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EDITORIAL

Perspective of future drugs targeting sterile 20/SPS1-related proline/alanine-rich kinase for blood pressure control

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Abstract

According to a genome-wide association study, intronic SNPs within the human sterile 20/SPS1-related proline/alanine-rich kinase (*SPAK*) gene was linked to 20% of the general population and may be associated with elevated blood pressure. As cell volume changes, mammalian SPAK kinases respond to phosphorylate and regulate cation-coupled chloride co-transporter activity. To our knowledge, phosphorylation of upstream with-no-lysine (K) (WNK) kinases would activate SPAK kinases. The activation of WNK-OSR1/SPAK cascade on the kidneys and aortic tissue is related to the development of hypertension. Several regulators of the WNK pathway such as the Kelch kinase protein 3 - Cullin 3 E3 ligase, hyperinsulinemia, and low potassium intake to mediate hypertension have been identified. In addition, the SPAK kinases may affect the action of renin-angiotensin-aldosterone system on blood pressure as well. In 2010, two SPAK knock-in and knock-out mouse models have clarified the pathogenesis of lowering blood pressure by influencing the receptors on the kidneys and aortic smooth muscle. More recently, two novel SPAK inhibitors for mice, Stock 1S-14279 and Closantel were discovered in 2014. Targeting of SPAK seems to be promising for future antihypertensive therapy. Therefore we raised some viewpoints for the issue for the antihypertensive therapy on the SPAK (gene or kinase).

Key words: With-no-lysine (K) kinase; Oxidative stress-responsive kinase 1/SPS1-related proline/alanine-rich kinase kinase; Na-Cl co-transporter; Na^+-K^+-$2Cl^-$ cotransporter; Hypertension

Core tip: According to a genome-wide association study, intronic SNPs within the human sterile 20/SPS1-related proline/alanine-rich kinase (*SPAK*) gene was linked to 20% of the general population and may be associated with elevated blood pressure. Based on current studies, targeting of SPAK seems to be promising for future antihypertensive therapy. Therefore, we raised some viewpoints regarding the issue for antihypertensive therapy on the SPAK (gene or kinase).

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THE WITH-NO-LYSINE (K) KINASES AND HYPERTENSION AND HYPERKALEMIA

Pseudohypoaldosteronism type Ⅱ, a disease characterizing hypertension with hyperkalemia has been caused by mutations in WNK [with-no-lysine (K)] 1 and WNK4[1]. WNK4 is predominantly produced in the kidneys and epithelial tissues and hence the expression of WNK4 is more restricted than that of WNK1. WNK4 has been shown as a potent inhibitor of diverse epithelial transporters including the renal outer medullary potassium ion channel and the thiazide-sensitive sodium chloride co-transporter (NCC)[2]. In addition, paracellular chloride ion flux is enhanced by WNK4 activity[2]. Importantly, mutations in WNK4 have divergent effects on these transport pathways. WNK4 mutations could increase the inhibition of the renal outer medullary potassium ion channel, relieve the inhibition of NCC, and further promote paracellular chloride ion flux[2,3]. These findings can support a model in which WNK4, as a molecular switch, can alter the balance between potassium ion secretion and chloride ion reabsorption and explain the physiological abnormalities in patients with pseudohypoaldosteronism type Ⅱ. Other WNK kinases also distribute in diverse epithelia throughout the body and are involved in chloride ion flux, suggesting that these kinases may generally participate in the regulation of chloride ion flux.

