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Investigation of Regulation of WNK Signal by Protein Degradation System

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Summary

Mutations in with-no-lysine kinase 1 (WNK1), WNK4, Kelch-like 3 (KLHL3) and Cullin 3 (CUL3) genes are reported to cause PHAII. Recently, we generated KLHL3 $^{R528H/+}$ mice and demonstrated that mutant KLHL3 resulted in defective degradations of WNK1 and WNK4, leading to PHAII, indicating that KLHL3/CUL3 ubiquitin ligase complex interacts and degradates WNK kinases. However, pathophysiological roles of KLHL3 other than PHAII is still unclear.

To answer these questions, we generated two KLHL3-/- mice lines; conventional KLHL3-/- mice and KLHL3-/- mice that express β-gal under endogenous KLHL3 promoter in this study. Using these mice, we sought to determine the tissue distribution of KLHL3 and its role in regulating WNK protein level in each tissue.

At first, we investigated the tissue distribution of KLHL3 using β-gal expression. Immunoblots of β-gal showed the strong expression in brain and kidney, and the lower expressions in eye, testis, heart, lung and pancreas. Strong LacZ staining was observed in hypocampus and distal tubules in brain and kidney, respectively. Next, we investigated the protein levels of WNK1, WNK3 and WNK4 in the whole tissues of KLHL3+/- and KLHL3-/- mice where KLHL3 expression was detected. In the brain and other tissues showing the lower expression levels of KLHL3, expression levels of WNK1, WNK3 and WNK4 were not increased in KLHL3+/- and KLHL3-/- mice. However, only in kidney, WNK1 and WNK4 was significantly increased in KLHL3-/- mice, but not in KLHL3 +/- mice. KLHL3-/- mice also showed PHAII-like phenotypes, but KLHL3+/- mice did not.

Our data clearly showed that the WNK protein levels in KLHL3-expressing tissues might not be governed only by KLHL3. Involvement of other KLHL protein could be possible, or the assessment of WNK protein levels at cell level, not in tissue level, might be necessary to determine the role of KLHL3 in specific types of cells in each tissue. Lack of PHAII phenotypes in KLHL3+/- mice clearly showed the heterozygous deletion of KLHL3 was not enough to cause PHAII in the kidney, indicating that PHAII phenotypes in KLHL3$^{R528H/+}$ heterozygous mice we previously observed are caused by the dominant-negative effect of R528H KLHL3 mutant. Dimer formation of wild-type and R528H KLHL3, which we could demonstrate, would explain the dominant-negative effect of this mutant. These findings could help us to further understand the physiological roles of KLHL3 and the pathophysiology of PHAII caused by mutant KLHL3.