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Decrease of WNK4 ubiquitination by disease-causing mutations of KLHL3 through different molecular mechanisms

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ABSTRACT

Recently, we demonstrated that WNK4 is a substrate for KLHL3–Cullin3 (CUL3) E3 ubiquitin ligase complexes and that impaired WNK4 ubiquitination is a common mechanism for pseudohypoaldosteronism type II (PHAII) caused by WNK4, KLHL3, and CUL3 mutations. Among the various KLHL3 mutations that cause PHAII, we demonstrated that the R528H mutation in the Kelch domain decreased the binding to WNK4, thereby causing less ubiquitination and increased intracellular levels of WNK4. However, the pathogenic mechanisms of PHAII caused by other KLHL3 mutants remain to be determined. In this study, we examined the pathogenic effects of three PHAII-causing mutations in different KLHL3 domains; the protein levels of these mutants significantly differed when they were transiently expressed in HEK293T cells. In particular, S410L expression was low even with increased plasmid expression. The cycloheximide chase assay revealed that an S410L mutation in the Kelch domain significantly decreased the intracellular stability. Mutations in E85A in the BTB domain and C164F in the BACK domain decreased the binding to CUL3, and S410L as well as R528H demonstrated less binding to WNK4. *In vitro* and *in vivo* assays revealed that these mutants decreased the ubiquitination and increased the intracellular levels of WNK4 compared with wild-type KLHL3. Therefore, the KLHL3 mutants causing PHAII investigated in this study exhibited less ability to ubiquitinate WNK4 because of KLHL3's low stability and/or decreased binding to CUL3 or WNK4.

1. Introduction

Pseudohypoaldosteronism type II (PHAII) is a hereditary disease characterized by hypertension, hyperkalemia, and metabolic acidosis [1]. Mutations in the with-no-lysine kinase 1 (WNK1) and WNK4 genes are known to be responsible for PHAII [2]. Several *in vitro* and *in vivo* studies have been performed to clarify the molecular pathogenesis of PHAII [3,4]. We discovered a novel phosphorylation signal cascade in the kidney that consisted of WNK-oxidative stress-responsive kinase 1 (OSR1) and STE20/SPS1-related proline/alkaline-rich kinase (SPAK)–NaCl cotransporter (NCC) [5–8]. The constitutive activation of this cascade is the pathogenic molecular mechanism for PHAII caused by WNK4.

In 2012, two new genes KLHL3 and Cullin3 (CUL3) were reported as being responsible for PHAII [9,10]. KLHL3 is a member of the BTB–BACK–Kelch family of proteins, some of which were reported to function as substrate adapters in CUL3-based E3 ubiquitin ligase complexes [11–13]. We have previously reported that WNK4 is a target for ubiquitination by the KLHL3–CUL3 E3 ligase complex [14] and that the PHAII-causing mutations of WNK4 and KLHL3 (i.e., D564A and R528H, respectively) impaired the binding of WNK4 and KLHL3, thereby reducing WNK4 ubiquitination and increasing the intracellular protein levels of WNK4. Although the role of WNK4 in NCC regulation is controversial, our recent study using WNK4 transgenic mice clearly indicated that increased WNK4 expression activated the WNK–OSR1/SPAK–NCC cascade and caused PHAII [14]. Taken together, these findings indicated that PHAII was caused by impaired ubiquitination of WNK4. Two recent reports have supported this conclusion [15,16]. Furthermore, it has been reported that the KLHL3–CUL3 E3 ligase complex ubiquitinates WNK1 *in vitro* [17], and very recently, we demonstrated that KLHL2, which possesses 84% amino acid identity with KLHL3 in the Kelch domain, also acts with CUL3 as an E3 ubiquitin ligase for WNK kinases [18]. Therefore, various combinations of KLHL2 and KLHL3 with the four types of WNK kinases (WNK1–4) may regulate the WNK signal cascades in different types of cells.

Because the above concept is based on the analysis of a single mutant of KLHL3 and WNK4 that causes PHAII [14], we conducted the current study to examine the pathogenic effects of other mutants of KLHL3 and further establish the molecular pathogenesis of PHAII. KLHL3 is composed of the BTB, BACK, and Kelch domains. The R528H mutation, which we previously characterized, is located

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in the Kelch domain. Consistent with its role of substrate binding in the Kelch domains, R528H revealed less binding to WNK4, thereby causing less ubiquitination and increased intracellular protein levels of WNK4 [14]. Therefore, we selected other mutants from different domains in this study (Fig. 1), namely E85A (BTB domain) and C164F (BACK domain). Moreover, we focused on another mutant in the Kelch domain, S410L, because it may have a novel pathogenic effect that is not observed with R528H.

