Methods: (1) HK-2 cells were cultured, divided into a normal control group, a TGF-β1 stimulation group, a PP2Ac overexpression + TGF-β1 stimulation group, and a PP2Ac shRNA + TGF-β1 stimulation group. Western blot detected the expression of total Smad3 protein, pSmad3 linker region protein pSmad3-L (Ser204) and pSmad3-L (Ser208). (2) HK-2 cells were randomly divided into a control group, a TGF-β1 stimulation group, and a TGF-β1 stimulation + NCTD group. Immunofluorescence was used to analyze the cellular location of pSmad3-L (Ser204) and pSmad3-L (Ser208). Western blots were used to detect the expression of PP2Ac protein and nuclear proteins pSmad3-L (Ser204) and pSmad3-L (Ser208).

Results: (1) Western blots showed HK-2 cells to be stimulated with TGF-β1 1 h after transfection with PP2Ac overexpression plasmid, while total Smad3 protein and the expression of pSmad3-L (Ser204) and pSmad3-L (Ser208) decreased. In HK-2 cells treated with TGF-β1 for 1 hour after transfection with PP2Ac shRNA, total Smad3 protein and the expression of pSmad3-L (Ser204) and pSmad3-L (Ser208) increased. (P < 0.05). (2) The distribution and expression of pSmad3-L (Ser204) and pSmad3-L (Ser208) in the nucleus of HK-2 cells stimulated by TGF-β1 was significantly elevated, with further upregulation by NCTD treatment.

Conclusion: PP2Ac inhibits Ser204 and Ser208 phosphorylation. Consequently, NCTD, a PP2Ac inhibitor, could abolish this kind of inhibition.

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0262 Antifibrotic Effect of Norcantharidin (NCTD) on Downregulating Protein Phosphatase 2 (PP2Ac) Expression

Y. Li, Q. Q. Xu, T. Hou, Z. Xiao, H. Liu, J. Li, S. B. Duan, L. Sun, Y. M. Peng, F. T. Liu
Department of Nephrology, Second Xiangya Hospital, Central South University, Changsha, China

The aim of this study was to investigate whether the antifibrotic effect of norcantharidin (NCTD) is dependent on its downregulating protein phosphatase 2 (PP2Ac) expression. Sixteen SD rats were randomly divided into four groups: (1) sham operation; (2) unilateral ureteral obstruction (UUO) day 3; (3) UUO day 7; and (4) UUO day 14. UUO was performed using an established protocol. The rats were killed at 1, 3, 7 and 14 days after UUO, and kidney tissues were harvested. In the second panel, another 15 SD rats were also randomly divided into three groups: (1) sham operation; (2) UUO; and (3) UUO+okadaic acid (OA) (30 μg/kg/day). OA was diluted with 1.8% alcohol and administered to rats through a gastric tube. Rats were killed 3 days after UUO, and the kidney tissues were removed. In the third panel, four groups of rats were used: (1) sham operation; (2) UUO; (3) UUO+CTD (0.05 mg/kg/day); and (4) UUO+NCTD (0.1 mg/kg/day). NCTD was dissolved in normal saline and administered to rats by intraperitoneal injection at the doses of 0.05 mg/kg/day. Rats were killed 14 days after UUO, and the kidney tissues were harvested. All these removed kidney tissues were used for various analyses. In vitro experiment, HK-2 was cultured, after reaching 80% confluence, HK-2 cells were serum-starved for 16 hours and pretreated with 2.5 μM NCTD or 40 nM OA for 0.5 hours, followed by incubation with or without recombinant TGF-β1 at 5 ng/ml for 24 h. The cells were then collected for real-time RT-PCR and Western blot analysis. For overexpression and depletion of PP2Ac, HK-2 cells were transfected with the plasmid pEGFP-N1-PP2Ac and SD11-PP2Ac shRNA. The vector-only plasmid pEGFP-N1 and SD11 were used as the negative controls, respectively. And the normal HK-2 cells without plasmid transfection were treated as the blank control.

