【Outstanding Academic Research Meeting 2-1】

Exploring the Pathogenesis of GS and PHAII: from Animal to Clinical Study

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Enhanced SPAK/OSR1-NCC signal cascade caused by mutations in Kelch-like 3 (KLHL3) or Cullin3 (Cul3) involved in WNK1/4 ubiquitination is known to cause pseudohypoaldosteronism type II (PHAII). It remains unclear which WNK kinases is the major regulator in the pathogenesis of KLHL3 mutation-causing PHAII. We generated mutant KLHL3M131V knockin mice (corresponding to human M78V) in BTB domain and WNK4 null mice. KLHL3M131V mutant mice were crossed with WNK4 null mice. Our finding concluded that KLHL3 knockin mice featuring PHAII with increased expression of WNK1 and WNK4 fail to correct the GS phenotypes of WNK4 null mice, indicating that WNK4 is the most paramount kinase in SPAK/OSR1-NCC cascade in the PHAII caused by KLHL3 mutation. We also know that mutations in Kelch-like 3 (KLHL3) are among the most common causative genes detected in patients with pseudohypoaldosteronism type II (PHAII). However, the molecular mechanisms by which kelch repeat domain of KLHL3 cause PHAII have not been fully investigated in vivo. We also generated and analyzed mutant Klhl3 knock-in (KI) mice carrying a nonsense W523X mutation in the kelch repeat domain (corresponding to human KLHL3 W470X mutation). Our finding supported that Klhl3W523X/+ KI mice feature typical PHAII with a simultaneous increase of WNK1 and WNK4 through directly impaired binding of the KLHL3 kelch domain to WNKs and indirectly unstable formation of E3 complex causing Cul3 reduction. Moreover, phosphorylation status at S433 of KLHL3 could affect the binding ability with WNK1/4 and thus regulate the WNK-SPAK/OSR1-NCC cascade. Several stimuli such as angiotensin II, insulin, calcineurin inhibitors or potassium (K+) diet were shown to alter KLHL3 S433 phosphorylation in vitro. The important role of S433 phosphorylation of KLHL3 has not been tested in vivo. We generated and analyzed two knock-in (KI) mice at KLHL3 S486 site (corresponding to human KLHL3 S433) in terms of phosphomimetic (S486D) and phosphodefective (S486G) mutation. We concluded that Phosphomimetic Klhl3S486D/+ mice featured a more severe PHAII phenotype than phosphodefective Klhl3S486G/+ mice. Missense KLHL3 S433 mutation per se together with active phosphorylation status might exert the in vivo synergic effect to cause the deleterious regulation of electrolyte homeostasis and blood pressure in the kidney.