ELUCIDATING THE MECHANISM OF HYPOXIA-INDUCIBLE FACTOR 1 (HIF-1) REGULATION IN KIDNEY

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Backgtound and Objectives:

- HIF-1 plays a critical role in tubular cells of both acute and chronic ischemic kidney.
- Its protective role against tubular injury has been suggested and HIF activation is a potential therapeutic option in kidney diseases.
- We aimed to identify a novel gene involved in regulation of HIF-1 in hypoxia of the kidney, a final common pathway to end stage kidney disease.

Methods:

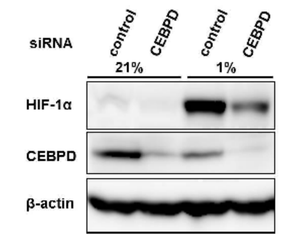
- Microarray analysis of the renal cortex of chronic hypoxia model, rat renal artery stenosis (RAS) model (day3 and day7), was used as *in vivo* screening for potential HIF-1 regulating genes.
- HeLa cells transfected with shRNA plasmids against continuously up-regulated genes in RAS kidney compared to sham kidney were evaluated for potency of HIF-1 regulation.
- Acute and chronic rat hypoxic models were analysed for this gene's expression to localise and identify its role in kidney.
- To confirm its relation to hypoxia in cultured human renal tubular cells, RNAi treated HK-2 cells under hypoxic condition were examined for HIF-1 regulation.
- Pathways regulating this gene up-regulation under hypoxia and mechanism of HIF-1 regulation by this gene were investigated.

Results:

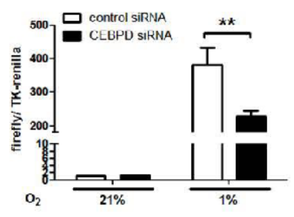
Fig1. Identification of HIF-1 upregulating genes

- 150 genes were extracted from microarray analysis.
- shRNA library experiment revealed CEBPD as the most promising HIF-1 regulator.

A. Immunoblot/ HeLa



B. HREluc/ HeLa



**p < 0.01 by two-way ANOVA, post-Bonferroni's test

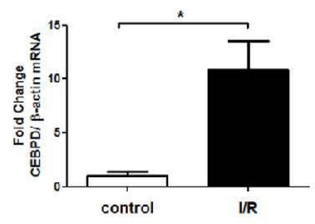
CCAAT/enhancer-binding protein delta (CEBPD)

- transcription factor
- Leucine zipper DNA-binding protein
- exhibits cell-type specific roles: acute inflammatory response in macrophages, tumor suppressor in certain cancer cells, differentiation factor in adipocytes, etc.

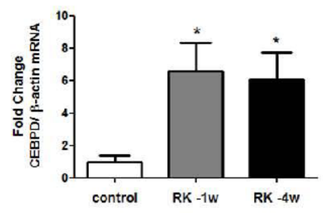
Fig2. CEBPD is expressed in tubular cells in acute and chronic hypoxic kidney.

RT-PCR (kidney cotex)

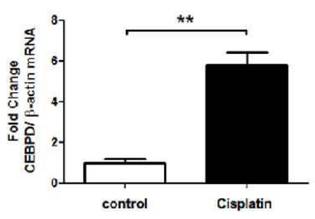
A. I/R injury



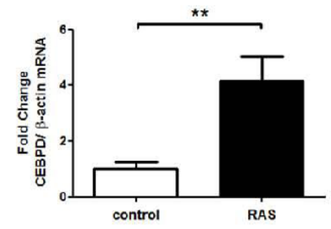
C. Remnant kidney (RK)



