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Cellular mechanisms of WNK4-mediated regulation of ion transport proteins in the distal tubule

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Gene mutations in WNK4 kinase cause a genetic form of hypertension by affecting multiple ion transport pathways through different mechanisms. Cai *et al.* report that the inhibitory effect of WNK4 on the thiazide-sensitive sodium-chloride cotransporter occurs through the lysosomal degradation pathway in mammalian cells.

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WNK (with no lysine (K)) kinases form a novel serine/threonine protein kinase family characterized by the absence of a conserved lysine residue in the kinase domain.[1] Mutations in the *WNK1* and *WNK4* genes, two members of a family of four, are linked to pseudohypoaldosteronism type II (PHA II; also known as familial hyperkalemic hypertension or Gordon's syndrome; Online Mendelian Inheritance in Man #145260), an autosomal dominant disease.[2] Increased expression of WNK1 caused by deletions in the first intron of the *WNK1* gene, and missense mutations in the *WNK4* gene close to its two coiled-coil domains outside the kinase region, lead to the development of PHA II.[2] Common features of PHA II include hyperkalemia, hypertension, mild metabolic acidosis, low renin, and normal glomerular filtration rate; this condition is a mirror image of Gitelman's syndrome, which is caused by loss of function in the thiazide-sensitive sodium-chloride cotransporter (NCC; SLC12A3, also known as TSC). This is especially true for patients carrying mutations in the *WNK4* gene, who exhibit marked hypercalciuria and sensitivity to thiazide diuretics.[3] These clinical features served as the rationale that led two groups[4,5] to independently report that WNK4 inhibits NCC when expressed in *Xenopus* oocytes, and that some disease-causing mutations of WNK4 exhibit decreased ability to inhibit NCC.

In addition to NCC, WNK4 has also been shown to inhibit renal epithelial ion channels, including the renal outer medullary potassium channel (ROMK)[6] and the transient receptor potential cation channel, subfamily V, member 4 (TRPV4).[7] Interestingly, WNK4 enhances the epithelial calcium channel TRPV5 (previously known as ECaC) (J-B Peng *et al.*, *J Am Soc Nephrol* 2004; **15**: 62A, abstr.), and it increases paracellular Cl^- permeability,[8,9] most likely through phosphorylation of claudins.[8] Most recently, it has been shown that WNK4 phosphorylates the STE20-related serine/threonine kinases SPAK and OSR1[10] and, in turn, regulates the cation-Cl^- cotransporters NKCC1 and KCC2[11] (Figure 1).

The mechanism of WNK4-mediated regulation of renal transporters and channels appears to be multifaceted (Figure 1). In contrast to the phosphorylation of claudins,[8] SPAK, and OSR1,[10] WNK4 regulates membrane transporters and ion channels by affecting plasma membrane abundance, as in the case of NCC,[4,5] ROMK,[6] TRPV4,[7] and TRPV5 (J-B Peng *et al.*, *J Am Soc Nephrol* 2004; **15**: 62A, abstr.). Obviously, the amount of surface expression of a transport protein is an important determinant of ion transport activity, especially with regard to constitutive activity of membrane transport proteins. Surface expression of ion transporters is determined by several steps: biosynthesis, processing, insertion in the plasma membrane, removal from the plasma membrane, and protein degradation. In the case of ROMK, WNK4 facilitates the retrieval of ROMK from the plasma membrane through a clathrin-dependent pathway.[6] This process is actually independent of kinase activity of WNK4, as the kinase-dead mutant exhibits the same effect on ROMK as the wild-type WNK4. In contrast, kinase-dead WNK4 does not affect NCC activity.[5] However, the WNK4 tail containing 222 amino acid residues without the kinase domain is capable of inhibiting NCC, which suggests that kinase activity is not required for the inhibitory effect of WNK4 on NCC.[12] Nevertheless, the cellular mechanism by which WNK4 decreases the membrane level of NCC has not been resolved.

To further understand the regulation of NCC expression by WNK4, Cai *et al.*[13] (this issue) examined the effect of WNK4 on NCC using African green monkey kidney Cos-7 and mouse cortical collecting duct M-1 cells. One advancement achieved in these studies was the use of a mammalian expression system that provided a more physiologically relevant model as compared with expression in *Xenopus* oocytes. The authors confirmed that wild-type WNK4 reduces the surface level of NCC in both Cos-7 and M-1 cells. Quantitative analysis of surface NCC by biotinylation indicated that disease-causing mutants R1185C and E562K partially failed and completely failed, respectively, to reduce the surface expression of NCC. Both wild-type WNK4 and E562K mutants co-immunoprecipitate NCC, indicating that the lack of an effect of

