Aldosterone, Hypertension, and Beyond

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Aldosterone, a mineralocorticoid hormone, has a well-known function on water balance and blood pressure homeostasis. Recently, its role in metabolic syndrome, insulin resistance, and obesity has come into a spotlight. Aldosterone induces inflammation and oxidative stress that are attenuated by mineralocorticoid receptor blockers such as spironolactone. Aldosterone exerts its effects via the epithelial sodium channel by non-genomic pathways, including serum and glucocorticoid kinase 1, neural precursor cell-expressed developmentally downregulated (gene 4) protein, and K-Ras, and genomic pathways via epigenetic mechanisms. Beyond regulating epithelial sodium channel, aldosterone induces cardiac hypertrophy, endothelial dysfunction, podocyte injury, and fibrosis. This opens new horizons for mineralocorticoid receptor antagonists and novel therapeutic targets such as serum-glucocorticoid regulated kinase 1.

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INTRODUCTION

First isolated by Simpson and colleagues in 1953,[1] aldosterone was synthesized from cholesterol in zona glomerulosa of the adrenal cortex. Its secretion is mainly regulated by renin-angiotensin system, plasma potassium concentration, and adrenocorticotropic hormone. Traditional functions of aldosterone include maintenance of salt and water balance and blood pressure. Correlation between hypertension and aldosterone excess became clear after defining aldosterone-producing mass in 1950s. Primary hyperaldosteronism was first reported by Conn in 1955 in a patient with hypertension, hypokalemia, hypernatremia, and a large adrenal adenoma.[2,3] Since then, various aldosterone excess syndromes have been described, including idiopathic hyperaldosteronism (bilateral adrenal hyperplasia), and aldosterone-producing adenoma being the most common ones (Table).[4]

Resurgence of idiopathic hyperaldosteronism was followed by its increased prevalence and diverse presentations. Compared with aldosterone-producing adenoma, patients with idiopathic hyperaldosteronism might have lower aldosterone concentrations and less suppressed plasma renin activity. In addition, they are more expected to have a normal serum potassium level. Postsaline infusion aldosterone level in patients with idiopathic hyperaldosteronism might be partially suppressed (5 ng/dL to 10 ng/dL).[4]

In a study between 1997 and 2001, four hundred and two patients with moderate to severe hypertension who were taking α-blockers were evaluated for different causes of secondary hypertension. Diagnostic criteria for primary hyperaldosteronism were as follows: (1) aldosterone-renin ratio of 50 ng/L and higher, (2) plasma renin activity of 0.7 ng/mL/h and lower, and (3) plasma aldosterone level of 150 ng/Land higher. In that study, the prevalence of primary hyperaldosteronism was 19%. Among those with

Aldosterone Excess Syndromes

Syndrome
Idiopathic hyperaldosteronism
Aldosterone-producing adenoma
Primary (unilateral) adrenal hyperplasia
Pure aldosterone-producing adrenocortical carcinoma
Familial hyperaldosteronism
Type I (glucocorticoid-remidiable aldosteronism)
Type II (aldosterone-producing adenoma or idiopathic hyperaldosteronism)
Ectopic aldosterone-producing adenoma or carcinoma

primary hyperaldosteronism, 42% had idiopathic hyperaldosteronism, 36% had aldosterone-producing adenoma, 7% had unilateral adrenal hyperplasia, 13% had unclassified primary hyperaldosteronism, and 2% had familial type I hyperaldosteronism.[5]

In another study on 1125 patients who were on calcium channel blockers and/or α-blockers, the curable forms of primary hyperaldosteronism in newly diagnosed hypertensive patients were evaluated.[6] The diagnostic criteria used for primary hyperaldosteronism included a baseline aldosterone-renin ratio of 40 ng/Land higher, an aldosterone-renin ratio after captopril of 40 ng/L and higher, and plasma renin activity less than 0.2 ng/mL/h. In this study, the prevalence of primary hyperaldosteronism was 11.2%. Patients with primary hyperaldosteronism were older and had high blood pressure levels than those without primary hyperaldosteronism. Among patients with primary hyperaldosteronism, 42.8% had aldosterone-producing adenoma, and the remaining 57.2% had idiopathic hyperaldosteronism.[6] In that study, the proportion of patients with primary hyperaldosteronism has significantly increased from 7.2% to 19.5%. In addition, the severity of hypertension has also been increased. The most common cause of primary hyperaldosteronism was idiopathic hyperaldosteronism. Incidental hypokalemia was detected in 48% of patients with aldosterone-producing adenoma and 16.8% of those with idiopathic hyperaldosteronism.[6]