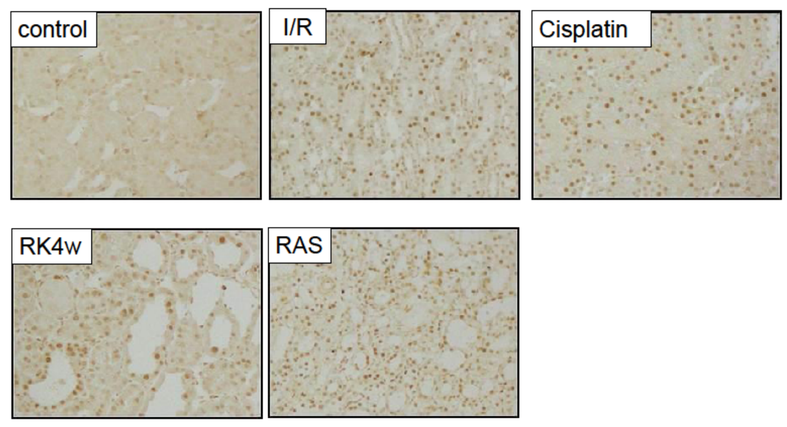
B. Cisplatin nephrotoxicity



D. RAS



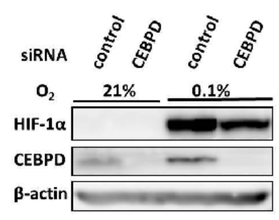
Immunohistochemistry (anti-CEBPD Ab)



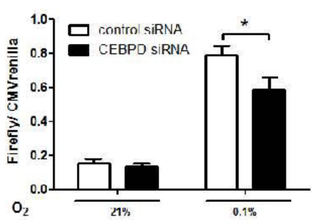
A. Ischemia-reperfusion (I/R) group: right nephrectomy and clamping of left renal artery and vein for 45 mins, sacrificed 24 hrs after operation. Control n=3, I/ R n=5. (*p < 0.05 vs control)
B. Cisplatin nephrotoxicity group: Cisplatin 6mg/kg ip, vehicle saline, sacrificed 3 days after treatment. Control n=5, Cisplatin n=6. (*p < 0.05 vs control)
C. Remnant kidney (RK) group: right nephrectomy followed by ligation of the posterior and two of the anterior branches of the left renal artery, sacrificed at indicated times. Control n=5, RK1w n=5, RK4w n=5. (*p < 0.05 vs control)
D. Renal artery stenosis (RAS): left renal artery clipped with U-shaped clip, sacrificed 7 days after treatment. Control n=5, RAS n=5. (**p < 0.01 vs control).

Fig3. CEBPD regulates HIF-1 in proximal tubular cells.

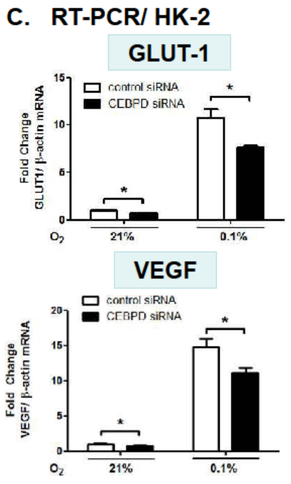
A. Immunoblot/ HK-2



B. HREluc/ HK-2



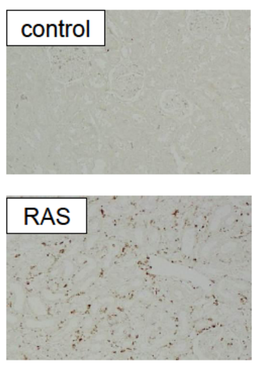
C. RT-PCR/ HK-2



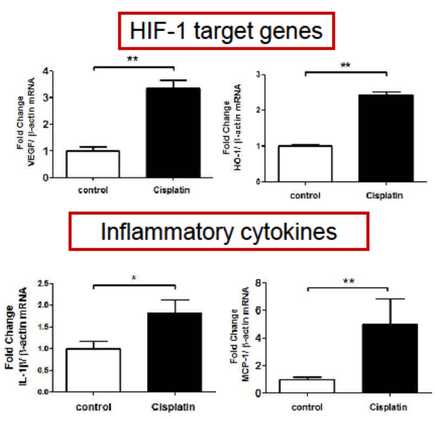
*p <0.05, *p < 0.01 by two-way ANOVA, post-Bonferroni's test

Fig4. Inflammation and hypoxia co-exist in tubulointerstitium.

