

Abstract No. : A77

Theme : Basic Research on Biocompatibility, Immunology, Inflammation and Fibrosis

## **Smad3 is a key mediator of TGF- $\beta$ signaling in peritoneal fibrosis in vitro.**

*Duan, W.J.<sup>1</sup>, Huang, X.R.<sup>1</sup>, Yu, X.Q.<sup>2</sup>, Lan, H.Y.<sup>1</sup>; Department of Medicine-Nephrology, Baylor College of Medicine, Houston, United States<sup>1</sup>, Department of Medicine-Nephrology, The First Affiliated Hospital, Sun Yat-Sen University, Guangzhou, China<sup>2</sup>*

**Background.** TGF- $\beta$ 1 is a key mediator in peritoneal fibrosis, which is a most severe complication of continuous ambulatory peritoneal dialysis (CAPD). It is known that TGF- $\beta$ 1 signals through Smad2 and Smad3 to mediate fibrosis. However, roles of Smad2 and/or Smad3 in peritoneal fibrosis in response to TGF- $\beta$ 1 remain unclear, which was investigated in peritoneal mesothelial cells (PMC) that lack Smad3 or have conditional knockout (KO) for Smad2.

**Methods.** Primary culture of PMC was obtained from Smad3 KO and wild-type (WT) mice or from mice with conditional deletion of Smad2 (Smad2 flox/flox) using adenovirus-mediated Cre/LoxP system. PMC were stimulated with different doses of TGF- $\beta$ 1 over 72 hours. Immunohistochemistry, Western blot, and real-time PCR were used to detect activation of Smad2 and Smad3 (phosphorylation and nuclear location) and expression of  $\alpha$ -SMA and collagen I at both mRNA and protein levels.

**Results.** TGF- $\beta$ 1 was able to activate Smad2/3 in PMC obtained from Smad3 WT or Smad2 flox/flox control mice in a time- and dose-dependent manner, which was associated with marked upregulation of  $\alpha$ -SMA and collagen I at both mRNA and protein levels. Most significantly, PMC null for Smad3 showed a complete protection against fibrosis in response to TGF- $\beta$ 1. Unexpectedly, conditional deletion of Smad2 showed no protective effect on peritoneal fibrosis induced by TGF- $\beta$ 1.

**Conclusions.** TGF- $\beta$ 1/Smad signaling plays a critical role in fibrosis in PMC in vitro. TGF- $\beta$ 1 signals through its downstream mediator Smad3, but not Smad2, to mediate peritoneal fibrosis. Blockade of the TGF- $\beta$ /Smad signaling pathway, specifically Smad3, may have therapeutic potential for peritoneal fibrosis.