As mentioned above, the role of high plasma aldosterone level in secondary hypertension is well known. However, it is not clear whether a normal plasma aldosterone level is a risk factor for essential hypertension. In order to answer this question, 1688 normotensive participants of the Framingham Offspring Study were categorized into 4 quartiles based on their baseline aldosterone level, and followed for 4 years from 1998 to 2001. The risk of an elevated blood pressure increased in the 2nd to 4th quartile relative to the 1st (lowest) quartile. Comparing the highest quartile group to the lowest, there was a 1.60 fold increased risk of an increase in blood pressure (95% confidence interval, 1.19 to 2.14) and 1.61 fold increased risk of hypertension in the latter group.[7]

Increased prevalence of metabolic syndrome in the recent 3 decades may play a role in increasing the prevalence of primary hyperaldosteronism. However, increased awareness, more accurate testing, and better screening for primary hyperaldosteronism have a contributing role. Individuals with metabolic syndrome have high plasma aldosterone levels. The missing link between hypertension and metabolic syndrome might be the adipose tissue. Adipose tissue not only is a fat depot, but also plays a role as an inflammatory organ. It releases inflammatory cytokines like tumor necrosis factor-α, interleukin-6, and renin, and also increases rennin-angiotensin-aldosterone system components, both of which leading to vasoconstriction. On the other hand, nonesterified free fatty acids may directly induce sympathetic overactivity. Adipose tissue causes insulin resistance by changing in the proportion of adiponectin, leptin, and resistin. Increased serum insulin level induces serum endothelin, which both result in sodium retention and intravascular volume expansion. Sympathetic overactivity and vasoconstriction along with volume expansion induce hypertension in obese individuals.[8]

Serum aldosterone level is usually controlled by renin activity and hyperkalemia. However, in obese population it would not be suppressed by high salt intake. Recent findings showed epoxy-keto derivative of linoleic acid stimulates aldosterone secretion independently, which is not sensitive to volume and sodium intake.[9] In obese population, both high serum aldosterone and angiotensin II levels increase oxidative stress. Increased oxidative stress level activates a serine kinase and phosphorylates insulin-related substrate, which in turn leads to decreased activation of phosphatidylinositol-3 kinase and protein kinase B, and downregulates insulin-responsive glucose transporter 4 gene expression on cell membrane, which induces insulin resistance. On the other hand, aldosterone, by inducing inflammation, has inhibitory effects on protein kinase B and insulin-responsive glucose transporter 4 gene. Thereby, mineralocorticoid receptor blockers like spironolactone can improve insulin sensitivity, reduce oxidative stress, enhance the endothelial-mediated vasodilatation, increase nitric oxide bioavailability, and decrease inflammation and fibrosis.[10]

ALDOSTERONE: EPITHELIAL SODIUM CHANNEL CROSS TALK

Expression of epithelial sodium channel (ENac) in collecting duct is mostly regulated by its

ubiquitylation, in which neural precursor cell-expressed developmentally downregulated (gene 4) (Nedd4) proteins (Nedd4-1 and Nedd4-2) play a major role. As in Liddle syndrome, mutation in PY motif at the C-terminal end of β and/or γ subunit of ENac prevents the attachment of Nedd4-2, and results in overexpression of ENac surface and hypertension. Aldosterone binds to its receptor that leads to release of mediators and expression of ENac. In the first 3 hours, these mediators will increase ENac trafficking via nontranscriptional regulation, and thereafter, will increase *ENAC* gene transcription.

Role of Aldosterone in Epithelial Sodium Channel Trafficking

K-Ras, a small G protein, is the first gene product that is induced by aldosterone. K-Ras has a dual effect on ENac activity. It decreases ENac activity through Raf-MEK-ERK pathway.[11] It also induces serum-glucocorticoid regulated kinase 1 (SGK1) by its phosphorylation via phosphoinositol kinase.[12] Phosphorylated SGK1, whose gene transcription is also induced by aldosterone, mediates Nedd4-2 phosphorylation and inhibition and provides a binding site for 14-3-3 protein. The 14-3-3 protein prevents binding on Nedd4-2 to ENac and impairs ENac ubiquitylation. Along with the 14-3-3, ubiquitin-specific protease (Usp2-45) and glucocorticoid-induced leucine zipper are also increased in response to the activation of aldosterone receptor. The role of Usp2-45 is deubiquitylation, while glucocorticoid-induced leucine zipper modulates Nedd4-2 and ENac interaction through inhibiting Raf-MEK-ERK pathway and neutralizing K-Ras action (Figure 1).[13]

