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Possible Mechanism of WNK Signal Activation by PHD Inhibitor/Hypoxia

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Summary

The inappropriate over-activation of with-no-lysine kinase (WNK)–STE20/SPS1–related proline/alanine-rich kinase (SPAK)–NaCl cotransporter (NCC) phosphorylation cascade increases sodium reabsorption in distal kidney nephrons, resulting in salt-sensitive hypertension. The discovery of the WNK phosphorylation cascade has implications not only for a rare monogenic disease (pseudohypoaldosteronism type II (PHAII)) but also for salt-sensitive hypertension associated with several (patho-)physiological conditions such as low-potassium diet and metabolic syndrome. Through the investigation of the molecular mechanism of these (patho-)physiological conditions, several regulatory factors of WNK signaling have been discovered.

Prolyl hydroxylase (PHD) inhibitors are recently approved therapy for renal anemia. Their function relies on the stabilization of hypoxia inducible factor (HIF) which plays an important role in response to tissue hypoxia. Interestingly, clinical study that proved the efficacy of PHD inhibitors reported that they observed hypertension, hyperkalemia and acidosis as side effects which are common manifestations of PHAII. However, the relationship between PHD inhibitor/tissue hypoxia and WNK signaling has not been assessed. Therefore, in this study, we assessed the effect of PHD inhibitor on WNK signaling in mice and cultured renal tubule epithelial cells to explore the potential role of HIF and tissue hypoxia as a regulator of WNK signaling.

First, we administered PHD inhibitor roxadustat (10mg/kg/day) to 8 week old male C57BL6/J mice for five days. Protein abundance of WNK1, WNK4 and phosphorylation of SPAK was increased in roxadustat treated mice, but phosphorylation of NCC was not different between two groups. We next assessed the mechanism of increased protein abundance of WNK1 and WNK4 and found that they were increased at the transcriptional level. To investigate the detailed mechanism of transcriptional activation of WNKs, cultured distal convoluted renal epithelial cells (mpkDCT cells) were treated with roxadustat. However, treated cells did not exhibit WNK-SPAK signaling activation. This is at least in part due to cell toxicity caused by roxadustat. As normal mice did not manifest increased phosphorylation of NCC in response to roxadustat treatment, we also assessed the effect of roxadustat on WNK signaling in mice fed with high salt diet that normally emphasizes the difference of WNK signaling activity between normal and salt-sensitive hypertensive states. However, on the contrary, mice fed with high salt diet did not manifest activation of WNK signaling in response to roxadustat. Based on the fact that patients in clinical trial were on PHD inhibitor at least several weeks until they develop hypertension, hyperkalemia and metabolic acidosis, we are currently extending the treatment period to see the long term effect of roxadustat on WNK signaling.

Altogether, we have shown that PHD inhibitor stimulates the transcription of WNK1 and WNK4 and increases the phosphorylation of SPAK accordingly. This phenomenon is interesting especially because transcriptional

regulation of WNK signaling has not been reported before. Moreover, as there are many renal conditions that are associated with both salt-sensitive hypertension and tissue hypoxia, WNK signaling activation by PHD inhibitors has potential implications for the mechanism of salt sensitive hypertension in these conditions. However, further research is still needed to optimize the *in vivo* and *in vitro* condition to conclude the effect of PHD inhibitors on WNK signaling. Urinary exosome based assessment of WNK signaling in human patients on PHD inhibitors that we previously established is also planned.