A. Immunohistochemistry (ED-1)



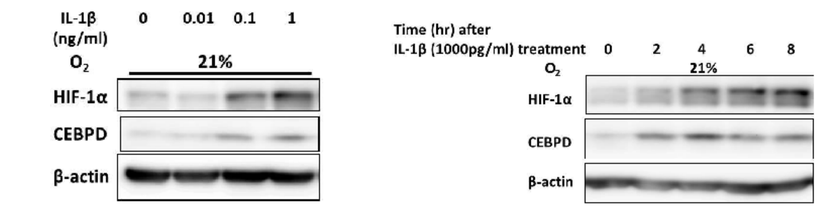
B. RT-PCR (renal cortex)



*p<0.05, **p< 0.01 vs control

Fig5. IL-1β activates CEBPD/HIF-1 pathway even under normoxia.

A. Immunoblot/HK-2



B. Immunoblot/HK-2

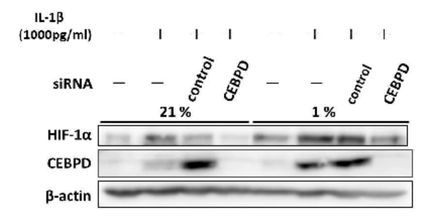
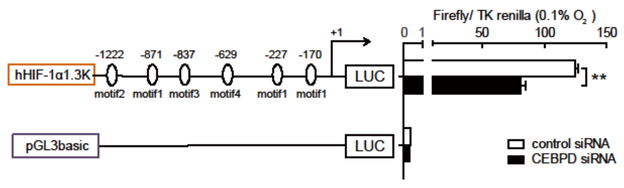
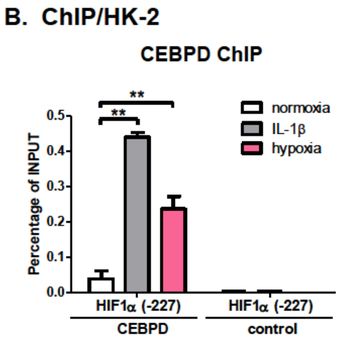


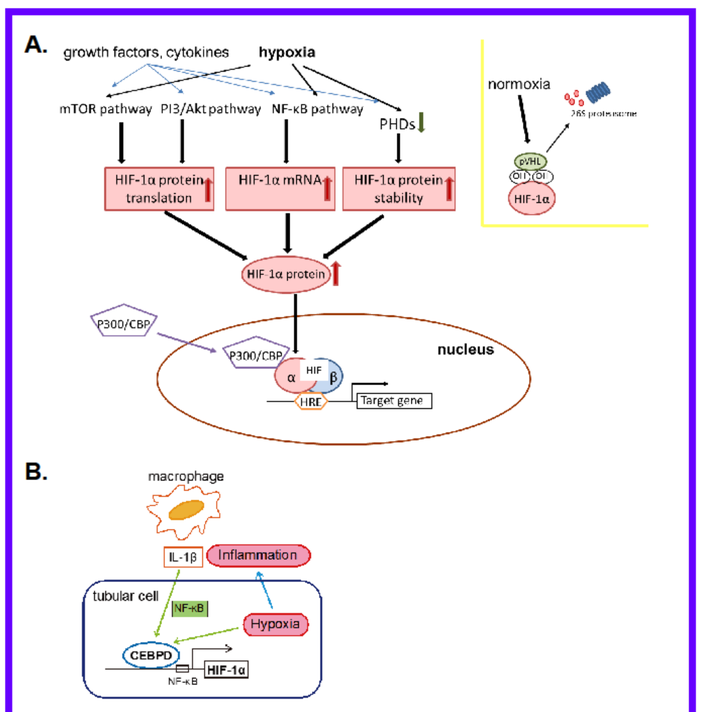
Fig6. CEBPD regulates HIF-1α at transcription level.

A. Human HIF-1α promoter assay/HK-2



B. ChIP/HK-2





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Conclusion:

- We identified CEBPD as a novel HIF-1 upregulator in kidney.
- We identified CEBPD's expression in tubular cells in acute and chronic hypoxic kidney.
- We found a potential role of CEBPD as a crosslinker of inflammation and hypoxia via HIF-1.
- CEBPD is induced by NF-κB, which then regulates HIF-1α at transcription/post-transcription level.
- CEBPD/HIF-1 pathway is a potential therapeutic target in kidney disease.

Reference:

- Kojima I, Tanaka T, Nangaku M: *Kidney Int 75: 268-77, 2009*
- Tanaka T, Kojima I, Nangaku M: *Am J Physiol Renal Physiol 289: F1123-33, 2005*

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