2. Materials and methods

2.1. Plasmids

Expression plasmids for 3×FLAG-tagged human WNK4 have been previously described [19]. The cDNA encoding Halo-tagged human wild-type (WT)-KLHL3 (Halo-WT-KLHL3) in the pFN21A vector was purchased from Promega. The disease-causing mutations (E85A, C164F, S410L, and R528H) were introduced using a QuickChange Site-directed Mutagenesis Kit (Stratagene). Human CUL3 cDNA was isolated using the reverse transcription–polymerase chain reaction (RT-PCR) using human prostate mRNA as a template and then cloned into the 3×FLAG-CMV10 vector (Sigma–Aldrich). The HA_4-tagged ubiquitin expression vector was kindly provided by T. Ohta (School of Medicine, St. Marianna University).

2.2. Cell culture and transfections

HEK293T cells were cultured at 37 °C in a humidified 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/ml penicillin, and 0.1 mg/ml streptomycin. HEK293T cells (3×10^5 cells/6-cm dish or 1×10^5 cells/3.5-cm dish) were transfected with the indicated amount of plasmid DNA using the Lipofectamine 2000 reagent (Invitrogen).

2.3. Immunoprecipitation

HEK293T cells transfected with the indicated amount of DNA were lysed in a buffer [50 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1% Nonidet P-40, 1 mM sodium orthovanadate, 50 mM sodium fluoride, and protease inhibitor cocktail] for 30 min at 4 °C. For proteasome inhibition, HEK293T cells were treated with 1 μM epoxomicin (Peptide Institute, Osaka, Japan) for 3 h before harvesting. After centrifugation at 12,000×*g* for 15 min, the protein concentration of the supernatants was measured, and equal amounts were used for immunoprecipitation with anti-FLAG M2 beads (Sigma–Aldrich) or anti-Halo beads (Promega) for 2 h at 4 °C. Thereafter, the immunoprecipitates were washed with the lysis buffer and eluted in SDS sample buffer after boiling for 5 min. To detect WNK4 ubiquitination in the denatured samples, the cells transfected with various plasmids were lysed in 2% SDS buffer [2% SDS, 150 mM NaCl, 10 mM Tris–HCl (pH 8.0), 2 mM sodium orthovanadate, 50 mM sodium fluoride, and 1× protease inhibitor cocktail] and then boiled for 10 min, followed by sonication. Before immunoprecipitation, the lysates were diluted at 1:10 in a dilution buffer [10 mM Tris–HCl (pH 8.0), 150 mM NaCl, 2 mM EDTA, and 1% Triton X-100], incubated at 4 °C for 1 h with rotation, and centrifuged at 12,000×*g* for 15 min.

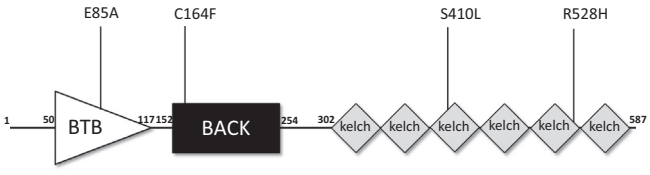

Fig. 1. Structure of human KLHL3 and PHAII mutations analyzed in this study. KLHL3 is composed of BTB, BACK, and Kelch repeat domains. We selected four PHAII-causing KLHL3 mutants for the purpose of evaluation in this study.

2.4. Antibodies

The blots were probed using the following primary antibodies: anti-FLAG (Sigma–Aldrich), anti-Halo (Promega), anti-HA (Merck Millipore), and anti-GAPDH (Cell Signaling Technology). Alkaline phosphatase-conjugated anti-IgG antibodies (Promega) were used as the secondary antibodies for immunoblotting, and the signals were detected by the Western blue Stabilized Substrate for Alkaline Phosphatase (Promega).

2.5. Cycloheximide chase assay

HEK293T cells (6×10^5 cells in 6-well plates) were transfected using the Lipofectamine 2000 reagent with 1 μg of Halo-WT-KLHL3, or 4 μg of either Halo-S410L or R528H–KLHL3. The cells were then cultured for 24 h, followed by incubation with 100 μM cycloheximide. After incubation for the indicated time periods, the cells were washed with PBS and suspended in 50-μl lysis buffer. Thereafter, the cleared lysate (10 μl) was subjected to immunoblot analyses with anti-Halo (Promega).

