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Investigation of the Roles of KLHL3/Cullin3 in the Regulation of WNK Signal

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Summary

Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by salt-sensitive hypertension due to increased sodium reabsorption in the kidney, which is the result of activation of the WNK kinase-OSR1/SPAK kinase-NaCl cotransporter (NCC) phosphorylation cascade. Activation of this WNK phosphorylation signaling cascade in the kidney leads to increased sodium reabsorption through NCC. Mutations in the with-no-lysine kinase 1 (WNK1) and WNK4 genes are known to be responsible for PHAII. Recently, two novel genes, KLHL3 and Cullin3, were identified as also being involved in PHAII pathology. KLHL3 is a member of the BTB–BACK–Kelch family, which is a substrate adapter protein of Cullin3-based E3 ubiquitin ligase complexes. We have previously reported that WNK4 is the substrate of KLHL3-Cullin3 E3 ligase-mediated ubiquitination. However, WNK1 and NCC were also reported to be targets of KLHL3-Cullin3 E3 ligase by other groups. Therefore, the targets of KLHL3 remain unclear, as well as their involvement in the pathogenesis of PHAII. Moreover, all of these studies were performed on cultured cells. Thus, it was necessary to clarify the pathophysiological role of KLHL3 in PHAII in an *in vivo* system.

In this study, we generated and analyzed KLHL3$^{R528H/+}$ knock-in mice, carrying the same mutation as PHAII patients, to determine the pathophysiological role of KLHL3 in PHAII *in vivo*. These mice exhibited salt-sensitive hypertension and hyperkalemia, indicating that the KLHL3$^{R528H/+}$ mouse is an ideal model of PHAII. Interestingly, we found increased protein expression levels of both WNK1 and WNK4 kinases in KLHL3$^{R528H/+}$ mouse kidney *in vivo*, resulting in the activation of WNK-OSR1/SPAK-NCC phosphorylation signaling. In addition, we confirmed that mutant KLHL3 R528H is not able to bind with the acidic motif of WNK1 and WNK4. Here, we clarified that KLHL3 mutation results in the accumulation of both WNK1 and WNK4 due to the loss of the ability of the Cullin3-KLHL3 E3 ligase complex to bind to WNK kinases *in vivo*. Thus, we demonstrated pathogenesis of KLHL3 causing PHAII *in vivo*, for the first time.

Importantly, these results also indicate that this novel KLHL3 mediated regulation of WNK signaling is an important physiological mechanism for sodium handling in the kidney. In this respect, we believe that our findings could prove extremely valuable in furthering understanding of the physiological mechanisms underlying sodium homeostasis in the kidney.