Control of electrolyte balance through ubiquitination

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The specific causes of elevated blood pressure cannot be determined in the vast majority of patients with hypertension. However, identification of genes causing rare Mendelian forms of hypertension has provided penetrating insights into the basic mechanisms of human hypertension, highlighting the key role of altered sodium handling by the kidney as a final common pathway in hypertension pathogenesis (1). Pseudohypoaldosteronism type II (PHAII) is one of these Mendelian syndromes, characterized by the unusual combination of hypertension and high levels of potassium in the blood (hyperkalemia). The Lifton laboratory had previously found mutations in four distinct genes that can cause PHAII (2, 3). In PNAS, Shibata et al. show that impaired ubiquitination of With no lysine kinases (WNKs), key molecular switches for regulating electrolyte flux in the distal nephron, explain clinical features of PHAII (4).

Several years ago, Lifton and colleagues identified causal mutations in the genes encoding WNK-1 and -4 in PHAII (3). This finding triggered intense study of these unique kinases, identifying roles for WNK-1 and -4 in regulation of sodium and potassium flux in the distal nephron. This role was primarily mediated through control of the relative levels and activities of the thiazide-sensitive sodium chloride cotransporter (NCC) and the renal outer medullary potassium channel (ROMK) (5, 6). NCC is a major pathway for sodium reabsorption in the distal nephron and the target for thiazide diuretics, which are effective and widely used antihypertensive agents (7). Thiazides are also a mainstay of treatment for PHAII, consistent with findings that NCC overactivity is a key feature of the disorder (8). ROMK is an important pathway for secretion and elimination of potassium in the urine, and relative inhibition of ROMK contributes into hyperkalemia in PHAII (8).

Although the ongoing delineation of WNK functions has provided significant insights into kidney physiology, only a small subset of patients with PHAII have mutations in WNK genes. Recently, Lifton and colleagues used exome sequencing to identify additional disease-causing mutations in the Kelch-like 3 (KLHL3) and Cullin 3 (CUL3) genes. Within a group of 52 families with PHAII, they found that mutations in these two genes accounted for disease in ∼80% of affected individuals (2). KLHL3 is one of a family of more than 50 bric-a-brac tramtrack broad complex (BTB)-containing kelch proteins, characterized by six-bladed, β-propeller domains for binding specific target proteins. CUL3 provides the scaffold for a complex including BTB domain proteins, such as KLHL3 and E3 ubiquitin ligase, targeting specific protein substrates for ubiquitination (9). Both dominant and recessive mutations in KLHL3 were found to cause PHAII. Dominant mutations in KLHL3 consisted of missense variants clustering in the kelch propeller domains involved in substrate binding or the BTB domains responsible for binding to CUL3, whereas recessive mutations were scattered throughout the protein and included premature deletions and frame-shifts, consistent with loss–of–function (2). On the other hand, the majority of the diverse CUL3 mutations in PHAII were de novo, dominant, and all caused an in-frame deletion of exon 9 (2). The observation that dominant mutations of KLHL3 and CUL3 phenocopy the loss–of–function, recessive mutations of KLHL3 suggested that disrupted ubiquitination of KLHL3-specific substrates might be a common mechanistic pathway in PHAII. The objective of the studies by Shibata et al. (4) was to test this hypothesis and to identify key substrates of the KLHL3–CUL3 ubiquitin ligase.

To this end, Shibata et al. (4) first used a combination of immunoprecipitation and protein mass spectrometry to identify binding partners of KLHL3. These studies confirmed the association between KLHL3 and CUL3, but also found that KLHL3 binds both WNK1 and WNK4, the two other proteins implicated in PHAII pathogenesis. When
the immunoprecipitation was carried out using proteins bearing the PHAII-causing mutations for either KLHL3 (R528H) or WNK4 (E559K and Q562E) as targets, the interaction with their corresponding wild-type binding partner was abrogated. A critical role of these interactions for ubiquitination was subsequently demonstrated by showing that KLHL3 is required for poly-ubiquitination of WNK4, whereas ubiquitination did not occur in cells expressing the R528H mutant of KLHL3. Furthermore, abrogation of this pathway for ubiquitination resulted in intracellular accumulation of WNK4. Accordingly, these studies indicate that the levels of WNK4 protein are regulated through ubiquitination by the CUL3-KLHL3 ubiquitin ligase complex. Mutations interfering with interactions between proteins in the complex impair WNK4 ubiquitination and subsequent degradation.

