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See related article, “Cubilin Is Essential for Albumin Reabsorption in the Renal Proximal Tubule,” on pages 1859–1867.

An Emerging Role for SPAK in NCC, NKCC, and Blood Pressure Regulation

Aylin R. Rodan and Chou-Long Huang
Division of Nephrology, Department of Medicine, University of Texas Southwestern Medical Center, Dallas, Texas

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The cloning of the related sodium chloride (NCC) and sodium potassium 2 chloride (NKCC) co-transporters in the early 1990s ushered in a new era for understanding the mechanisms of renal salt handling. Recent research has focused on the phosphorylation of NCC and the two NKCC co-transporters: NKCC1, which is widely expressed, and NKCC2, which is renal specific. Two closely related kinases, STE20/SPS1-related proline/alanine-rich kinase (SPAK) and oxidative stress-responsive kinase-1 (OSR1), seem to play a key role in NCC, NKCC1, and NKCC2 phosphorylation. All three co-transporters are phosphorylated by SPAK and OSR1 on N-terminal cytoplasmic serines and threonines that are conserved among NCC, NKCC1, and NKCC2, and phosphorylation of these residues results in co-transporter activation.

The importance of these phosphorylation events for NCC activity was first demonstrated in Xenopus oocytes, in which mutation of these residues to nonphosphorylatable alanines reduced baseline NCC activity as well as stimulation of NCC by intracellular chloride depletion. Similar results were then observed in mammalian cells by Richardson et al., who also showed that, like NCC, SPAK and OSR1 were activated by low intracellular chloride, and mutation of the SPAK/OSR1-binding site of NCC abolished NCC phosphorylation. These results suggest that specific stimuli activate SPAK and OSR1, which in turn activate NCC by phosphorylating N-terminal threonines and serines.

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Correspondence: Dr. Aylin R. Rodan, Department of Medicine, UT Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75390-8856. Phone: 214-648-8627; Fax: 214-648-2071; E-mail: aylin.rodan@utsouthwestern.edu

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The link between SPAK and NCC is strengthened by the report of Yang et al. in this issue of JASN. The authors generated SPAK null mice to demonstrate decreased levels of total NCC as well as decreased NCC phosphorylation on the N-terminal serines and threonines. This would predict a decrease in NCC activity. Consistent with this idea, SPAK null mice exhibit a phenotype similar to patients with Gitelman syndrome caused by mutations in NCC: Low BP, elevated aldosterone levels, hypokalemia with increased fractional excretion of potassium, hypochloremia, hypomagnesemia with increased fractional excretion of magnesium, and hypocalciuria. Further evidence supporting a decrease in renal NCC activity is the lack of response to hydrochlorothiazide in the SPAK null mice.

Because SPAK is also known to phosphorylate and activate NKCC1 and NKCC2, the authors investigated these co-transporters as well. NKCC1 phosphorylation in aorta decreased in the SPAK null mice, and aortic rings from these animals demonstrate decreased contractile force in response to phenylephrine compared with aortic rings from wild-type mice. The authors propose that this deficiency contributes to the hypotension observed in the SPAK null mice, although more definitive proof of this would require tissue-specific SPAK knockout in the vasculature.

Surprisingly, the authors also found that NKCC2 phosphorylation is increased. They attribute this finding to compensatory upregulation of OSR1. However, in two recent reports in which SPAK was unable to be activated or is missorted, phosphorylation of NKCC2 was decreased. Indeed, mice in which SPAK is missorted in thick ascending limb as a result of absence of the intracellular sorting receptor SORLA have a selective decrease in NKCC2 phosphorylation, whereas NCC phosphorylation is preserved. These mice have a phenotype reminiscent of human Bartter syndrome, which can be caused by loss-of-function mutations in NKCC2, suggesting that loss of NKCC2 phosphorylation results in decreased NKCC2 activity.

What are the upstream events leading to NCC phosphorylation? Stimuli that activate renal salt retention, such as angiotensin II, aldosterone, vasopressin, and low-salt diet, result in N-terminal phosphorylation of NCC in rodents. Furthermore, these stimuli also result in the phosphorylation of SPAK/OSR1 on a conserved threonine in the activation loop. In vitro, cell culture, and Xenopus oocyte studies show that members of the with-no-lysine [K] (WNK) family of kinases, including WNK1 and WNK4 and likely WNK3, phosphorylate SPAK and OSR1 on the activation loop threonine, thereby activating SPAK and OSR1. This finding suggests that WNKs are positive regulators of SPAK and OSR1, which then are positive regulators of NCC and NKCCs.

Consistent with the positive regulation of SPAK and OSR1 by WNKs, mice with a hypomorphic loss-of-function allele of WNK4 have modestly decreased levels of OSR1 phosphorylation (SPAK phosphorylation was not examined), decreased NCC...
phosphorylation, renal sodium wasting, and low BP when awake. In addition, knock-in mice, in which the activation loop threonine of endogenous SPAK is mutated to alanine, thereby abolishing the ability of WNKs to activate SPAK, have low BP, decreased NCC phosphorylation, and a Gitelman phenotype, with renal salt wasting, hypomagnesemia, hypokalemia, and hypocalciuria.