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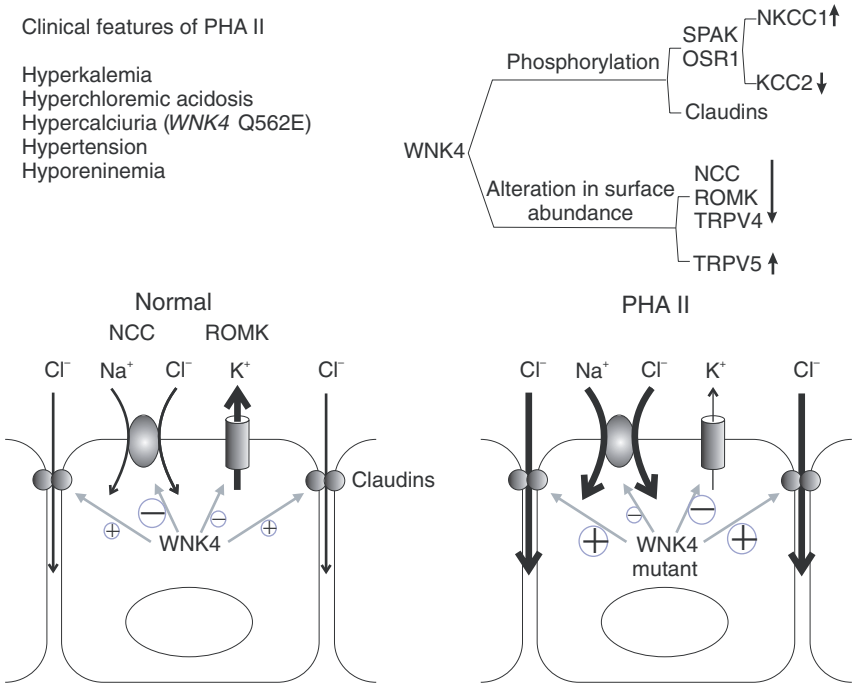

Figure 1 | Connection between clinical manifestation of pseudohypoaldosteronism type II (PHA II) and WNK4 function. WNK4 has been shown to regulate certain ion transport proteins expressed in the kidney, as shown in the upper left. Among them, differential effects between wild-type WNK4 and its disease-causing mutants have been observed for sodium-chloride cotransporter (NCC), renal outer medullary potassium channel (ROMK), and paracellular chloride permeability, as shown in the lower panel. There are different cellular mechanisms of WNK4 regulation of ion transport proteins (see text): NCC is inhibited by WNK4 through enhancement of its lysosomal degradation; ROMK is inhibited by enhancement of the clathrin-dependent endocytosis; and claudins are phosphorylated in the presence of WNK4. Ion transport activity is indicated by the thickness of the lines; the magnitude of WNK4's effect is indicated by the size of the plus and minus symbols. For simplicity, NCC and ROMK are shown in the same cell.

E562K on WNK4 was not due to a loss of interaction. The authors next examined whether clathrin-mediated endocytosis of NCC was affected by WNK4 using co-transfection of dynamin 2 and its dominant-negative mutant K44A, which arrest clathrin-coated pits at the plasma membrane. Interestingly, the surface level of NCC was not affected by either dynamin 2 or the K44A mutant in the presence or absence of WNK4. Therefore, the clathrin-dependent pathway does not appear to be involved in the effects of WNK4 on NCC. Lastly, bafilomycin, a vacuolar proton pump inhibitor, which may disrupt the acidification of lysosomes, dose-dependently diminished the inhibitory effect of WNK4 on the steady-state level of NCC expression. Thus it is likely that WNK4 causes the degradation of NCC before it is inserted into the plasma membrane. Hence, Cai *et al.*[13] provide evidence that the action of WNK4 on NCC is through facilitation of degradation of this protein but not through the clathrin-dependent endocytosis. This is in striking contrast to the action of WNK4 on ROMK.[6]

The study by Cai *et al.*[13] suggests a novel mechanism of WNK4-mediated regulation of an ion transporter. It remains to be determined how WNK4 acts on the lysosomal pathway, and what type of interaction occurs between WNK4 and NCC in this process. Ellison and co-workers reported that a 222-amino acid WNK4 carboxyl-terminal fragment could inhibit NCC.[12] How the E562K mutation, which is upstream of the carboxyl terminal, could affect the lysosomal pathway remains an issue to be investigated.

