Life and death of the distal nephron: WNK4 and NCC as major players

Missense mutations in the WNK4 gene lead to the development of familial hyperkalemic hypertension, a rare form of human hypertension. It was shown in vitro that WNK4 regulates the surface expression and activity of a number of ion channels and transporters. The in vivo analysis of wild-type and mutant WNK4 overexpression in transgenic mice models demonstrated that this serine-threonine kinase controls ion handling in the kidney mainly, and probably exclusively, through the regulation of the NaCl cotransporter NCC activity.

Familial hyperkalemic hypertension (FHHt), also called Pseudohypoaldosteronism type II (PHAII), is a rare autosomal dominant form of arterial hypertension, characterized by hypertension, hyperkalemia, mild hyperchloremia, and metabolic acidosis despite normal renal glomerular filtration (Gordon et al., 1995). In 2001, the genetic analysis of a few affected families led to the identification of two new and unsuspected potential regulators of blood pressure, WNK1 and WNK4 (Wilson et al., 2001). They belong to a recently identified family of serine-threonine kinases, the with no lysine (K) (WNK) kinase family (Xu et al., 2000). In addition to the kinase domain, these proteins contain an autoinhibitory domain, two coiled-coil domains, and three proline-rich domains. WNK4 mutations responsible for FHHt correspond to amino acid changes located in small (about 10 amino acids) and highly conserved sequences, just downstream of the two coiled-coil domains (Wilson et al., 2001). Coiled-coil domains being generally described as protein-protein interaction domains, the mutations are supposed to affect the interaction of WNK4 with its partners.

The clinical and biological phenotype of FHHt corresponds to an altered ionic transport in the renal distal tubule. Affected subjects are highly sensitive to thiazide diuretics suggesting a deregulation of NCC activity, the thiazide-sensitive sodium-chloride cotransporter. Indeed, it was shown that WNK4 inhibits NCC activity in Xenopus oocytes by decreasing its surface expression and that mutated WNK4 lost this ability to regulate NCC (Wilson et al., 2003). Other in vitro studies, in Xenopus oocytes or cultured renal cells, showed that the regulatory role of WNK4 extends to the regulation of a wide range of transport systems expressed in the distal nephron (Hadchouel et al., 2006). WNK4 could inhibit the activity of the renal apical K+ channel ROMK, the basolateral isoform of the Na⁺-K⁺-2Cl⁻ cotransporter (NKCC1), the apical Cl⁻/HCO₃⁻ exchanger CFEX and TRPV4 (Fu et al., 2006), again by decreasing their surface expression. Interestingly, FHHt mutants have a stronger inhibitory effect on ROMK than wild-type WNK4. Finally, WNK4 was shown to stimulate the paracellular chloride transport, via phosphorylation of members of the claudin family that encode tight junction proteins. Taken together, these studies suggest that WNK4 plays a new and unsuspected role in the regulation of a number of renal ion transporters and channels. However, this hypothesis and the hierarchy between the variety of effects shown in vitro awaited in vivo confirmation. The group of Richard Lifton very recently published such an in vivo study and thus provided the first integrated view of WNK4 role in vivo (Lalioti et al., 2006).

The authors demonstrated that overexpression of a mutant (Q562E) WNK4 (WNK4PHAII transgene) in transgenic mice reproduces the phenotype observed in FHHt patients, i.e., hypertension, hyperkalemia, hyperchloremia, metabolic acidosis and hypercalciuria. In contrast, overexpression of a wild-type WNK4 (WNK4WT transgene) led to hypotension, hypocalciuria, a modest reduction in chloremia and a tendency to hypokalemia. Moreover, the authors showed that overexpression of WNK4, wild-type or mutated, had consequences on the morphology of the distal convoluted tubule (DCT) and the surface expression of NCC. There was a marked hyperplasia in the DCT accompanied by increased apical expression of NCC in mice overexpressing WNK4PHAII and a marked hypoplasia in those overexpressing WNK4WT, similar to that observed in mice lacking NCC (Loffing et al., 2004; Schultheis et al., 1998). However, there was no change in ROMK expression in WNK4PHAII mice while overexpression of WNK4WT led to an increase in ROMK expression at the apical surface. The impaired regulation of plasma potassium in these transgenic animals could therefore be due to altered ionic cellular concentration in the distal tubule following variations of NCC activity rather than to a direct effect of WNK4 on ROMK (Figure 1).