Role of Aldosterone on Epithelial Sodium Channel Transcription: Epigenetic Mechanisms

Epigenetics is about changes in gene activity without altering a DNA sequence. Major epigenetic mechanisms are DNA modification, chromatin modification, and micro-RNA. Chromatin consists of nucleosomes that are DNA strands wrapped around histones. Several reversible modifications can occur on histone such as acetylation, methylation, phosphorylation, and ubiquitylation. Methylated DNA and deacetylated histone repress gene transcription. Histone arginine methylation activates gene transcription, while histone lysine

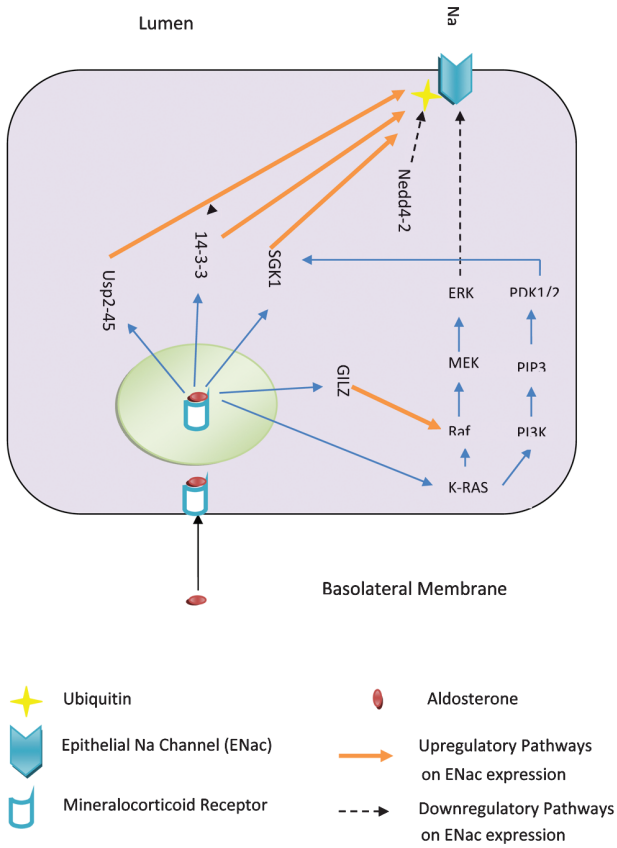

Figure 1. Role of aldosterone in endothelial sodium channel (ENac) trafficking. Aldosterone induces serum and glucocorticoid-regulated kinase 1 (SGK1), 14-3-3, Usp2-45, glucocorticoid-induced leucine zipper and K-ras. K-ras downregulates ENac via Raf pathway. On the other hand, it upregulates ENac by phosphorylating SGK1. Serum and glucocorticoid-regulated kinase 1, ubiquitin-specific protease (Usp2-45), and 14-3-3 deubiquitylate ENac. neural precursor cell-expressed developmentally downregulated (gene 4) protein (Nedd4-2) ubiquitylates ENac.

(Lys) methylation in position 79 inhibits gene transcription. To begin an active transcription, an open chromatin structure, unmethylated DNA and acetylated histone are necessary.

Disruptor of telomere silencing 1 (Dot-1) is a member of histone methyltransferase family. It methylates Lys-79 in histone and suppresses gene transcription. Dot1α-AF9 complex helps histone hypermethylation. Under basal circumstances, *ENAC* gene transcription is constrained. It can be induced by aldosterone and SGK1. Aldosterone diminishes Dot1α and AF9 expression and leads to histone hypomethylation and *ENAC* gene transcription. Serum and glucocorticoid-regulated kinase phosphorylates AF9 at Ser435 and disrupts Dot1α-AF9 complex and increases ENac synthesis.[14]

CLINICAL RELEVANCY

The risk of hypertension increases with the mutations in mineralocorticoid receptor (MR). The MR polymorphism has been associated with aggravated hypertension in pregnancy. In women with gestational hypertension, aldosterone level was lower and progesterone level was higher than those with normal pregnancy. The S810L polymorphism of MR has been reported in up to 12% of women with pregnancy induced hypertension.[15] This mutation constitutively activates the MR and alters receptor's specificity. Progesterone and other steroids lacking 21-hydroxyl groups, which are normally MR antagonists, become MR agonists and induce hypertension.[16]