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0266 AngⅡ via SM22α pkc-α/p47phox Mediates Podocyte Oxidative Stress

Xingzhi Wang, Lirong Hao
First Affiliated Hospital of Harbin Medical University, Harbin, Heilongjiang, China

Our previous study had shown that kinetic change of the expression level of SM22α in the animal model of podocyte injury was related with the induction of oxidative stress. Angiotensin II (AngII) induces oxidative stress. This induction is regulated by PKCδ-p47phox pathway. Our hypothesis is that SM22α maybe regulate oxidative stress through PKCδ-p47phox pathway, which results in the damage of podocyte. In this study we will explore how SM22α will regulate the oxidative stress and identify the role of SM22α in the podocyte damage process. We hope to block the oxidative stress and provide early intervention to the podocyte damage.

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0276 HBx Induces Apoptosis of Podocytes Through β3/1 Integrin and the Ratio of Bcl-2/Bax Downregulation

He Ping, Li Detian
Shengjing Hospital of China Medical University, Shenyang, China

Objective: The hepatitis B virus X protein (HBx) regulates numerous signaling pathways, including those that modulate apoptosis. The purpose of this study was to investigate the mechanism of HBx-induced apoptosis of podocytes.

Methods: An HBx expression vector (pc-DNA3.1(+)-HBx) was used to transfect podocytes to establish an HBx overexpression model. One control group was not transfected and the other control group was transfected with plasmid lacking the HBx-encoding insert. The rate of apoptosis was determined by flow cytometry. The expression of β3 integrin, β1 integrin, Bcl-2, and Bax were determined by western blotting.

Results: Transfection of podocytes with pc-DNA3.1(+)-HBx increased apoptosis relative to the controls. Transfected cells had increased protein expression of Bax and decreased protein expression of β3 integrin, β1 integrin, Bcl-2 relative to the controls.

Conclusion: Our results suggest that HBx induces apoptosis in podocytes, at least in part through decreased protein expression of β3 integrin, β1 integrin and the ratio of Bcl-2/Bax.

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0277 Effects of Endothelin-1 on the Morphological Changes of Human Renal Proximal Tubule Epithelial Cells and Expression of Transforming Growth Factor-β1

Wei Li, Yichuan Wang, Le Zhou
Affiliated Hospital of Shandong University of Traditional Chinese Medicine, Jinan, China

Objective: To investigate the effects of endothelin-1 (ET-1) on the morphological changes of human renal proximal tubule epithelial cells and expression of transforming growth factor-β1 (TGF-β1).

Methods: Add ET-1 into the human proximal tubular epithelial cell line (HK-2) which cultured in vitro to make the TEMT cell model, then BQ123 (ETAR antagonist), BQ788 (ETBr antagonist) and BQ123 + BQ788 (ETAR and ETBr double antagonism) were added to models respectively, set up blank control group, scanning electron microscope was used to observe the changes of cell morphology. Real-time quantitative PCR and Western blot were used to test the expression of TGF-β1 mRNA and protein in each group.

Results: ET-1-induced HK-2 cells were significantly irregular shape, with cytoplasm reduced, cell shrinkage, surface microvilli and intercellular connections significantly reduced, the expression of mRNA and protein of TGF-β1 were significantly up-regulated (P < 0.05); the cell morphology was improved in BQ123 group and BQ788 group, the expression of mRNA and protein of TGF-β1 were significantly lower than those in the ET-1 induced group (P < 0.05), the inhibitory effect of BQ788 was more obvious in the two groups, but there was no significant difference between the two groups.

Conclusion: ET-1 may lead to a significant increase in profibrotic factor TGF-β1 and have a significant effect in inducing TEMT, blocking ET-1 receptor A or B can significantly inhibit this effect, especially ETBr antagonist BQ788.

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