2.6. Fluorescence correlation spectroscopy (FCS)

Fluorescent TAMRA-labeled WNK4 peptides covering the PHAII mutation sites were prepared (Hokkaido System Science Co., Ltd., Hokkaido, Japan). The sequences of these peptides have been previously reported [14]. Human full-length KLHL3 (wild-type and mutants) was cloned into pGEX6P-1 vectors. The recombinant GST-fusion KLHL3 protein expressed in the BL21 *Escherichia coli* cells was purified using glutathione Sepharose beads. The TAMRA-labeled WNK4 peptides were incubated at room temperature for 30 min with different concentrations of GST-KLHL3s (0–2 μM) in 1× PBS containing 0.05% Tween 20 (reaction buffer). Further, FCS was performed to measure single-molecule fluorescence using the FluoroPoint-light analytical system (Olympus, Tokyo, Japan) [14,18,20]. The assays were performed in 384-well plates, with all the experiments including 10s of data acquisition. The measurements were repeated five times per sample.

2.7. In vitro ubiquitination assay

cDNA encoding human WNK4 (490–626) with a C-terminal His-tag was amplified by PCR and cloned into a pGEX6p-1 vector. Recombinant GST-fusion WNK4 protein expressed in BL21 *E. coli* cells was purified using glutathione Sepharose beads. KLHL3–CUL3 complexes were immunoprecipitated from the lysates of HEK293T cells transiently expressing Halo-KLHL3. We have previously reported that CUL3 overexpression is not necessary for the *in vitro* ubiquitination assay, because HEL293T cells contain abundant endogenous CUL3 [18]. Thereafter, the complexes were incubated in a 20-μl reaction buffer [50 mM Tris–HCl (pH 7.4), 2.5 mM $MgCl_2$, 0.5 mM DTT, 2 mM ATP] for 2 h at 30 °C with purified GST-WNK4-His (1 μg), 100 ng recombinant human E1 (Boston Biochem.), 500 ng recombinant human UbcH5a/UBE2D1 (Boston Biochem.), and 2.5 μg recombinant HA-tagged human ubiquitin (Boston Biochem.). The reaction was terminated by addition of SDS–PAGE sample buffer, followed by boiling for 5 min. The reaction mixtures were then subjected to immunoblot analyses with anti-Ub (Cell Signaling Technology) or His (Abcam) antibodies.

2.8. Statistical analysis

Comparisons between the two groups were performed using the unpaired*t*-tests. Analysis of variance with the Tukey's post hoc test was used to evaluate the statistical significance of the comparisons between multiple groups. *P* values <0.05 were considered statistically significant. Data are presented as the mean ± S.E.M.

3. Results and discussion

3.1. Low expression levels of S410L and R528H in HEK293T cells

To analyze each mutant causing PHAII (Fig. 1), we transiently expressed the Halo-tagged KLHL3 (wild-type and mutant) in HEK293T cells. We observed that the expression levels of S410L and R528H appeared to be lower compared with those in the wild-type (WT) and other mutants (Fig. 2A). To confirm this finding, we compared the expression levels of the wild-type and the abovementioned two mutants using different amounts of expression plasmids. As presented in Fig. 2B, the expression levels of these mutants, particularly S410L, was considerably lower compared with that in the wild-type. Because all the KLHL3 expression vectors were the same, we suspected that the missense mutants may have degraded more rapidly in the cells, probably by an endoplasmic reticulum quality control system.

3.2. Significantly decreased stability of S410L

To evaluate the mechanism responsible for the low expression levels of S410L and R528H, we measured the stability of these mutants using the cycloheximide chase assay. As presented in Fig. 2C, S410L was degraded more rapidly than WT–KLHL3. The half-life of S410L was about 6 h, whereas the protein levels of WT–KLHL3 were stable for at least 12 h after cycloheximide treatment. Contrary to our expectations, the stability of R528H was not decreased compared with that of WT–KLHL3. These results suggest that the pathogenic effects of the S410L mutation may be attributable to the decreased intracellular expression levels resulting from decreased stability. It is possible that the low R528H expression may be attributable to its low translation efficiency, although this remains to be established.