In 1502 Spanish participants, functional polymorphism of the MR (*NR3C2*) gene was associated with the risk of hypertension. In addition, genotype GG of the rs5522 had protective effects against hypertension (odds ratio, 0.1; 95% confidence interval, 0.02 to 0.56).[17]

As mentioned in the previous section, Nedd4-2 has an important role in ENac ubiquitination. Mutations in *NEDD4L* gene, which lead to production of new isoform I, are associated with the increased risk of hypertension due to the abnormally increased sodium reabsorption.[18,19]

Apart from SGK1 action on ENac, SGK1 increases sodium reabsorption via various transporters, thereby it influences the blood pressure level. It stimulates Na/H exchanger in the proximal tubule, Na/K/2Cl in the loop of Henle, and Na/K ATPase in different nephron segments.[20] The SGK1 is also activated by insulin and insulin-like growth factor 1 (IGF1) via PI3 kinase and activation of the serine/threonine kinases PDK1 or PDK2. Therefore, SGK1 might have a role in increased sodium reabsorption and hypertension in acromegaly. There is also a significant linkage of the *SGK1* gene locus to diastolic blood pressure ($P < .001$).[11] In the presence of high salt intake, *SGK1* expression should be downregulated, which is impaired in salt sensitive hypertensive patients.[21]

IS IT JUST EPITHELIAL SODIUM CHANNEL REGULATED BY ALDOSTRONE?

Aldosterone release is under the influence of both hyperkalemia and hypovolemia. These effects are regulated by a multigene kinase network. In hyperkalemia or potassium load, renin and angiotensin II levels are low and plasma aldosterone is high. This activates kidney-specific with no lysine kinase 1 (KSWNK1), induces potassium channel expression and Na/Cl cotransporter (NCC) inactivation in the distal collecting tubule. Therefore, more sodium ion reaches the distal parts and would be reabsorbed in exchange with potassium and results in kaliuresis.

In hypovolemic states, both aldosterone and angiotensin II levels are high, leading to long WNK-1 (LWNK1) activation and potassium channel down regulation. SGK1 phosphorylates WNK4 and blocks its inhibitory effects on NCC, which is also phosphorylated by SGK1 (Figure 2). An

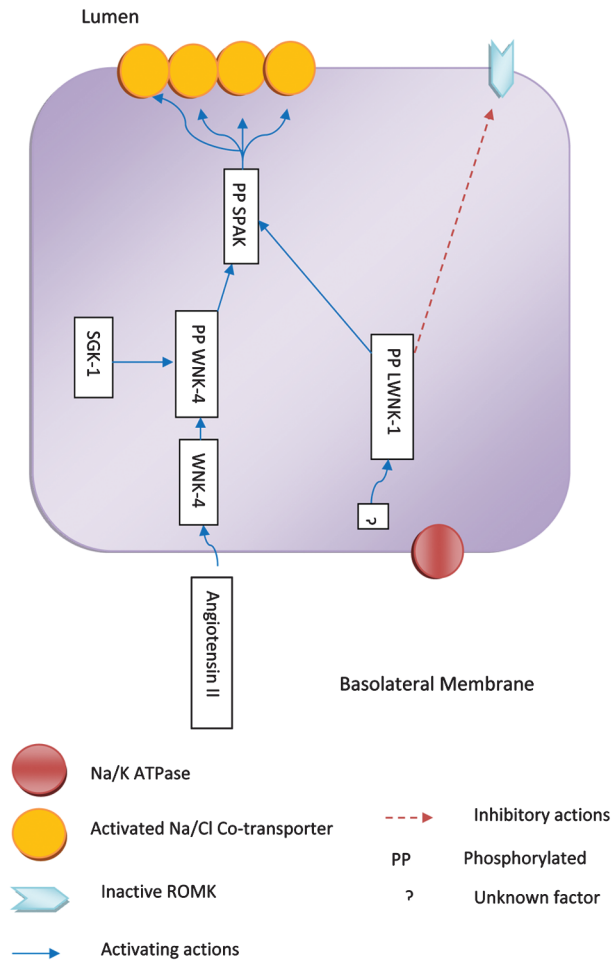

Figure 2. Action of aldosterone in hypovolemia. Elevated level of angiotensin II in the setting of hypovolemia induces WNK-4 phosphorylation, which in turn phosphorylates SPAK (serine threonine proline-alanine rich kinase). Phosphorylated SPAK activates Na/Cl cotransporter. On the other hand, an unknown factor phosphorylates LWNK-1 which downregulates ROMK channel. Therefore, potassium secretion is inhibited despite elevated aldosterone level.

