

## 근위 세뇨관 상피 세포에서 Hypoxia Inducible Factor-1 $\alpha$ 가 NF $\kappa$ B dependent Inflammation에 미치는 영향

분당서울대병원 신장내과

오세원, 하수정, 이윤미, 진호준, 채동완, 나기영

### Hypoxia Inducible Factor-1 $\alpha$ Attenuates NF $\kappa$ B-dependent Inflammation in Renal Tubule Cells

Se-Won Oh, Soo-Jung Ha, Yun-Mi Lee, Ho-Jun Chin, Dong-Wan Chae, Ki-Young Na

Department of Internal Medicine Seoul National University  
Bundang Hospital Seoul National University College of Medicine

**Purpose:** Renal inflammation is an invariable pathologic finding in almost all forms of chronic kidney disease irrespective of its cause. Hypoxia-inducible factor (HIF) is a transcription factor that regulates cellular hypoxic responses. The activation of HIF has been proved to be effective in various kidney disease models. The aim of this study is to investigate the anti-inflammatory mechanism of HIF in renal tubular epithelial cells.

**Methods:** Renal tubular epithelial cells (HK-2 cells) were exposed to tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) at 5 ng/mL and interferon- $\gamma$  (INF- $\gamma$ ) at 50 ng/mL for 24 hours, and cobalt chloride (CoCl<sub>2</sub>) 150  $\mu$ M was added. The RANTES (Regulated upon Activation, Normal T cell expressed and Secreted), MCP-1 (monocyte chemoattractant protein-1) levels were investigated by enzyme-linked immunosorbent assay. Nuclear factor kappa B (NF $\kappa$ B) p65, phosphorylated NF- $\kappa$ B p65, inhibitor of NF- $\kappa$ B (I $\kappa$ B) $\alpha$ , phosphorylated I $\kappa$ B $\alpha$  were determined by western blotting. NF- $\kappa$ B p50 DNA binding was detected by ELISA using Transfactor p50 kit. NF- $\kappa$ B transcription activity was evaluated by luciferase reporter gene assay.

**Results:** Stimulation of HK-2 cells by combination of TNF- $\alpha$  and INF- $\gamma$  for 24 hrs results in strong production of RANTES (813.0 $\pm$ 21.7 pg/mL) and MCP-1 (305.9 $\pm$ 91.4 pg/mL), these levels were decreased by pre-treatment of pyrrolidine dithiocarbamate 10 mM (190.1, 131.2 pg/mL, respectively), and also attenuated by CoCl<sub>2</sub> treatment (RANTES, 242.9 $\pm$ 187.2 pg/mL,  $p$ <0.001; MCP-1, 65.5 $\pm$ 21.6 pg/mL,  $p$ =0.001).

Combination of TNF- $\alpha$  and INF- $\gamma$  induced NF $\kappa$ B p65 phosphorylation, it was attenuated by CoCl<sub>2</sub> treatment in nuclear fraction ( $p$ =0.023). Combination of TNF- $\alpha$  and INF- $\gamma$  caused decreased expression of I $\kappa$ B $\alpha$  and it was recovered in cells with CoCl<sub>2</sub> treatment ( $p$ <0.001). NF- $\kappa$ B p50 DNA binding activity in HK-2 cell treated for TNF- $\alpha$  and INF- $\gamma$  was 1.8-fold, in the presence of CoCl<sub>2</sub> was 1.4-fold, compared with control ( $p$ =0.039). Luciferase reporter gene assay showed that TNF- $\alpha$  and INF- $\gamma$  increased NF- $\kappa$ B transcription activity by 8.1 fold, and the increased transcription activity decreased to 4.5-fold by addition of CoCl<sub>2</sub>, compared with the control ( $p$ <0.001).

**Conclusion:** Treatment of CoCl<sub>2</sub> attenuates cytokine production of RANTES and MCP-1, phosphorylation of NF $\kappa$ B p65 and degradation of I $\kappa$ B $\alpha$ . These data suggest that HIF-1 $\alpha$  attenuate TNF- $\alpha$  and INF- $\gamma$  induced inflammation, by preventing degradation of I $\kappa$ B $\alpha$  in renal tubular cells.

**Key Words:** 저산소 유도 인자 1, NF-kappa B, 염증

Hypoxia-inducible factor 1, NF-kappa B, Inflammation