3.3. Impaired binding of the mutant KLHL3 to WNK4 or CUL3

Using immunoprecipitation, we previously demonstrated that the R528H mutation in the Kelch domain decreased its binding to the acidic domain of WNK4 [14]. The current study involved direct measurements of the binding ability of various KLHL3 mutants, including R528H, to WNK4. These measurements were performed using our recently developed FCS method [14,18]. We measured the diffusion time of the TAMRA-labeled WNK4 peptide using FCS in the presence of different concentrations of GST-fusion proteins of WT and the mutant KLHL3; the binding of KLHL3 to this peptide was assessed by the increased diffusion time of the fluorescent peptide [14,18]. In agreement with our previous study [14], we demonstrated that WT–KLHL3 increased the diffusion time of the TAMRA-labeled peptide (Fig. 3). However, S410L and R528H had little effect on the diffusion time, similar to that observed for GST alone. This indicated that both S410L and R528L decreased the ability to bind to WNK4. Therefore, S410L may have two pathogenic roles in the cells, namely increased degradation and decreased binding to WNK4. These changes may be related to the more severe phenotype of patients with this mutation. For example, Louis-Dit-Picard et al. [10] reported a 29-year-old male patient with a heterozygous S410L mutation, who revealed a blood pressure of 165/98 mm Hg and serum potassium levels of 6.7 mmol/L even under hydrochlorothiazide treatment.

We observed that the behavior of E85A (BTB domain mutant) and C164F (BACK domain mutant) was similar to that of

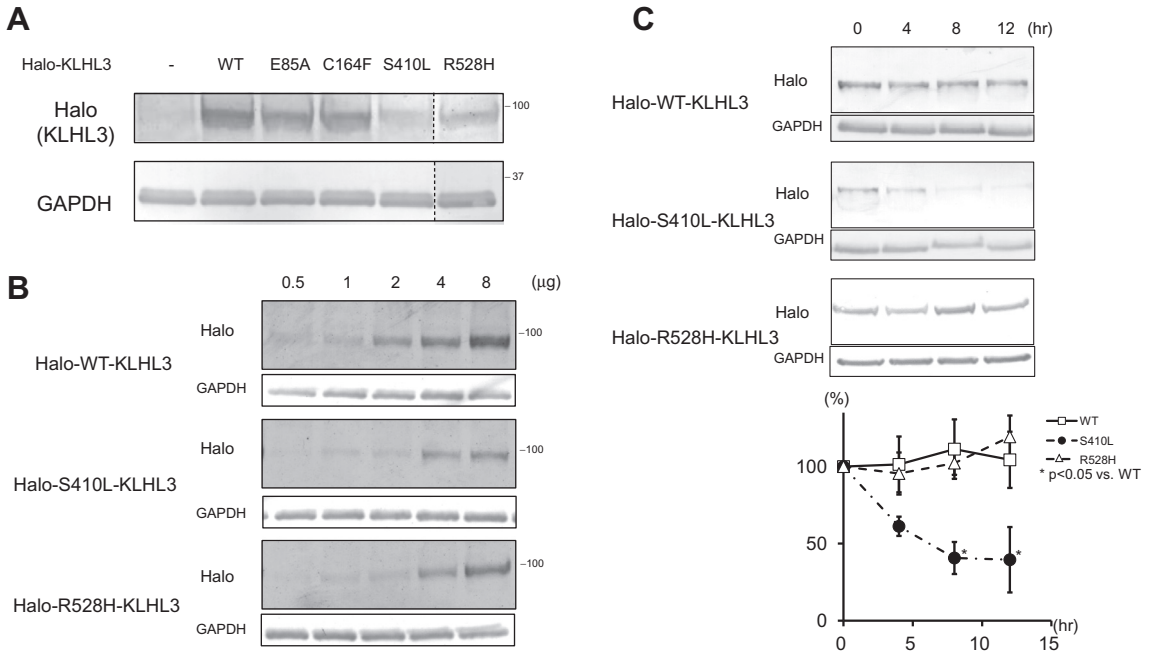

Fig. 2. Transient expression and stability assay of Halo-tagged KLHL3 mutants in the HEK239T cells. (A) We transfected HEK293T cells (1×10^5 cells/3.5-cm dish) with a Halo-KLHL3-pFN21A vector (using 2 μg plasmid). The expression levels of S410L and R528H appeared to be lower compared with those for the other genes. (B) Wild-type, S410L, and R528H KLHL3 were expressed in HEK293T cells (1×10^5 cells/3.5-cm dish) by different amounts of the expression plasmids. The expression of S410L and R528H was lower compared with that of the wild-type KLHL3. (C) Cycloheximide chase assay of Halo-WT-KLHL3, Halo-S410L-KLHL3, and Halo-R528H-KLHL3. Halo-S410L-KLHL3 was degraded more rapidly compared with Halo-WT-KLHL3 and Halo-R528H-KLHL3. The half-life of Halo-S410L-KLHL3 was approximately 6 h.

