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Effect of TGF- β_1 stimulation on the Smad signal transduction pathway of human peritoneal mesothelial cells

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Objective: To prove whether the SMAD signal transduction pathway in human peritoneal mesothelial cells (HPMCs) influenced the process of human peritoneal fibrosis stimulated by TGF- β_1 .Methods HPMCs were isolated from normal human omentum and the third generation cells were stimulated by 5 ng/ml TGF- β_1 .Immunohistochemistry, Western blotting, ELISA and RT-PCR were employed to investigate the protein expression of p-Smad2/3 and the protein and mRNA expressions of SMAD 7, fibronectin(FN) and collagen-I(COL1).Results The protein expression of p-Smad2/3 in HPMCs was remarkably increased 15 min (29% p-Smad2/3-positive cells) after TGF- β_1 stimulation, peaking from 30 min (81%) to 1 h (84%) and dropping after 2 h (37%); Meanwhile, p-Smad2/3 mainly distributed in cytoplasm at 15 min, concentrated in cell nucleus and peri-nucleus from 30 min to 1 h, and distributed in cytoplasm again at 2 h. The protein expression of SMAD 7 in cells was obviously increased 24 h after TGF- β_1 stimulation, peaking at 48 h. The mRNA expression of SMAD7 was time-dependently increased. The expressions of extracellular FN protein, intracellular FN mRNA, as well as intracellular COL1 protein and mRNA were significantly increased and all of them displayed time dependency. Conclusions The SMAD signal transduction pathway of HPMCs can be specifically activated by TGF- β_1 and influence the process of human peritoneal fibrosis. The protein and mRNA expression of SMAD 7 (an inhibitor of SMAD pathway) are significantly increased as a result of feedback.

KEY WORDS: mesothelial cells; TGF- β_1 ; Smad2/3; Smad7; extracellular matrix

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