influential factor in this kinase pathway is serine threonine proline-alanine rich kinase (SPAK), which is coded by STK39 gene. STK39 is now known as a hypertension susceptibility gene.[22] Increased SPAK expression leads to a higher blood pressure.[23]

ALDOSTERONE BEYOND EPITHELIAL SODIUM CHANNEL

Apart from the classic role of aldosterone in sodium and potassium homeostasis, recently, the new aspects of its action on the cardiovascular system have come to attention. Aldosterone has been shown to induce ventricular hypertrophy as well as cardiac fibrosis.[24] It seems that the effects of aldosterone on the left cardiac ventricular mass are beyond its effects on intravascular volume and hemodynamic status.[25]

Aldosterone has both genomic and nongenomic effects on the vascular system, including endothelial and smooth muscle cells as well as atherosclerosis.[26,27] Aldosterone produces an inflammatory response by inducing reactive oxygen species,[28] MCP1, interleukin-6, and interleukin-1β.[29,30] Moreover, it increases plasminogen activator inhibitor-1 level and promotes extracellular matrix accumulation. On the other hand, aldosterone promotes fibrosis through increasing growth factors such as tumor growth factor-β, connective tissue growth factor (CTGF), and fibroblast growth factor, all result in glomerulosclerosis, tubulointerstitial fibrosis, and renal scarring.[31]

Podocytes were recognized as a target of aldosterone. In a study conducted in mice that were continuously infused with aldosterone, transcription of nephrin and podocin were downregulated, mean arterial pressure was elevated, and there was evidence of increased level of oxidative stress and *SGK1* overexpression, in addition to higher amounts of proteinuria. Infusion of eplerenone prevented podocyte damage and decreased blood pressure and oxidative stress. Aldosterone induced high-grade glomerulosclerosis, which was prevented by eplerenone.[32]

Dot-1α and AF9 have inhibitory effects not only on *ENAC* expression, but also on the *CTGF* gene and plasma aldosterone level. Diminished expression leads to overproduction of CTGF and tissue fibrosis.[33] Serum and glucocorticoid-regulated kinase appears to play a key role in mineralocorticoid-induced fibrosis, especially cardiac fibrosis. Serum and glucocorticoid-regulated kinase-knockout mice in comparison with the wild type were protected against cardiac fibrosis after administration of deoxy corticosterone acetate along with high-salt water. In wild type mice, *CTGF* expression and fibrosis were increased, and also, NFκB was more activated. This suggests a critical role for SGK1 in fibrosis.[34]

CONCLUSIONS

Today, aldosterone has come to attention not only for its roles in maintenance of volume and electrolyte balance, but also for its nontraditional aspects. It has been known to play a major role in *ENAC* expression, inflammation, metabolic syndrome and hypertension, insulin resistance, and cardiovascular and renal fibrosis. It seems that therapeutic targeting of the mediators such as SGK1 along with aldosterone receptor blockers might help to prevent efficiently from these disorders.

CONFLICT OF INTEREST

None declared.

REFERENCES

1. Simpson SA, Tait JF, Wettstein A, et al. Constitution of aldosterone, a new mineralocorticoid. Experientia. 1954;10:132-3.
2. Conn JW. Primary aldosterosim. J Lab Clin Med. 1955;45:661-4.
3. Britton KE, Goodwin TJ, Peart W, Snell M. Adrenal aldosterone-producing adenoma: use of colonic potential in diagnosis and subtraction scanning technique for localization. BMJ. 1976;2:11-4.
4. Young WF. Primary aldosteronism: renaissance of a syndrome. Clin Endocrinol. 2007;66:607-18.
5. Strauch B, Zelinka T, Hampf M, Bernhardt R, Widimsky J. Prevalence of primary hyperaldosteronism in moderately to severe hypertension in central Europe region. Journal of Human Hypertension. 2003;17:349-52.
6. Rossi GP, Bernini G, Caliumi Ch, et al. A prospective study of the prevalence of primary aldosteronism in 1125 hypertensive patients. J Am Coll Cardiol. 2006;48:2293-300.
7. Vasan RS, Evance JC, Larson MG, et al. Serum aldosterone and the incidence of hypertension in nonhypertensive persons. N Engl J Med. 2004;351:33-41.
8. Bochud M, Nuddberger J, Bovet P, et al. Plasma aldosteronism is independently associated with the metabolic syndrome. Hypertension. 2006;48:239-45.
9. Goodfriend TH, Ball D, Egan B, Campell W, Nithipatikom K. Epoxy-keto derivative of linoleic acid stimulates aldosterone secretion. Hypertension. 2004;43:358-63.
10. Sowers J, Whaley-Connell A, Epstein M. Narrative

review: the emerging clinical implications of the role of aldosterone in the metabolic syndrome and resistant hypertension. Ann Intern Med. 2009;150:776-83.