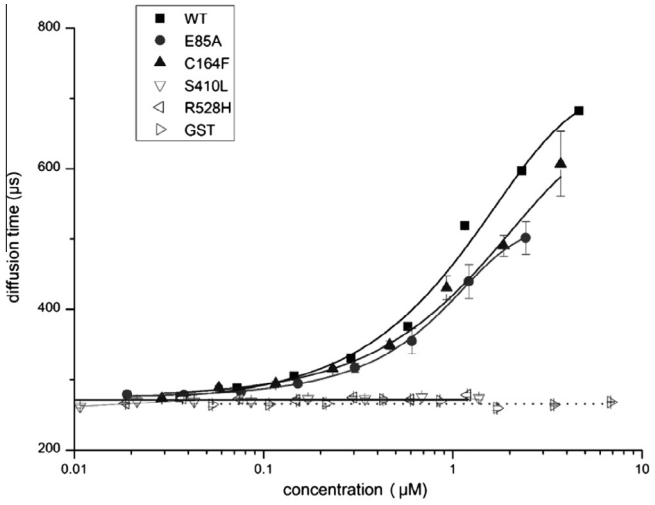

Fig. 3. FCS assay of the binding of KLHL3 and WNK4. The diffusion time of the TAMRA-labeled WNK4 peptide containing the acidic motif (a binding domain to KLHL3) was measured using FCS in the presence of different concentrations of GST fusion proteins of the wild-type and the KLHL3 mutant. The WNK4 peptide bound to the wild-type, E85A, and C164F GST-KLHL3. On the other hand, GST alone and S410L and R528H did not affect the diffusion time, indicating that these mutants could not bind to WNK4.

WT–KLHL3 in the FCS assay, indicating that these mutants maintained the ability to bind to WNK4. This result was not unexpected because the BTB and BACK domains of KLHL proteins are both thought to be involved in binding, not to the substrates (WNK kinases) but to CUL3 [17,21,22]. As presented in Supplementary Fig. 1, we confirmed the decreased binding of these mutants to CUL3 using a coimmunoprecipitation experiment.

3.4. Decreased ability of KLHL3 mutants causing PHAII to ubiquitinate WNK4 and to decrease the intracellular WNK4 protein levels

We previously reported that the expression of WT–KLHL3 significantly decreased the coexpressed protein levels of WNK4, but this effect of KLHL3 was impaired in a PHAII-causing R528H mutant [14]. Wu et al. recently reported a similar effect in other mutants associated with PHAII, including R528H and C164F, in Xenopus oocytes [16]. As presented in Fig. 4A, coexpression of PHAII-causing mutants revealed less ability to decrease the intracellular WNK4 protein levels compared with that of WT–KLHL3. Therefore, we performed *in vitro* and *in vivo* assays to evaluate WNK4 ubiquitination using the KLHL3 mutants E85A, C164F, and S410L. We have previously demonstrated that the GST-WNK4-His protein (residue from 490 to 626 containing the binding domain to KLHL3) was directly ubiquitinated by the KLHL3–CUL3 complex *in vitro* and that the R528H mutation caused a significant decrease in WNK4 ubiquitination [14]. Further, as presented in Fig. 4B, the ability of the mutant KLHL3–CUL3 complexes (E85A, C164F, and S410L) to ubiquitinate WNK4 was lesser compared with that of the wild-type KLHL3. In addition, we performed an *in vivo* ubiquitination assay to confirm this finding. Following the coexpression of FLAG-tagged WNK4, Halo-tagged KLHL3, and hemagglutinin (HA)-tagged ubiquitin in HEK293T cells, the cells were treated with 1 μM epoxomicin, and WNK4 immunoprecipitated with the FLAG antibody. In order to exclude the ubiquitination signals from other proteins that coimmunoprecipitated with WNK4, immunoprecipitation was performed under denaturing conditions. The ubiquitination signals were observed as a smear band of over approximately 200 kDa, which is the apparent molecular size of

