

February 20, 2015



Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14674-editorial.doc).

Title: A Perspective of Future Drugs Targeting SPAK for Blood Pressure Control

Author: Gen-Min Lin, Pang-Yen Liu, Ching-Fen Wu, Wen-Been Wang, Chih-Lu Han

Name of Journal: *World Journal of Cardiology*

ESPS Manuscript NO: 14674

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

- (1) **Reviewer 1:** In this review article, authors review some viewpoints for the issue regarding the antihypertensive therapy on the SPAK (gene or kinase). Although this review article is well-written and suitable for publication to WJC, I will propose that you provide some scheme which explain the mechanism of antihypertensive therapy on the SPAK (gene or kinase), if possible. Minor comment: Reference number 1 has no title. The title is 'Human hypertension caused by mutations in WNK kinases'. You should add the title.

Response: Thank you for your great suggestions. A scheme has been added in the revised manuscript as Figure 1 to explain the mechanism of antihypertensive therapy on the SPAK. In addition, the missing title of reference 1 has been added.

- (2) **Reviewer 2:** This short editorial is quite clear and mostly well written. A small amount of editing should improve the manuscripts readability, and I have made some suggestions in the uploaded pdf file.

Response: Thank you so much to make these adjustments for the manuscript to be more readable. We have revised the paper as your suggestions.

- (3) **Reviewer 3:** This is an interesting review article discussing the authors' views on SPAK as a target for hypertension. There are quite some very nice thoughts, which are certainly worth publishing. I have some specific recommendations. 1. It might be a good idea to provide a diagram to illustrate the relationships among WNK, SPKA/OSR1, NKSS/NCC, and blood pressure control. 2. The main comments made by the authors are on two research articles published in 2010. Are there any more recent findings on this topic? 3. The writing could have been significantly improved. Some sentences are too long and it is not very easy to follow.

Response: Thank you for your great suggestion. 1. A scheme (Figure 1) has been added to illustrate the relationships among WNK-SPAK/OSR1-NCC/NKCC to regulate blood pressure. 2. We added many latest researches (references 6-13 and 22) regarding this topic which are described as follow.

From the last paragraph on page 6 to the beginning on page 7:

“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. Chávez-Canales et al 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. SPAK mediates the effect of vasopressin on sodium reabsorption along the distal nephron as well. Figure 1 shows the potential mechanisms of hypertension related to the WNK-SPAK/OSR1-NCC/NKCC cascade.”

On page 10, lines 5-11

....“Recently, Kikuchi et al 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”

- (4) **Reviewer 4:** In current Editorial titled “A Perspective of Future Drugs Targeting on the SPAK for Blood Pressure Control”, authors reviewed recent progress on SPAK kinase and its potential role in blood pressure control. Major concerns, 1. There are quite a few similar reviews published in the recent years, for example, the most recent one is published on Science Signal on July, 2014, “The WNK-SPAK/OSR1 pathway: Master regulator of cation-chloride cotransporters”. Compare to these published reviews, is there any new progress discussed in current editorial? The references the author cited are up to 2010, as an editorial, the author should introduce/discuss the most recent progress and give some new insights that are not covered by already published articles. 2. It will be very helpful if the authors could show a summarized picture of SPAK signal pathway including the participating molecules and how they effect on blood pressure. Minor concerns, the writing is good, but there are still a few places need to be corrected/improved.

Response: [1]. Thank you very much for your great suggestions. The latest researches for this topic has been added in the manuscript (references 6-13, and 22) which are described as follow.

From the last paragraph on page 6 to the beginning on page 7:

“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. Chávez-Canales et al 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. SPAK mediates the effect of vasopressin on sodium reabsorption along the distal nephron as well. Figure 1 shows the potential mechanisms of hypertension related to the WNK-SPAK/OSR1-NCC/NKCC cascade."

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...."Recently, Kikuchi et al 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."

[2]. A figure of WNK-SPAK/OSR1-NCC/NKCC signal pathway on controlling blood pressure has been added.

[3]. The grammar and word's errors have been corrected in the revised manuscript.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Cardiology*

Sincerely yours,

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