MOLECULAR PATHWAYS FOR WNK-SPAK/OSR1-NCC/NKCC TO CONTROL BLOOD PRESSURE

As cell volume changes, mammalian SPAK (SPS1-related proline/alanine-rich kinase) and OSR1 (oxidative stress-responsive kinase 1) kinases respond to phosphorylate and regulate cation-coupled chloride cotransporter activity[4]. Phosphorylation of upstream WNK kinases would activate SPAK and OSR1. There are four mammalian WNK kinases: WNK1-WNK4. In humans, WNK1 and WNK4 mutations result in hyperkalemia and hypertension partly by altering renal sodium and potassium transport. WNK1 and WNK4 recruit an endocytic scaffold protein, intersectin, and thereby stimulate endocytosis of ROMK1. This recruitment occurs between the PXXP motif of the WNKs and the SH3 domain of intersectin which is independent of WNK kinase activity. WNKs regulate cation-chloride-coupled cotransporters, Na^+-K^+-2Cl^- cotransporter (NKCC) 1 and NKCC2 (and NCC, under some conditions) dependent on kinase activity[5]. OSR1 and SPAK, two Ste20-related protein kinases, which are bound with and phosphorylated by WNK1 and WNK4, in turn bind with and phosphorylate cation-chloride-coupled cotransporters to increase their activity. Binding of OSR1/SPAK to upstream WNKs and downstream cation-chloride-coupled cotransporters are both mediated by a docking site in the C-terminus of OSR1/SPAK and RFX[V/I] motifs present in WNKs or in NKCCs and NCC[5].

Several regulators of the activation of WNK kinase have been identified in recent animal studies as the Kelch kinase protein 3-Cullin 3 E3 ligase, low potassium intake, hyperinsulinemia, and some hormones (angiotensin Ⅱ, aldosterone and vasopressin), which may act on the kidneys or aortic tissues to affect blood pressure[6-10]. Chávez-Canales *et al*[11] showed that WNK4 could decrease the WNK1 and WNK3-mediated activation of NCC in the kidneys. This finding suggests that WNK kinases form a network in which WNK4 associates with WNK1 and WNK3 to regulate NCC. In addition, the activity of OSR1/SPAK in the kidneys could be enhanced by AMP-activated protein kinase resulting in sodium retention *via* phosphorylation of NKCC2 in obesity[12]. The effect of vasopressin on sodium reabsorption is mediated by SPAK along the distal nephron to control blood pressure as well[13]. Figure 1 shows the potential mechanisms of hypertension related to the WNK-SPAK/OSR1-NCC/NKCC cascade.

SPAK KNOCK-IN AND KNOCK-OUT MOUSE MODELS AND THE EXPRESSION AND FUNCTION OF NCC/NKCC IN THE KIDNEY AND AORTIC TISSUE

Since intronic SNPs within the human *SPAK* gene (also known as *Stk*39) was linked to 20% of the general population and may be associated with hypertension in a genome-wide association study, targeting of SPAK seems to be promising for future