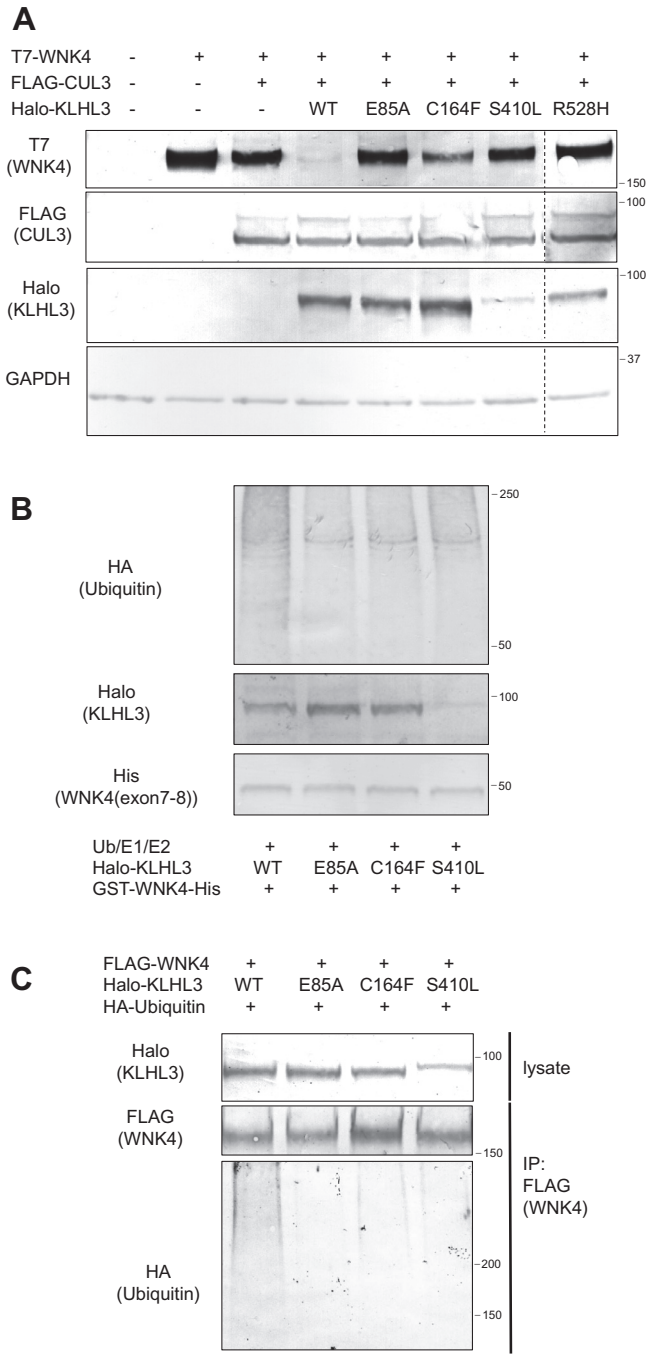

Fig. 4. Effect of the wild-type and mutant KLHL3 on the protein levels and ubiquitination of WNK4. (A) T7-tagged WNK4 and FLAG-tagged CUL3 were coexpressed with Halo-tagged KLHL3. Coexpression of the wild-type KLHL3 caused a significant decrease in the abundance of WNK4, but the KLHL3 mutants were less able to reduce WNK4. (B) *In vitro* ubiquitination assay of WNK4. As demonstrated in our previous report (14, 18), the wild-type KLHL3 complex ubiquitinated the GST-WNK4-His protein. However, the KLHL3 mutant failed to ubiquitinate WNK4 as efficiently as wild-type KLHL3. (C) *In vivo* ubiquitination assay of WNK4. Wild-type KLHL3 coexpression increased the WNK4 ubiquitination. However, the KLHL3 mutants were less able to ubiquitinate WNK4.

WNK4. As presented in Fig. 4C, WNK4 ubiquitination by these KLHL3 mutants was lower compared with that by WT–KLHL3.

Following our publication that impaired WNK4 ubiquitination was a common molecular mechanism in PHAII and was caused by mutations in WNK4 (D564A), KLHL3 (R528H), and CUL3 (skipping of exon 9), Shibata et al. analyzed the same R528H mutant

and reported similar results [15]. In addition to R528H, Wu et al. examined other PHAII-causing mutants (A77E, C164F, Q309R, and L387P) in Xenopus oocytes and confirmed that they were less effective in decreasing the WNK4 protein levels compared with WT–KLHL3 [16]. However, with the exception of R528H, the effect of these mutations on WNK4 ubiquitination has not been investigated. A more thorough investigation of these disease-causing mutants of the KLHL3 gene is therefore necessary to establish the pathogenesis of PHAII. Evidence to date indicates these mutations are distributed in different functional domains of the KLHL3 protein and that KLHL proteins other than E3 ligase (e.g., actin-binding protein) may have a pathogenic role in PHAII [10]. The current study clearly demonstrated that each mutation associated with PHAII led to the common consequence of decreased WNK4 ubiquitination, with this effect being mediated through different molecular mechanisms. The mutations in the BTB and BACK domains affected the interaction with CUL3, whereas those in the Kelch domains affected the binding to WNK4. In addition to the impaired binding to WNK4 or CUL3, analysis of S410L in this study suggested that some mutants may be unstable within the cells, which would be expected to impair the functions of KLHL3. Therefore, the data obtained in this study clearly establishes that impaired WNK4 ubiquitination by KLHL3 mutations causes PHAII. Recently, we reported that KLHL2, the KLHL protein member that is closest to KLHL3, could also behave as an E3-ubiquitine ligase with CUL3 [18]. In addition, we observed that KLHL2 had the ability to bind to all WNK kinases, although Ohta et al. reported that KLHL3 could bind and ubiquitinate WNK1 [17]. In this regard, the regulation of WNK kinases by KLHL2 and KLHL3 may occur in various types of cells in the body and may be involved not only in the pathogenesis of PHAII but also in other pathophysiological conditions.