In contrast, several groups have shown that WNK4 is a negative regulator of NCC in Xenopus oocytes and mammalian cells. Specifically, WNK4 coexpression with NCC results in decreased cell surface NCC, and overexpression of WNK4 in a transgenic mouse model results in hypotension (reviewed in reference 4). Thus, it seems that WNK4 can have either an inhibitory effect on NCC by decreasing its phosphorylation, or a stimulatory effect through activation of SPAK/OSR1 and phosphorylation of NCC. The article by Yang et al., together with the findings of the WNK4 hypomorphic loss-of-function mice and the nonactivating SPAK knock-in mice, support the idea that WNK4 is a positive regulator of NCC through SPAK/OSR1 in vivo. Further experimentation is required to advance this hypothesis. Stimulation of negatively charged glutamate for the activation loop threonine, a phosphomimicking mutation, results in SPAK/OSR1 activation. If such a mutant rescues phenotypes caused by loss-of-function WNK alleles, then this would strongly suggest a pathway in which WNKs activate SPAK in vivo. Indeed, this is already demonstrated in Cae- norhabditis elegans. There is also debate as to whether mutations in WNK4 causing pseudohypoaldosteronism type II (PHAII), an autosomal dominant disorder characterized by hypertension and hyperkalemia as a result, at least in part, of NCC overactivity, result in an increase or a decrease in normal WNK4 function. Because the PHAII phenotype is the mirror image of the Gitelman phenotype and SPAK loss-of-function results in a Gitelman phenotype, one would predict that SPAK activity would increase in PHAII. Indeed, knock-in mice carrying a point mutation in WNK4 homologous to a human PHAII mutation have increased phosphorylation of SPAK and OSR1, consistent with increases in SPAK and OSR1 activation. These PHAII mice could be crossed to the SPAK loss-of-function mice. If the phenotypes observed recapitulate those seen in the SPAK loss-of-function mice, then this would suggest that PHAII WNK4 mutations have their pathogenic effect through activation of SPAK. Several other unanswered questions remain. Oocyte and cell culture studies suggest complex interactions among WNK1, WNK3, and WNK4, despite similar effects on SPAK and OSR1. How this plays out in vivo has not been determined and is complicated by the multiple WNK and SPAK/OSR1 family members. There are also additional regulators of SPAK, including PKCδ, PKCβ, and the intracellular sorting receptor SORLA; how and whether these intersect with the WNK-SPAK pathway is unclear. Finally, the mechanisms by which signals such as angiotensin II, aldosterone, or vasopressin results in WNK and/or SPAK activation need study. In summary, the article by Yang et al. adds to our growing understanding of the role of SPAK in the pathophysiology of hypertension and may allow the eventual development of novel therapeutics.

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DISCLOSURES

None.

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Rate of Kidney Function Decline Associates with Increased Risk of Death

Csaba P. Kovesdy
Division of Nephrology, Salem Veterans Affairs Medical Center, Salem, Virginia; and Division of Nephrology, University of Virginia, Charlottesville, Virginia

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The current classification scheme for chronic kidney disease (CKD) does not differentiate between patients who show a progressive course of CKD and those whose kidney function remains stable over a long period. There is an association of faster progression of CKD with worse mortality, suggesting that an individual’s slope of estimated GFR (eGFR) over time could help us refine the prognosis for patients with any given stage of CKD. Many questions still need to be answered before the mainstream application of the slopes of eGFR is possible.

The estimation formula-based CKD classification by the Kidney Disease Outcomes Quality Initiative (K/DOQI) has proved to be an eye opener to the medical community and has raised awareness about the common frequency of decreased eGFR and the high mortality associated with lower eGFR.

The immediate impact of this awareness has been a significant increase of clinical referrals to nephrologists from various practitioners who suddenly discovered that many of their patients had CKD, a condition that became easy to diagnose but whose management still befuddles many nonspecialists. Those of us on the receiving end of these consultations have quickly come to realize that some patients who are labeled as having CKD received a misdiagnosis because of deficiencies in our estimation formulas or of standardization of serum creatinine measurements, and many patients, of course, who indeed have a GFR of <60 ml/min per 1.73 m² display a stable and asymptomatic course with no change in metabolic disturbances characteristic of CKD. How to deal with the latter group has been a challenge from both a prognostic and a therapeutic point of view.

Prognostic studies that show decreased kidney function to associate with increased mortality are mostly based on a static definition of CKD and bundle together patients who might have had progressive or nonprogressive disease. More important, though, nephrologists assessing patients with mild and nonprogressive CKD are often faced with questions about how much to treat, because interventions aimed at delaying progression are obviously moot and the utility of therapies targeting mild metabolic derangements of CKD is questionable.

A practical solution to these deficiencies of the static K/DOQI classification for CKD is to identify patients with progressive loss of kidney function by using past serial measurements of their serum creatinine. Plotting slopes of 1/creatinine or eGFR versus time has long been a tool of nephrologists, and slopes have been used as an outcome variable in clinical studies, yet they have attracted surprisingly little attention as independent predictors of clinical outcomes such as mortality or risk for ESRD.

The study by Al-Aly et al. in this issue of *JASN* fills a void in this sense because it expands to a select group of patients with early stage 3 CKD (estimated GFR 45 to 60 ml/min per