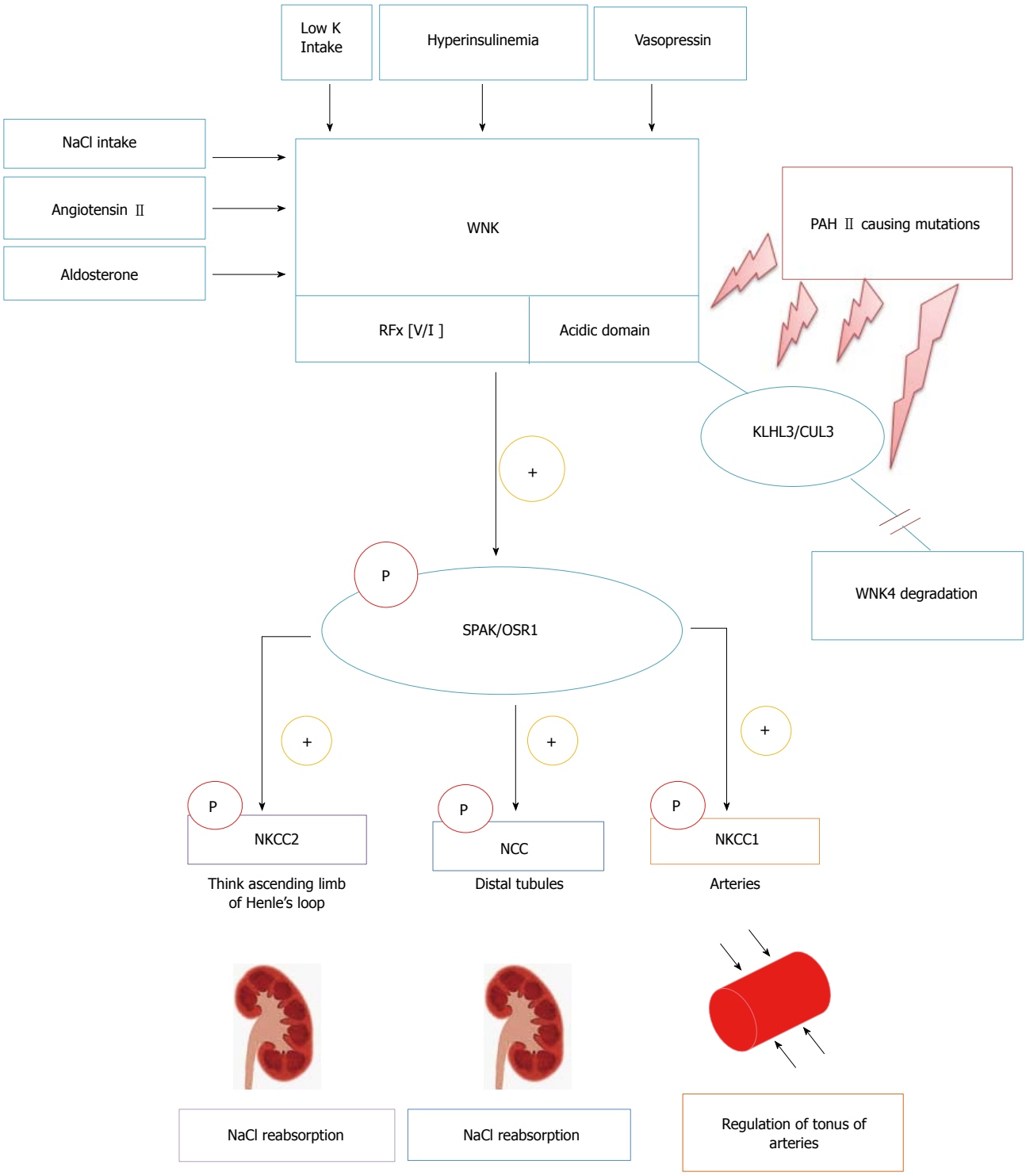

Figure 1 Potential mechanisms of With-no-lysine (K) - SPS1-related proline/alanine-rich kinase/oxidative stress-responsive kinase 1 - Na-Cl co-transporter/ Na^+-K^+-2Cl ($^-$) cotransporter to contribute to hypertension. Several regulators of the activation of WNK cascade, such as KLHL3/CUL3, low potassium intake, hyperinsulinemia and some hormones (angiotensin Ⅱ, aldosterone and vasopressin) may act on the kidneys or aortic tissues. SPAK and OSR1 are activated *via* phosphorylation by upstream WNK kinases using docking site in the RFX (V/I). As a result, SPAK/OSR1 may regulate cation-chloride-coupled cotransporters in kidneys, tonus of aortic tissues, and blood pressure. PAH Ⅱ causing mutations in acidic domain of WNK4, KLHL3 and Cullin 3 activate SPAK/OSR1-NCC signaling by decreasing WNK4 degradation and accumulation of WNK4. KLHL3: Kelch kinase protein-3; CUL3: Cullin3; PAH Ⅱ: Pseudohypoaldosteronism type Ⅱ; WNK: With-no-lysine (K); SPAK: SPS1-related proline/alanine-rich kinase; NCC: Na-Cl co-transporter; NKCC: Na^+-K^+-2Cl ($^-$) cotransporter; OSR1: Oxidative stress-responsive kinase 1.

antihypertensive therapy[14]. In 2010, Yang *et al*[15] generated SPAK null mice in which exons 9 and 10 of the *Stk*39 gene were deleted to investigate its role in the kidneys and aortic blood vessels[15]. Earlier, Rafiqi *et al*[16] had generated knock-in mice in which SPAK could not respond to the WNK kinases. Both

the homozygous SPAK knock-in ($SPAK^{243A/243A}$) and knock-out mice ($SPAK^{-/-}$) demonstrated the same phenotype of hypotension. Rafiqi *et al*[16] accounted for the mechanisms of hypotension in knock-out mice as possibly by lowering expression and phosphorylation of NKCC2 and NCC in the kidneys. Yang *et al*[15] further pointed out that the impaired vasoconstriction may be caused by both reduced function in aortic tissues and NKCC1 phosphorylation in addition to defects of NCC in the kidneys leading to hypotension in their SPAK null mice. However, some different characteristics are present between the SPAK knock-in and knock-out mice that need to be explained. For example, Yang *et al*[15] reasoned the increased NKCC2 phosphorylation in the SPAK null mice due to compensatory up-regulation of OSR1 in the kidneys, which is contrary to the decreased NKCC2 phosphorylation and normal activity of OSR1 in the SPAK inactivated mice.