In summary, we clarified the pathogenic mechanisms of PHAII-causing mutations in different domains in KLHL3. Although the molecular mechanisms were different, impaired WNK4 ubiquitination is a common consequence of these mutations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2013.08.035.

References

[1] R.D. Gordon, The syndrome of hypertension and hyperkalemia with normal glomerular filtration rate: Gordon's syndrome, Aust. NZ J. Med. 16 (1986) 183–184.
[2] F.H. Wilson, S. Disse-Nicodeme, K.A. Choate, K. Ishikawa, C. Nelson-Williams, I. Desitter, M. Gunel, D.V. Milford, G.W. Lipkin, J.M. Achard, M.P. Feely, B. Dussol, Y. Berland, R.J. Unwin, H. Mayan, D.B. Simon, Z. Farfel, X. Jeunemaitre, R.P. Lifton, Human hypertension caused by mutations in WNK kinases, Science 293 (2001) 1107–1112.
[3] C. Richardson, D.R. Alessi, The regulation of salt transport and blood pressure by the WNK-SPAK/OSR1 signalling pathway, J. Cell Sci. 121 (2008) 3293–3304.
[4] J.A. McCormick, D.H. Ellison, The WNKs: atypical protein kinases with pleiotropic actions, Physiol. Rev. 91 (2011) 177–219.
[5] S.S. Yang, T. Morimoto, T. Rai, M. Chiga, E. Sohara, M. Ohno, K. Uchida, S.H. Lin, T. Moriguchi, H. Shibuya, Y. Kondo, S. Sasaki, S. Uchida, Molecular pathogenesis of pseudohypoaldosteronism type II: generation and analysis of a WNK4 (D561A/+) knockin mouse model, Cell Metab. 5 (2007) 331–344.
[6] M. Chiga, T. Rai, S.S. Yang, A. Ohta, T. Takizawa, S. Sasaki, S. Uchida, Dietary salt regulates the phosphorylation of OSR1/SPAK kinases and the sodium chloride cotransporter through aldosterone, Kidney Int. 74 (2008) 1403–1409.
[7] A. Ohta, T. Rai, N. Yui, M. Chiga, S.S. Yang, S.H. Lin, E. Sohara, S. Sasaki, S. Uchida, Targeted disruption of the WNK4 gene decreases phosphorylation of Na–Cl cotransporter, increases Na excretion and lowers blood pressure, Hum. Mol. Genet. 18 (2009) 3978–3986.
[8] M. Chiga, F.H. Rafiqi, D.R. Alessi, E. Sohara, A. Ohta, T. Rai, S. Sasaki, S. Uchida, Phenotypes of pseudohypoaldosteronism type II caused by the WNK4 D561A missense mutation are dependent on the WNK-OSR1/SPAK kinase cascade, J. Cell Sci. 124 (2011) 1391–1395.
[9] L.M. Boyden, M. Choi, K.A. Choate, C.J. Nelson-Williams, A. Farhi, H.R. Toka, I.R. Tikhonova, R. Bjornson, S.M. Mane, G. Colussi, M. Lebel, R.D. Gordon, B.A. Semmekrot, A. Poujol, M.J. Valimaki, M.E. De Ferrari, S.A. Sanjad, M. Gutkin, F.E. Karet, J.R. Tucci, J.R. Stockigt, K.M. Keppler-Noreuil, C.C. Porter, S.K. Anand, M.L. Whiteford, I.D. Davis, S.B. Dewar, A. Bettinelli, J.J. Fadrowski, C.W. Belsha, T.E. Hunley, R.D. Nelson, H. Trachtman, T.R. Cole, M. Pinsk, D. Bockenhauer, M. Shenoy, P. Vaidyanathan, J.W. Foreman, M. Rasoulpour, F. Thameem, H.Z. Al-Shahrouri, J. Radhakrishnan, A.G. Gharavi, B. Goilav, R.P. Lifton, Mutations in Kelch-like 3 and Cullin3 cause hypertension and electrolyte abnormalities, Nature 482 (2012) 98–102.
