induced EMT was blocked not only by inhibition of TGF- $\beta$ 1 but also by antioxidants or inhibitors of mitogen-activated protein kinases (MAPK). Since MAPKs are downstream target molecules of reactive oxygen species (ROS), these data suggest that high glucose-induced generation of ROS and subsequent MAPK activation mediate high glucose-induced EMT in HPMC. We and others also observed that bone morphogenetic protein-7 (BMP-7) prevented EMT in HPMC. Glucose degradation products (GDP) were shown to play a role in inducing EMT. Involvement of a mammalian target of rapamycin (mTOR) in TGF- $\beta$ 1-induced EMT has also been proposed in cultured HPMC. A better understanding of the precise mechanisms involved in EMT of HPMC may provide new therapeutic strategies for inhibiting peritoneal fibrosis in long-term PD patients.

**Key Words :** Bone Morphogenetic Protein-7; Fibrosis; Mitogen-Activated Protein Kinases; Reactive Oxygen Species; Peritoneal Dialysis

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## INTRODUCTION

Long-term peritoneal dialysis (PD) is associated with progressive increase in the thickness of peritoneal membrane, predominantly in the submesothelial compact collagenous zone (1), and membrane hyperpermeability (2). The mechanisms involved in these structural and functional changes remain unclear, but prolonged exposure of the membrane to conventional PD solution containing high concentrations of glucose and glucose degradation products (GDP) may play an important role. Epithelial-mesenchymal transition (EMT) of epithelial cells, characterized by loss of epithelial cell characteristics and gain of ECM-producing myfibroblast characteristics, is an important mechanism involved in tissue fibrosis (3-6). Recent data (7-9) suggest that human peritoneal mesothelial cells (HPMC) undergo EMT during PD and that EMT may play an important role in the development and progression of peritoneal fibrosis leading to failure of peritoneal membrane function. This brief review will discuss the mechanisms of EMT and suggest strategies for the prevention of EMT and preservation of peritoneum during long-term PD.

## EMT AND PERITONEAL FIBROSIS

Accumulating evidence implicates EMT as a potential mechanism for the development and progression of peritoneal fibrosis during long-term PD leading to failure of peritoneal membrane function. Yanez-Mo et al. (7) first reported that peritoneal mesothelial cells undergo a transition from an epithelial phenotype to a mesenchymal phenotype soon after PD was initiated with a decrease in the expression of cytokeratin and E-cadherin through induction of the transcriptional repressor Snail. Margetts et al. (8) demonstrated that intra-peritoneal administration of adenovirus-mediated transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) increases peritoneal expression of genes associated with fibrosis and EMT including collagen I,  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA), and Snail, suggesting that EMT occurs in vivo after TGF- $\beta$ 1 overexpression in the peritoneum. Nonepithelioid mesothelial cells from spent dialysate produces a greater amount of VEGF ex vivo than epithelioid-like mesothelial cells (9), suggesting that EMT may also have a role in peritoneal hyperpermeability. It is therefore important to clearly understand the

mechanisms involved in EMT during PD in order to provide a novel therapeutic strategy to prevent EMT and peritoneal fibrosis.

### MECHANISMS INVOLVED IN EMT OF HPMC DURING PD

We have shown that high glucose (10) and glucose-based PD solution (11) induce generation of reactive oxygen species (ROS) in cultured HPMC and that ROS generated by glucose-based conventional PD solution is responsible for peritoneal neoangiogenesis, membrane hyperpermeability, and peritoneal fibrosis in rats treated with glucose-based PD solutions (12). We (13) recently reported that 1) high D-glucose,  $H_2O_2$ , and glucose-based PD solutions upregulate  $\alpha$ -SMA and downregulate E-cadherin in HPMC, 2) antioxidants, N-acetylcystein (NAC) and catalase, effectively reverse high glucose-induced  $\alpha$ -SMA and E-cadherin expression in HPMC, and that 3) prolonged exposure of rat peritoneum to glucose-based PD solution upregulates  $\alpha$ -SMA expression in the peritoneum, which is effectively inhibited by NAC. All these data suggest that ROS plays a major role in peritoneal EMT induced by high glucose and glucose-based PD solutions. This is consistent with our previous observation that ROS is involved in TGF- $\beta$ 1-induced EMT in renal tubular epithelial cells (14).

