Endothelial Cell derived Endothelin-1 Knockdown Attenuates Kidney Fibrosis via Reducing Peritubular Capillary Loss and Pericyte to Myofibroblast Transition

Nur Arfian[1], Dwi Aris A. Nugrahaningsih[1], Anggoro B. Hartopo[1], Nicholas V. Zellweger[2], Susi Heiden[2], Kazuhiko Nakayama[2], Keiko Yagi[2], Masashi Yanagisawa[3], Ken-ichi Hirata[1], Noriaki Emoto[1,2]. [1]Kobe University Graduate School of Medicine, Cardiovascular Division Internal Medicine, 650-0017, Japan, [2]Kobe Pharmaceutical University, Clinical Pharmacy Department,658-8558, Japan, [3]Howard Hughes Medical Institute, University of Texas Southwestern Medical Centre, Molecular Genetic Department,75390, United States.

Regardless to the causes, chronic kidney diseases lead to kidney fibrosis, characterized by myofibroblast formation as a key regulator. Targeting cells that are responsible for myofibroblast formation, such as pericyte may represent the best way to treat kidney fibrosis. ET-1 is not only a potent vasoconstrictor but also has role in fibrosis. Interaction of ET-1 from endothelial cells and endothelin A receptor (ETA) in pericyte may play role in kidney fibrosis progression and myofibroblast formation.

Unilateral ureteral obstruction (UUO) and sham operation (SO) were performed in female, 6-8 weeks old, vascular endothelial endothelin-1 knock out (VEETKO, n=28) and wild type (WT, n=28) mice. Before sacrificed in day 3 or 14, renal blood flow were observed by laser speckle blood flow imaging (LSBFI). Sirius Red, Alpha SMA, and PAS staining were performed, afterwards of 12-15 randomly fields of each kidney from SO, contralateral and UUO groups were scored for the degree of fibrosis, myofibroblast area, and tubular injury. Peritubular capillary number and pericyte expansion were examined by immunofluorescence staining for endothelial cell (CD31) and pericyte (NG2 and Platelet Derived Growth Factor Receptor β /PDGFR β). Double staining of alpha SMA and NG2, alpha SMA and ETA, and CD31 and NG2 were also done. Expression of VEGF, VE-Cadherin, Alpha SMA, NG2, PDGFR β, and ETA were examined by western blot. ET-1 and ETA were quantified by real time PCR. Significance was assessed by $P<0.05$.

Lower fibrosis and myofibroblast areas were apparently observed in VEETKO mice than in WT mice in 14 days after UUO. Renal architecture could be partially preserved in VEETKO mice rather than in WT mice as shown by lower tubular injury score. These findings were associated with higher renal blood flow, peritubular capillary number, and VE-Cadherin and VEGF expressions in VEETKO. ET-1 and ETA increased after UUO both in WT and VEETKO; however VEETKO had lower ET-1 and ETA expressions. VEETKO preserved pericyte detachment rather than in WT after UUO proved by lower NG2/DAPI fraction area and NG2 positive cells that co-express Alpha SMA. This finding might be driven by ETA activation since ETA was expressed by myofibroblast.

ET-1 knockdown from endothelial cells could attenuate kidney fibrosis via renal blood flow and renal microvessel improvement, and myofibroblast reduction. Interaction between ET-1 from endothelial cell and ETA in pericyte might play role in pericyte to myofibroblast transition. Targeting this interaction might represent an alternative approach for kidney fibrosis therapy.