It is intriguing that WNK4 acts on different membrane transport proteins through different mechanisms. It is conceivable that the effects of WNK4 depend, at least to some extent, on the specific regulatory mechanisms that govern the activity and distribution of individual transporters. For example, WNK4 regulates ROMK through the clathrin-dependent endocytosis pathway, as ROMK is constitutively regulated by this pathway in the absence of WNK4. On the other hand, as Cai *et al.* show,[13] NCC is not significantly affected by the clathrin-dependent endocytosis pathway in the absence or presence of WNK4. WNK4 regulates NCC through the lysosomal pathway. This may also help to explain the results obtained in the *Xenopus* oocyte expression system, where NCC is negatively regulated by WNK4 whereas TRPV5 is positively regulated by the same kinase. These opposite effects of WNK4 on NCC and TRPV5 are consistent with the well-known inverse relationship between sodium and calcium transport in the distal tubule. This suggests that the nature of regulation by WNK4 is, at least in part, coded within the transport protein.

Lessons learned from *Xenopus* oocyte and mammalian cell experiments serve to help our understanding of the molecular mechanism of PHA II (Figure 1). The increase in NCC plasma membrane expression that results from disease-causing WNK4 mutations as shown by Cai *et al.*[13] as well as previous studies[4,5,12] supports the idea that NCC regulation plays an important role in the pathogenesis of PHA II. The disease-causing mutants of WNK4 enhance NCC-mediated sodium chloride reabsorption and decrease ROMK-mediated potassium secretion, leading to volume expansion, hyperkalemia, and hypertension. In addition, it is worth noting that the paracellular pathway for ion transport is also affected by WNK4. The chloride-shunt hypothesis proposed by Schambelan *et al.*[14] has gained experimental support by the demonstration that disease-causing mutants of WNK4 lead to an increase in chloride permeability in MDCK cells.[8,9] According to this hypothesis, the increased chloride permeability in the distal tubule would decrease the electrical driving force for potassium and proton secretion, which would lead to hyperkalemia and acidosis.

Hypercalciuria is a feature of patients carrying the *WNK4* Q562E mutation and is not present in patients carrying *WNK1* mutations. As disease-causing mutations of WNK4 enhance TRPV5 to an extent comparable to that of wild-type WNK4 (J-B Peng *et al.*, *J Am Soc Nephrol* 2004; **15**: 62A, abstr.), it may be that other factors come into play in the hypercalciuria seen in PHA II. Distal tubular calcium reabsorption is inversely related to sodium reabsorption, and therefore the increase in calcium excretion may be linked to the elevation in distal tubular sodium reabsorption. In addition, the transcellular epithelial calcium channels TRPV5 and TRPV6 are also inhibited by acidosis.

Finally, WNK1 is also a regulator of the ion transporters ENaC and ROMK, which may provide a basis for the role of WNK1 in the molecular pathogenesis of PHA II. New lines of evidence indicate that WNK1 and WNK4 are upstream of two STE20-type kinases, SPAK and OSR1, that regulate members of the SLC12A family of electroneutral cation-Cl^- cotransporters.[10,11] Thus, identifying at a cellular and molecular level the differences between wild-type WNK4 and disease-causing mutants and their effects on transport protein activity and expression levels should provide further insight into the mechanisms underlying the pathogenesis of PHA II.

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Chronic kidney disease in the elderly: is it really a premise for overwhelming renal failure?

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Increasingly, the majority of patients being diagnosed as affected by chronic kidney disease (CKD) are elderly. Nonetheless, only a rather small proportion of elderly CKD patients actually progress to end-stage renal disease, whereas many more die before this stage is reached, largely because of cardiovascular disease. This underscores the urgent need for cardiovascular prevention, even more importantly than for renoprotection, among elderly patients with CKD.

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Chronic kidney disease (CKD) is becoming increasingly common. The United States Renal Data System has reliably estimated that the number of patients on maintenance dialysis in the United States will double over the next few years,[1] and a relatively large number of newly diagnosed CKD patients every year (incident CKD patients) are elderly. In Europe, an epidemiological survey of the Île-de-France area showed a striking age-related increase in the annual incidence of CKD (that is, the first evidence of serum creatinine levels greater than 2 mg per dl in the course of 1 year): the incidence rate among patients aged more than 75 years was almost seven times that of patients aged 20–39 years (619 versus 92 new cases per million population) and more than twice that of patients aged 40–59 years (619 versus 264 new cases per million population).[2] The increased incidence of CKD among the elderly translates into a similarly increased prevalence: the Third National Health and Nutrition Examination Survey (NHANES III) of a nationally representative sample of adults in the United States between 1988 and 1994 found that 7.6% of the individuals aged 60–69 years, and 25.9% of those aged at least 75 years, had a glomerular

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