GDP have been suggested to play a role in inducing EMT. Spent dialysate obtained at 12 months after the initiation of PD using solutions containing low GDP had a significantly fewer number of fibroblast-dominant cells compared to effluent obtained from patients using PD solutions containing high GDP (15). Given that GDP signal through ROS (16), it is conceivable that ROS mediate GDP-induced EMT in the peritoneum.

Rapamycin was shown to effectively inhibit TGF- $\beta$ 1-induced EMT in cultured HPMC, suggesting the involvement of a mammalian target of rapamycin (mTOR) in EMT (17). The phosphatidylinositol 3-kinase (PI3-K)/Akt/mTOR pathway has recently been recognized as an important pathway in diabetic renal injury. High glucose activates the Akt/mTOR pathway in mesangial cells (18), both Akt and mTOR are increased in diabetic kidneys (19), and low-dose rapamycin slows the progression of diabetic renal injury including  $\alpha$ -SMA overexpression and matrix accumulation (20).

### STRATEGIES FOR PREVENTING EMT DURING PD

In cultured HPMC, treatment with antioxidants effectively inhibited high glucose-induced ROS generation (10) and EMT (13). In an animal model of PD, intraperitoneal administration of NAC effectively prevented  $\alpha$ -SMA expres-

sion in the peritoneum (13) as well as peritoneal membrane thickening, neoangiogenesis, and hyperpermeability (12). These observations strongly suggest that ROS is an important therapeutic target in peritoneal EMT and structural and functional alterations in peritoneum during long-term PD.

Since TGF- $\beta$ 1 is the major inducer of EMT, strategies inhibiting TGF- $\beta$ 1 signaling is a plausible way to prevent EMT during PD. Bone morphogenetic protein-7 (BMP-7), a 35 kDa homodimeric protein and a member of TGF- $\beta$  superfamily, is an endogenous antifibrotic protein that prevents renal fibrosis in ureteral obstruction (21), diabetic nephropathy (22), and nephrotoxic serum nephritis (23). We (24) recently observed that HPMC constitutively express BMP-7, that high glucose, glucose-based PD solution, and TGF- $\beta$ 1 downregulated BMP-7 expression in HPMC, and that overexpression of BMP-7 in HPMC prevented EMT induced by TGF- $\beta$ 1, suggesting BMP-7 as a potential therapeutic strategy for preventing EMT of HPMC. This is consistent with a recent report (25) that ex vivo treatment with BMP-7 reversed in vivo and ex vivo EMT of HPMC. BMP-7 significantly inhibited TGF- $\beta$ 1-induced phosphorylation of Smad 2/3 and MAPKs in HPMC (24).

Rapamycin can prevent TGF- $\beta$ 1-induced EMT in HPMC (17) presumably through inhibition of high glucose-induced activation of the mTOR pathway (18).

Clinical trials are needed to prove the efficacy of these experimental strategies in PD patients.

### CONCLUSION

Cell culture and animal studies suggest that high glucose, GDP, glucose-based PD solution, TGF- $\beta$ 1, loss of BMP-7, and activation of the mTOR pathway induce EMT of HPMC and that antioxidants, BMP-7, and rapamycin may prevent EMT and allow better preservation of structural and functional integrity of the peritoneal membrane during long-term PD. Further studies elucidating the mechanisms involved in EMT of HPMC and clinical trials may provide new therapeutic strategies for inhibiting peritoneal fibrosis during long-term PD.

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