To link changes in WNK4 levels to physiological pathways relevant to PHAII, the impact of WNK ubiquitination on ROMK expression and trafficking to the cell membrane was examined. As shown previously (6), expression of wild-type WNK4 alone reduced the amount of cell-surface-associated ROMK, which in kidney tubules would have the consequence of reducing excretion of potassium in urine. On the other hand, coexpression of KLHL3 abrogated the inhibitory effect of WNK4, resulting in increased abundance of ROMK at the cell membrane. In contrast, coexpression of the KLHL3 R528H together with WNK4, the inhibitory effect of WNK4 on ROMK abundance was unaffected. This result emphasizes the importance of specific ubiquitination of WNK4 through its interaction with KLHL3 in regulating ROMK expression, thereby controlling potassium excretion in the urine (Fig. 1).

As a final step, to determine whether the pathway for WNK4 ubiquitination has relevance in vivo, the Shibata et al. (4) turned to a transgenic mouse model of PHAII (8). In this model, mice with a single-copy BAC transgene including the human genomic locus containing the WNK4 Q562E allele develop hypertension and hyperkalemia, similar to PHAII patients with this mutation. The authors found that mice bearing the WNK4 Q562E allele also have significantly higher levels of WNK4 protein in the distal nephron of the kidney compared with mice with a wild-type WNK allele. This finding indicates that the mutation impairs degradation of WNK4 in vivo, resulting in enhanced levels of WNK4 leading to the PHAII phenotype.

This work represents a substantive step forward in understanding the physiological regulation of blood pressure and potassium homeostasis, establishing an important role for the CUL3-KLHL3 ubiquitin ligase complex in controlling the levels and activity of WNK4, thereby modulating ROMK and potassium excretion. New clarity in understanding the genetic mechanisms of PHAII is also provided, demonstrating a unifying biochemical pathway of disrupted interactions between mutant KLHL3 and WNK4 and their wild-type binding partners, all resulting in impaired ubiquitination. Shibata et al. (4) suggest that this model may also provide insights into genotype-phenotype correlations observed in patients with PHAII. For example, the milder phenotype of patients with dominant WNK4 mutations may be explained by protective effects of the single wild-type allele, which would still be amenable to regulation by CUL3-KLHL3 ubiquitin ligase. On the other hand, in the more severe disease associated with KLHL3 mutations, all WNK4 proteins would be protected from degradation, and ubiquitination of WNK1 might also be attenuated, potentially promoting a more extreme phenotype.

These studies also raise a number of interesting questions for future research. The work by Shibata et al. (4) directly demonstrates the capacity of the CUL3-KLHL3-WNK4 pathway to regulate ROMK. However, disordered potassium homeostasis is only one of the features of PHAII. How does this impact NCC, the other transporter that is dysregulated in PHAII? In this regard, previous studies have suggested a more complex scheme for regulation of NCC by WNKs (6). Although the authors found that KLHL3 also binds WNK1 and this binding is abolished with the KLHL3 R528H mutant, it will be interesting to explore whether this has consequences for WNK1 ubiquitination and clearance, and if so, how this affects its downstream targets. Additionally, one wonders how this pathway fits into the fabric of classic mechanisms for regulating electrolyte excretion in the distal nephron. Do powerful hormonal mediators, such as aldosterone and angiotensin II, influence the actions of the CUL3-KLHL3 complex?

Finally, the relevance of these findings to more common forms of hypertension is worth considering. In the first instance, expanded understanding of the molecular basis of a pathway with the capacity to cause hypertension in humans has obvious value as a template for future work to determine its potential contribution to the pathogenesis of essential hypertension. Furthermore, as discussed above, the use of thiazide diuretics, which lower blood pressure by targeting the NCC, is a cornerstone of modern antihypertensive therapy. As such, pathways regulating NCC expression and potassium excretion might be exploited therapeutically to enhance efficacy or to minimize adverse side effects, such as the low serum potassium levels that frequently accompany thiazide diuretic therapy.

The work by Shibata et al. directly demonstrates the capacity of the CUL3-KLHL3-WNK4 pathway to regulate ROMK.

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