To test this hypothesis, the authors used two strategies. They first described the effect of the WNK4PHAII transgene on blood pressure level and ion handling in SLC12A3−/− mice, the SLC12A3 gene encoding NCC. They showed that kalemia was restored to normal. In contrast, the animals presented a hypochloremia and a severe hypercalciuria. Moreover, they were hypotensive, with blood pressure level similar to that of WNK4WT animals. Finally, DCT morphology remained as seen in SLC12A3−/− mice, with the proximal part of the DCT being virtually eliminated. The phenotype seen in the Tg(WNK4PHAII)/SLC12A3−/− animals is therefore similar to that of SLC12A3−/− mice (Loffing et al., 2004; Schultheis et al., 1998). In parallel, transgenic WNK4PHAII animals received a short-term treatment with hydrochlorothiazide, an inhibitor of NCC, which corrected both the hyperkalemia and the hypercalciuria. DCT morphology was not analyzed nor was the blood pressure measured in these animals. Finally, the authors showed that wild-type and mutated WNK4 interact by coimmunoprecipitation, suggesting that WNK4 mutants probably have a dominant negative effect on the wild-type protein activity.

Taken together, these transgenic models indicate that FHHt clinical and biochemical phenotype is mainly, if not exclusively, the consequence of a deregulation of NCC activity. This is particularly
interesting in the context of the regulation of potassium secretion. While in vitro studies suggested that WNK4 could directly regulate ROMK surface expression and activity, these in vivo studies imply that impaired K+ secretion could result from a decreased sodium reabsorption by the epithelial sodium channel ENaC following increased NCC activity (Figure 1). From a more general point of view, this study has very important repercussions for the understanding of water/salt homeostasis and electrolyte handling by the kidney since it may provide a response to what the authors call a ‘physiological paradox’. They suggest that WNK4 could be a determinant of the choice faced by the kidney between maximal NaCl reabsorption and K+ secretion in response to aldosterone secretion and that this determination could be mediated by the modulation of NCC activity only. O’Reilly et al. recently described the effect of sodium or potassium dietary challenges as well as aldosterone infusion on WNK4 expression (O’Reilly et al., 2006). Using real-time PCR and semiquantitative in situ hybridisation, they showed that WNK4 expression is upregulated when K+ intake is increased. In contrast, chronic aldosterone excess or a low sodium diet has no effect on WNK4 expression. This is surprising since these three challenges induce a hyperaldosteronism. Lalioti et al. (2006) suggest a role for angiotensin II in the regulation of WNK4 expression or activity but this hypothesis does not explain why an infusion of aldosterone has no effect on WNK4 while a high-K+ diet does since AngII level is similar in both conditions. Deciphering the mechanisms by which these different stimuli regulate WNK4 expression is required to better understand the role played by this kinase in the coordination of ion handling by the kidney.

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Selected reading

Figure 1. Effect of WNK4 surexpression on ionic transport and morphology of the distal convoluted tubule

A) In physiological conditions, WNK4 regulates the balance between apical and cytoplasmic NCC. Na+-reabsorption is then mediated in part by NCC and in part by ENaC. The latter creates a lumen-negative potential, which drives K+-secretion by ROMK.

B) When mutant WNK4 is overexpressed, NCC apical expression and activity are increased resulting in a decrease in Na+ reabsorption by ENaC and the concomittant K+ secretion by ROMK. Moreover, the DCT becomes hyperplastic.