A PERSPECTIVE FOR DRUG DEVELOPMENT TARGETING OF SPAK TO LOWER BLOOD PRESSURE

To our best knowledge, the SPAK knock-in mice ($SPAK^{+/243A}$/$SPAK^{243A/243A}$) have partial or complete inactivated SPAK function together with WNK1/4 when binding to a cluster of conserved Thr residues which are located at the N-terminal cytosolic domain of the electroneutral cation-coupled chloride cotransporters (SCL2). Because OSR1 binds to a similar cytosolic site on SCL2 with SPAK, to design a drug blocking the binding site between SPAK/OSR1 and SCL2 may affect OSR1 function and result in a hazardous effect. Therefore, the SPAK knock-in mice are more like a model for developing a new drug to target the SPAK protein instead of the binding site of SCL2. As drugs within the cells would inactivate SPAK, they would be competitive antagonist for the site of the N-terminal cytosolic domain of SCL2 with OSR1. As a result, the activity of OSR1 would not be enhanced in SPAK knock-in mice, would subsequently lead to reduced activation of NKCC2 in the kidneys when all the SPAK is inactivated. From this point of view, could we be convinced whether targeting the protein component of SPAK is a promising route? The answer may be derived partly from the blood pressure of $SPAK^{+/243A}$ knock-in mice, which was not reported by Rafiqi *et al*[16]. Although $SPAK^{+/-}$ knock-out mice were observed to have the phenotype of hypotension, this result could not be translated to the knock-in mice directly. Since the SPAK knock-out mice had secondary hyperaldosteronism implying an aldosterone-resistant status which the SPAK knock-in mice did not have, hypotension in SPAK null mice may be associated with this condition rather than the reduction of NKCC1 activity that Yang *et al*[15] proposed. A more definite proof of this would require tissue-specific SPAK knock-out in the vasculature[17], the distribution of SPAK in reference to OSR1 in the arterial vessels in mice should also be estimated. Given that the $SPAK^{+/243A}$ knock-in mice had either a normal range or only a little lower than normal blood pressure, drugs targeting SPAK would work ineffectively. Apparently, the importance of SPAK for the activation of different SCL2 is variable according to their affinity (Kd, dissociation constant) and distributions in tissues. Pharmacokinetically, a drug should be bound to at least half of the SPAK contents to achieve 20% reduction of the epithelial functional NCC and 20% up-regulation of the functional NKCC2 in the kidneys and 40% down regulation of functional NKCC1 in the kidneys and vasculature similar to $SPAK^{+/243A}$ knock-in model[16]. How to determine the optimal drug concentration to obtain the goal of lowering blood pressure would also be a challenge due to different SPAK contents in the tissues and the competition from OSR1.

Alternatively, targeting the gene of *SPAK* in the kidneys and vasculature to produce the heterogeneous knock-out genotype of $SPAK^{+/-}$ with the phenotype of hypotension is a more difficult task. Secondary hyprerreninemia and hyperaldosteronism standing for an aldosterone-resistant status should be highlighted in which they may be harmful to the heart with predominant OSR1 and less SPAK[16,18]. In addition, whether it is useful in people with primary or secondary hyperaldosteronsim should be tested by a hypertensive mouse model with hyperaldosteronism.

Finally, there are some uncertainties regarding the inhibition of SPAK to control blood pressure including the adverse effects of infertility and reduced gastrointestinal glands secretion ability and the protective benefits from sepsis associated with the reduction of NKCC1[19-21]. Recently, Kikuchi *et al*[22] have discovered one small-molecule compound (Stock 1S-14279) and an antiparasitic agent (Closantel) that could inhibit SPAK-regulated phosphorylation and activation of NCC and NKCC1 *in vitro* and in mice[22].

The safety and efficacy of these novel SPAK inhibitors for mice and SPAK knock-in or knock-out mice could provide future models for the control of blood pressure and drug design for human beings. In summary, targeting of the gene or protein of SPAK should be evaluated systematically and the interactions among WNT, OSR1, SCL2 and Renin-Angiotensin-Aldosterone system would need further investigations.

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