[10] H. Louis-Dit-Picard, J. Barc, D. Trujillano, S. Miserey-Lenkei, N. Bouatia-Naji, O. Pylypenko, G. Beaurain, A. Bonnefond, O. Sand, C. Simian, E. Vidal-Petiot, C. Soukaseum, C. Mandet, F. Broux, O. Chabre, M. Delahousse, V. Esnault, B. Fiquet, P. Houillier, C.I. Bagnis, J. Koenig, M. Konrad, P. Landais, C. Mourani, P. Niaudet, V. Probst, C. Thauvin, R.J. Unwin, S.D. Soroka, G. Ehret, S. Ossowski, M. Caulfield, P. Bruneval, X. Estivill, P. Froguel, J. Hadchouel, J.J. Schott, X. Jeunemaitre, I.C.f.B.P. (ICBP), KLHL3 mutations cause familial hyperkalemic hypertension by impairing ion transport in the distal nephron, Nat. Genet. 44 (2012) 456–460. S451–453.
[11] M. Furukawa, Y. Xiong, BTB protein keap1 targets antioxidant transcription factor Nrf2 for ubiquitination by the Cullin3-Roc1 ligase, Mol. Cell Biol. 25 (2005) 162–171.
[12] Y.R. Lee, W.C. Yuan, H.C. Ho, C.H. Chen, H.M. Shih, R.H. Chen, The Cullin3 substrate adaptor KLHL20 mediates DAPK ubiquitination to control interferon responses, EMBO J. 29 (2010) 1748–1761.
[13] M.Y. Lin, Y.M. Lin, T.C. Kao, H.H. Chuang, R.H. Chen, PDZ-RhoGEF ubiquitination by Cullin3-KLHL20 controls neurotrophin-induced neurite outgrowth, J. Cell Biol. 193 (2011) 985–994.
[14] M. Wakabayashi, T. Mori, K. Isobe, E. Sohara, K. Susa, Y. Araki, M. Chiga, E. Kikuchi, N. Nomura, Y. Mori, H. Matsuo, T. Murata, S. Nomura, T. Asano, H. Kawaguchi, S. Nonoyama, T. Rai, S. Sasaki, S. Uchida, Impaired KLHL3-mediated ubiquitination of WNK4 causes human hypertension, Cell Rep. 3 (2013) 858–868.
[15] S. Shibata, J. Zhang, J. Puthumana, K.L. Stone, R.P. Lifton, Kelch-like 3 and Cullin3 regulate electrolyte homeostasis via ubiquitination and degradation of WNK4, Proc. Natl. Acad. Sci. USA 110 (2013) 7838–7843.
[16] G. Wu, J.B. Peng, Disease-causing mutations in KLHL3 impair its effect on WNK4 degradation, FEBS Lett. (2013).
[17] A. Ohta, F.R. Schumacher, Y. Mehellou, C. Johnson, A. Knebel, T.J. Macartney, N.T. Wood, D.R. Alessi, T. Kurz, The CUL3–KLHL3 E3 ligase complex mutated in Gordon's hypertension syndrome interacts with and ubiquitylates WNK isoforms: disease-causing mutations in KLHL3 and WNK4 disrupt interaction, Biochem. J. 451 (2013) 111–122.
[18] D. Takahashi, T. Mori, M. Wakabayashi, Y. Mori, K. Susa, M. Zeniya, E. Sohara, T. Rai, S. Sasaki, S. Uchida, KLHL2 interacts with and ubiquitinates WNK kinases, Biochem. Biophys. Res. Commun. (2013).
[19] K. Yamauchi, T. Rai, K. Kobayashi, E. Sohara, T. Suzuki, T. Itoh, S. Suda, A. Hayama, S. Sasaki, S. Uchida, Disease-causing mutant WNK4 increases paracellular chloride permeability and phosphorylates claudins, Proc. Natl. Acad. Sci. USA 101 (2004) 4690–4694.
[20] K. Kuroki, S. Kobayashi, M. Shiroishi, M. Kajikawa, N. Okamoto, D. Kohda, K. Maenaka, Detection of weak ligand interactions of leukocyte Ig-like receptor B1 by fluorescence correlation spectroscopy, J. Immunol. Methods 320 (2007) 172–176.
[21] A.X. Ji, G.G. Privé, Crystal Structure of KLHL3 in complex with Cullin3, PLoS One 8 (2013) e60445.
[22] Y. Kigoshi, F. Tsuruta, T. Chiba, Ubiquitin ligase activity of CUL3–KLHL7 protein is attenuated by autosomal dominant retinitis pigmentosa causative mutation, J. Biol. Chem. 286 (2011) 33613–33621.