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복막 중피세포에서 소포체 스트레스를 새로운 타겟함으로써 중간엽 세포 전이와 세포사멸을 완화한다

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신현수, 류은선, 최학선, 류동렬, 김승정, 최규복, 강덕희

Endoplasmic Reticulum Stress as a Novel Target to Meliorate Epithelial-to-Mesenchymal Transition (EMT) & Apoptosis in Human Peritoneal Mesothelial Cells (HPMC)

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Objectives: Endoplasmic reticulum (ER) stress is known to be implicated in both apoptosis and Epithelial-to-Mesenchymal Transition (EMT) of epithelial cells from lung and kidney. The aims of this study were to investigate the role of preconditioning of ER stress as well as ER stress per se in EMT of Human Peritoneal Mesothelial Cells (HPMC). We characterized the pattern of ER stress-induced EMT and apoptosis with an elucidation of mechanisms of protective effect of ER stress preconditioning on TGF-β1-induced EMT.

Methods: EMT was evaluated by morphological changes of HPMCs and the expressions of E-cadherin and α-smooth muscle actin after treatment with ER stress inducer tunicamycin (TM) or thapsigargin (TG). Apoptosis was assessed by FACscan. Effect of pretreatment TM (0.01ng/ml) or TG (0.01nM) on TGF-β (1 ng/mL)-induced EMT was also evaluated. Mechanisms suggested for peritoneal EMT such as phosphorylation of Smad2/3, snail and nuclear translocation of β-catenin were investigated by WB and ICC. EMT was evaluated by the expressions of E-cadherin and α-smooth muscle actin (α-SMA) after stimulation of TGF-β1 with or without a blocker of ER stress, tauroursodeoxycholic acid (TUDCA) by western blotting.

Results: TM and TG at the concentration inducing EMT of HPMC did not lead to apoptosis in HPMC up to 48 hours. TM or TG enhanced the phosphorylation of Smad2/3 and increased a nuclear translocation of β-catenin and Snail expression. TGF-β1 induced ER stress in HPMC, which was expressed as an increase in the expression of GRP78/94 and ATF6 with XBP-1 splicing, which was blocked by TUDCA, an endogenous bile acid to attenuate ER stress. Interestingly, TGF-β did not induce the phosphorylation of PERK, eIF2α or ATF4. TGF-β induced EMT of HPMC at 24 and 48 hours, which was confirmed by a transition of cell morphology and altered expression of epithelial and mesenchymal cell markers. TGF-β1 also induced apoptosis of HPMC. TUDCA blocked TGF-β1-induced EMT and apoptosis in HPMCs. Apoptosis of HPMCs by higher concentration of ER stress inducers was associated with a persistent increase in the expression of C/EBP homologous protein (CHOP), a UPR marker that was known to be implicated in apoptosis. However, a mild and transient up-regulation of CHOP was observed with a lower concentration of TM or TG. Pre-treatment with TM or TG for 4 hours protected the cells from TGF-β1-induced EMT and apoptosis in HPMCs.

Conclusions: ER preconditioning ameliorated TGF-β-induced EMT and apoptosis, demonstrating the role of ER stress as an adaptive response that served to protect HPMC against ER stress-induced apoptosis. Therefore, modulation of ER stress in peritoneal mesothelial cells could serve as a novel approach to ameliorate EMT and apoptosis in the pathogenesis of peritoneal fibrosis.

Key Words: 소포체 스트레스, 중간엽 세포전이, 세포사멸
Endoplasmic reticulum stress, Epithelial-to-mesenchymal transition (EMT), Apoptosis