0296

Effect of Macrophage 2c on Human Renal Tubular Epithelial Cell Activation

Fengming Dong, Rongguo Fu, Qiaoling Yu, Yanyan Yang, Yixin Zou, Fumeng Huang, Zhengmou Wang
Department of Nephrology, Second Affiliated Hospital, Xi'an Jiaotong University, Xi'an, China

Objective: To explore the effect of polarized macrophage 2c (M2c) on the activation of human renal proximal tubular epithelial cells.
Methods: M2c were induced by treating with TPA (20 ng/ml) and IL-10 (20 ng/ml) for 0 h, 12 h, 24 h respectively, and then M2c were co-cultured with human kidney proximal tubular epithelial cells for 48 h. The mRNA and protein expression levels of fibrosis indexes. α-SMA, E-cadherin and vimentin in proximal tubular epithelial cells were tested by RT-PCR, immunofluorescence and Western blotting. Migration assay was conducted to measure the migrated cells after co-cultured.
Results: Compared with normal control group, the mRNA levels of M2c markers CXCL13, B7H4 were significantly increased ($P < 0.01$) after co-culturing for 12 h and 24 h. Compared with normal control group, the mRNA and protein expressions of α-SMA, vimentin were higher and E-cadherin were lower in the proximal tubular epithelial cells which were co-cultured with M2c for 12 h and 24 h, especially in 24 h ($P < 0.01$). Migration assay indicated that the migration ability of proximal tubular epithelial cells was influenced by M2c, especially in 24 h ($P < 0.01$).
Conclusion: Polarized M2c can induce the phenotype changes of human renal proximal tubular epithelial cells and increase the migration ability. Polarized M2c plays a role in the process of human renal proximal tubular epithelial cells activation.
This work was supported by NSFC No. 81300581, 81470968, 81400740, 81100530.

http://dx.doi.org/10.1016/j.hkjn.2015.09.123

0301

Expression of HIF-1α After Exposure to an Altitude of 4600 m in Rat Kidney

Chunhua Wang, Wenbo Hu
The Qinghai Provincial People's Hospital, Qinghai, China

Objective: In order to discuss expression of HIF-1 on high altitude hypoxia.
Methods: To transported 50 male wistar rats from Lanzhou (1500 m) to Ke-ke-xi-li Natural Reservation Areas (4600 m) in Qinghai province by truck. The testing group was anestheiezd and all time sample were gained at different time points including 1d, 3d, 7d, 14d and 21d. We used the automatic biochemistry analzer to examine the kidney function of rats, and immunohistochemiscal streptavidin-perosidase (SP) staining was used to evaluate the levels of HIF-1α. Meanwhile, the control group was set up in Lanzhou (1500 m).
Results: The rats' renal functions were increased at first and then decreased after entered the plateau area. There was significant difference between the control group and testing groups. The level of HIF-1α in rats increased at first after exposure altitude (0.37 ± 0.12), and reached its peak at 3d (3.68 ± 0.20). Then it decreased gradually and was similar to the control group at 21d. The immunohistochemical staining demonstrated that HIF-1α distributed mainly in proximal, distal and tubular epithelial cells in medulla more than in cortex. There was less expression in glomerulus.
Conclusion: The variation reveals that the expression of HIF-1 of rat can be induced by high altitude hypoxia and the kidney was guarded through this procedure. Our results provided the theoretical foundation for the prevention and treatment of kidney injury induced by high altitude hypoxia.

http://dx.doi.org/10.1016/j.hkjn.2015.09.124

0312

Mitochondrial ROS-activated RAS Promotes Expression and Function of NCC in Proteinuric Kidney Disease

J. Yu, S. M. Huang, Y. B. Zhuang, G. X. Ding, Z. J. Jia, A. H. Zhang
Nanjing Children's Hospital, Nanjing Medical University, Nanjing, China

