Methods: Wild-type (EP3+/+) and EP1 gene-deficient (EP1−/−) mice were divided into 4 groups: (1) EP3+/− Sham-operated group; (2) EP3+/− 5/6 nephrectomy group; (3) EP3−/− Sham-operated group; (4) EP3−/− 5/6 nephrectomy group. The serum levels of blood urea nitrogen (BUN), serum creatinine (SCr), and urine osmolality in mice were measured. Kidney tissues were taken and fixed in 4% phosphate-buffered paraformaldehyde, embedded in paraffin and cut into slices after eight weeks. Pathological changes of renal tissue were observed by HE and PAS-Masson stain. The expression of FN, Col1, COX2, TGF, CTGF, Erbin were detected by immunohistochemistry.

Results: Compared with the respective sham group, 5/6 nephrectomy model group had higher levels of serum creatinine and BUN but lower urine osmolality. Whereas the changes of EP3+/− 5/6 nephrectomy group were significantly lower than those in the EP3+/− 5/6 nephrectomy group (P < 0.05). In 5/6 nephrectomy mice, the interstitial fibrosis including tubular atrophy, loss and dilatation, inflammatory cell infiltration and interstitial matrix deposition was prominent. Compared with the respective sham group, 5/6 nephrectomy group mesangial cells proliferation and extracellular matrix significantly higher, whereas the changes of EP3−/− 5/6 nephrectomy group were significantly lower than those in EP3+/− 5/6 nephrectomy group (P < 0.05). Immunohistochemistry technique showed that, compared with the each Sham-operated group, the expressions of FN, COL1, COX2, TGF, CTGF and Erbin in renal tissues significantly higher in 5/6 nephrectomy group, whereas the FN values in EP3+/− 5/6 nephrectomy group were significantly lower than those in EP3+/− 5/6 nephrectomy group (P < 0.05).

Conclusion: Targeted disruption of the EP3 can attenuate the pathological state of 5/6 nephrectomize induced renal fibrosis, and it implying that EP3 may take an important role in the process of renal fibrosis.

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0216

1,25(OH)2D3 Attenuates High Glucose-induced EMT, Oxidative Stress and Inflammation in Human Peritoneal Mesothelial Cells via TGFβ/Smad Pathway

Lina Yang, Jianfei Ma

Department of Nephrology, The First Affiliated Hospital of China Medical University, Shenyang, Liaoning, China

Objective: Epithelial-mesenchymal transition (EMT) is recognized to accelerate peritoneal membrane dysfunction. 1,25(OH)2D3 plays an important role in preventing many types of EMT in vivo. However, its function on the EMT, oxidative stress and inflammation of human peritoneal mesothelial cells (HPMCs) remains unknown. Therefore, we studied the effects of 1,25(OH)2D3 on high glucose (HG)-induced EMT, oxidative stress and inflammation in HPMC s and examined the underlying molecular mechanisms.

Methods: We used HG stimuli to reproduce the damage of peritoneal membrane injury in vitro, and examined the effect of 1,25(OH)2D3 on EMT oxidative stress and inflammation in HPMCs. The expressions of E-cadherin, α-SMA, FN, TGFβ, IL-6, phospho-Smad3 and Smad3 in HPMCs were evaluated by western blot analysis, and α-SMA was determined by immunofluorescence staining. GH5 levels were measured using ELISA kit. Intracellular accumulation of ROS was measured using 2,7-dichlorofluorescein diacetate (DCF-DA).

Results: We found that HG decreased GSH expression, and increased TGFβ, IL-6, ROS and Smad3 expression, and promoted EMT, as shown by decreased E-cadherin expression (epithelial marker), and increased α-SMA and FN expression (mesenchymal markers). 1,25(OH)2D3 pretreatment attenuated the HG-induced EMT and oxidative stress, inhibited the inflammatory cytokines (TGFβ and IL-6) in HPMCs, partly through inhibition of TGFβ/Smad pathways.

Conclusion: These results indicate that 1,25(OH)2D3 attenuated the HG-induced EMT, oxidative stress and inflammation, partly through inhibition of TGFβ/Smad pathways.

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0246

PPARβ Augments IL-1β-induced Expression of COX2 and Production of PGE2 in Human Mesangial Cells via Sirt1 Pathway

Rong Cao 1, Youfei Guan 2

1The First Affiliated Hospital of Shenzhen University, Shenzhen, Guangdong, China
2Dalian Medical University, Dalian, Liao ning, China

PPARβ, a ligand-activated nuclear receptor, plays important roles in the regulation of lipid and glucose metabolism, cell proliferation and differentiation. Growing evidence indicates that PPARβ also exerts anti-inflammatory properties by suppressing proinflammatory cytokines production. Previously, our studies showed that PPARβ was expressed in human mesangial cells. However, the role of PPARβ in human mesangial cell inflammation has not been elucidated. Excessive production of prostaglandin resulting from increased COX-2 expression is involved in various pathophysiological conditions, such as cancer and inflammation. Recently, it has been reported that activation of PPARβ up-regulates the expression of COX-2 and increases production of PGE2 in tumor cells. The present study aimed to investigate the potential role of PPARβ in COX-2 expression and PGE2 synthesis in human mesangial cells, particularly in the presence of IL-1β. Our studies showed that PPARβ was functionally expressed in human mesangial cells, and its expression was increased by IL-1β in human mesangial cells. Treatment with GW0742, a selective agonist of PPARβ, or viral-mediated overexpression of PPARβ significantly upregulated expression of IL-1β-induced COX-2 expression and increased cellular PGE2 content in human mesangial cells. Importantly, activation of PPARβ or PPARβ overexpression significantly augmented IL-1β-induced COX-2 expression and PGE2 production in human mesangial cells. In addition, PPARβ-β increased the expression of Sirt1, while knockdown of Sirt1 gene or inhibition Sirt1 activity by nicotinamide and EX527 partly abolished the effect of PPARβ on IL-1β-induced expression of COX-2 and production of PGE2 in human mesangial cells. Taken together, PPARβ augmented IL-1β-induced expression of COX-2 and production of PGE2 in human mesangial cells, at least in part, via the Sirt1 pathway. More importantly, PPARβ may represent a novel target for the treatment of renal inflammatory diseases.

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0258

Effect of Protein Phosphatase 2Ac and Norcantharidin on Smad3 Linker Region Phosphorylation in HK-2 Cells

Ying Li, Z. Xiao, Z. Y. Zhao, Q. Q. Xu, H. Liu, J. Li, S. B. Duan, L. Sun, Y. M. Peng, F. Y. Liu

The Second Xiangya Hospital of Central South University, Changsha, China

Objective: We assessed the role of PP2Ac and NCTD on the phosphorylation of Smad3 linker region in human renal proximal tubule cell lines (HK-2 cells).

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