C) In contrast, when wild-type WNK4 is overexpressed, NCC apical expression and activity are decreased, the DCT becomes hypoplastic and Na+ is reabsorbed mainly by ENaC. The resulting lumen-negative potential causes an increase in K+ secretion by ROMK.
Banking on ATM as a new target in metabolic syndrome

In this issue of Cell Metabolism, Semenkovich and his colleagues show that ATM, a protein well known for its roles in the cellular response to DNA breaks, may also be linked to metabolic and cardiovascular diseases (Schneider et al., 2006). ATM seemingly does this by inhibiting JNK, a stress kinase involved in inflammation with related effects in insulin resistance and atherosclerosis. In an interesting twist, the authors show that chloroquine, an antimalarial drug, also activates ATM, which seemingly does this by inhibiting JNK, a stress kinase involved in inflammation with related effects in insulin resistance and cardiovascular effects. These findings provide potential new insights into the pathogenesis and treatment of metabolic syndrome.

Ataxia telangiectasia (A-T) is a progressive, neurodegenerative disease that typically presents itself in the first decade with delayed development of motor skills, including poor balance and slurred speech. About 20% of patients with A-T develop cancer, most often acute lymphocytic lymphoma or leukemia, and many are immunodeficient and sensitive to radiation. A-T may be associated with varying degrees of insulin resistance and a relatively mild impairment in glycemc control (Ristow, 2004).

A-T is incurable and at this point there is not even a good way to slow progression of the disease. A-T is caused by mutations in Atm (ataxia telangiectasia mutated), the gene that encodes ATM (Savitsky et al., 1995). Over 500 mutations causing A-T have been identified; most affected individuals are compound heterozygotes. ATM is a large, modular protein with a serine/threonine kinase domain and a binding site for the tumor suppressor, p53. ATM senses breaks in double-stranded DNA and coordinates cell cycle checks prior to repair.

Schneider et al. (2006) examine the relationship between ATM deficiency and metabolism in mice, looking specifically at aspects of the metabolic syndrome such as insulin resistance, adiposity, blood pressure, circulating cholesterol and lipid levels, and atherosclerosis. Convincing data show that even a 50% reduction in gene dosage and probably protein concentration affects a large number of metabolic parameters. Fat mass, blood pressure, fasting, and postchallenge glucose and insulin concentrations were elevated, insulin sensitivity was decreased, and atherosclerotic lesions were more prominent in Atm−/+ and Atm−/− mice.

These pathophysiological changes were accompanied by concordant biochemical changes in a variety of tissues and cell types. For example, decreased insulin signaling accompanied reduced ATM activity in muscle, liver, and isolated macrophages. In aorta, there were decreases in p38, AKT, and ERK activities, but increases in inhibited IRS-1 and JNK activity, a serine/threonine kinase that inhibits IRS-1 (Figure 1).

The investigators next took advantage of an interesting in vitro finding, that the antimalarial chloroquine stimulates ATM activity in the absence of DNA strand breaks (Bakkenist and Kastan, 2003). They took this a step further by using a small dose of chloroquine to activate ATM in mice, which decreased atherosclerotic lesion area in Apoe−/− mice and insulin and glucose levels in ob/ob and db/db mice. Consistent with ATM as the pharmacological target, chloroquine treatment decreased JNK activity in macrophages from Atm−/+ but not Atm−/− mice, and improved blood pressure and glycemic control in Atm−/+ but not Atm−/− mice.

It is noteworthy that a 50% reduction in gene dose in Atm−/+ mice significantly affected several metabolic parameters, and it is undoubtedly important from a mechanistic viewpoint that many of the parameters studied in heterozygous and homozygous mice were equivalently affected. This is in distinct contrast with what is seen in patients with A-T, where both alleles are mutated and heterozygous carriers are unaffected. It is doubtful that drugs such as chloroquine would benefit patients with A-T. Heterozygous carriers do however have an increased risk for developing certain cancers, most notably breast cancer (Swift et al., 1987). These new findings (Schneider et al., 2006) suggest that drugs such as chloroquine that upregulate ATM activity might provide prophylactic benefit to carriers by decreasing cancer risk. Of course this needs to be formally tested, but this could be important for a substantial portion of the population, as 0.5%–2.0% are suspected of being carriers.

The increase in metabolic and cardiovascular abnormalities seen in Atm−/+ mice predicts a greater than previously appreciated risk for diabetes, hypertension, and cardiovascular disease in A-T family members and other carriers. One would expect that these individuals too...