The disorder of fluid metabolism is a common complication of kidney disease, whose pathogenic mechanisms remain unclear. Using an albumin overload mouse model (i.p injection of albumin for 12 days), we found that albuminuria strikingly increased NCC expression by 2.3-fold in mouse kidneys. To further evaluate the functional change of NCC in mice exposed to albumin overload, a specific NCC inhibitor hydrochlorothiazide (10 mg/kg) was administered via a single i.p injection. The response to hydrochlorothiazide was significantly enhanced in mice with albumin overload. In consideration of the established role of renin-angiotensin system (RAS) in the regulation of renal sodium transporters, we examined the key components of RAS and found a striking elevation of angiotensinogen (AGT), angiotensin converting enzyme (ACE) by 2−3 folds, in line with enhanced urinary AngII excretion. In proteinuric patients, we also observed a 4-fold upregulation of NCC and remarkable stimulation of ACE detected by immunohistochemistry in accord with significant increment of urinary Ang II excretion. To further investigate the role of RAS in albuminuria-mediated NCC upregulation, we performed primary culture of renal tubular cells and observed that albumin directly stimulated NCC expression in parallel with the induction of AGT and ACE in cells, and elevated Ang II secretion in medium. Strikingly, administration of a specific ACE inhibitor captopril to the cells remarkably abolished albumin-induced enhancement of NCC and RAS components. Additionally, albumin overload significantly reduced mitochondrial superoxide dismutase (SOD2) by 60%, and administration of a SOD2 mimic (MnTBAP) entirely abolished the stimulation of NCC, AGT, and ACE in mice with albumin overload. Taken together, these novel findings demonstrated that albuminuria is of vital importance in upregulating NCC expression through a mitochondrial oxidative stress-initiated stimulation of AGT/ACE/Ang II cascade. This upregulation of NCC may contribute to the fluid retention effect in proteinuric kidney disease at least to some extent.

http://dx.doi.org/10.1016/j.hkjn.2015.09.125

0313

Proteinuria Enhances NHE3 Expression via a Mitochondrial Oxidative Stress/RAS Axis

J. Yu, Y. B. Zhuang, G. X. Ding, S. M. Huang, Z. J. Jia, A. H. Zhang
Nanjing Children's Hospital, Nanjing Medical University, Nanjing, China

Imbalance of salt and water is a frequent and challenging complication of kidney disease, whose pathogenic mechanisms remain elusive. Employing an albumin overload mouse model (i.p injection of albumin for 12 days), we discovered that albuminuria increased NHE3 expression by 2-fold in mouse kidneys, as determined by Western blotting and qRT-PCR. Considering the known role of renin-angiotensin system (RAS) in modulating renal sodium transporters, we examined the key components of RAS and found a striking elevation of angiotensinogen (AGT, +2.1 folds), angiotensin converting enzyme (ACE, +3.3 folds), and urinary antgiotensin II (Ang II, +70%). In proteinuric patients, we detected a 1.9-fold upregulation of NHE3 and 3-fold increase of ACE determined by immunohistochemistry in line with a 2-fold increment of urinary Ang II excretion. To further investigate the role of RAS stimulation in NHE3 upregulation in the present experimental setting, we performed primary cultures of renal tubular cells and observed that albumin directly stimulated angiotensinogen (AGT)/angiotensin converting enzyme (ACE)/antgiotensin (Ang) II axis and NHE3 expression, which was remarkably abolished by an ACE inhibitor captopril, indicating a key role of RAS in mediating albuminuria effect on NHE3 upregulation. More interestingly, albumin overload significantly induced mitochondrial oxidative stress as evidenced by reduced mitochondrial superoxide dismutase (SOD2, −60%) and elevated ROS production. Notably, administration of a SOD2 mimic (MnTBAP) completely normalized the upregulation of NHE3 as well as AGT/ACE/Ang II cascade affected by albumin overload in mice. In sum, these novel findings demonstrated that albuminuria is of vital importance in upregulating NHE3 expression via mitochondrial oxidative stress-initiated stimulation of AGT/ACE/Ang II cascade. This may also offer novel therapeutic targets for dealing with fluid retention in proteinuric renal diseases.

http://dx.doi.org/10.1016/j.hkjn.2015.09.126