11. Busjahn A, Aydin A, Uhlmann R, et al. Serum- and glucocorticoid-regulated kinase (SGK1) gene and blood pressure. Hypertension. 2002;40:256-60.
12. Brennan F, Fuller P. Mammalian K-ras2 is a corticosteroid-induced gene in vivo. Endocrinology. 2006;147:2809-16.
13. Verrey F, Fakitsas P, Adam G , Staub O. Early transcriptional control of ENac (de) ubiquitylation by aldosterone. Kidney Int. 2008;73:691-6.
14. Zhang D, Yu Z, Cruz P, Kong Q, Li Sh, Kone B. Epigenetics and the control of epithelial sodium channel expression in collecting duct. Kidney Int. 2009;75:260-7.
15. Ramirez-Salazar M, Romero-Gutierrez G, Zaina S, Malacara JM, Kornhauser C, Perez-Luque E. Relationship of aldosterone synthase gene (C-344T) and mineralocorticoid receptor (S810L) polymorphisms and gestational hypertension. J Hum Hypertens. 2010 [In press].
16. Geller D, Farhi A, Pinkerton N, et al. Activating mineralocorticoid receptor mutation in hypertension exacerbated by pregnancy. Science. 2000;289:119-23.
17. Martinez F, Mansego M, Escudero J, et al. Association of a mineralocorticoid receptor gene polymorphism with hypertension in a Spanish population. American Journal of Hypertension. 2009;22:649-55.
18. Araki N, Umemura M, Miyagi Y, et al. Expression, transcription, and possible antagonistic interaction of human Nedd4L gene variant implication for essential hypertension. Hypertension. 2008;51:773-7.
19. Fava C, von Wowern F, Berglund G, et al. 24-h ambulatory blood pressure is linked to chromosome 18q21-22 and genetic variations of Nedd4L associated with cross-sectional and longitudinal blood pressure in Swedes. Kidney Int. 2006;70:562-9.
20. Vallon V, Lang F. New insights into the role of serum- and glucocorticoid-inducible kinase SGK1 in the regulation of renal function and blood pressure. Curr Opin Nephrol and Hypertens. 2005;14:59-66.
21. Farjah M, Roxas B, Geenen D, Danziger R. Dietary salt regulates renal SGK1 abundance relevance to salt sensitivity in the Dahl rat. Hypertension. 2003;41:874-8.
22. Welling P, Chang Y, Delpire E, Wade J. Multigene kinase network, kidney transport, and salt in essential hypertension. Kidney Int. 2010;77:1063-9.
23. Wang Y, O'Connell J, McArdle P, et al. Whole-genome association study identifies STK39 as a hypertension susceptibility gene. PNAS. 2009;106:226-31.
24. Ngarmukos Ch, Grekin R. Nontraditional aspects of aldosterone physiology. Am J Physiol Endocrinol Metab. 2001;281:1122-7.
25. Diez J. Effects of aldosterone on the heart. Beyond systemic hemodynamic? Hypertension. 2008;52:462-4.
26. Schiffrin E. Effects of aldosterone on the vasculature. Hypertension. 2006;47:312-318.
27. Young M. Mechanisms of mineralocorticoid receptor-mediated cardiac fibrosis and vascular inflammation. Curr Opin Nephrol Hypertension. 2008;17:174-80.
28. Nishiyama A, Yao L, Nagai Y, et al. Possible contributions of reactive oxygen species and mitogen-activated protein kinase to renal injury in aldosterone/salt-induced hypertensive rats. Hypertension. 2004;43:841-8.
29. Blasi E, Rocha R, Rudolph A, Blomme E, Polly M, McMahon E. Aldosterone/salt induces renal inflammation and fibrosis in hypertensive rats. Kidney Int. 2003;63:1791-800.
30. Leroy V, Seigneux S, Agassiz V, et al. Aldosterone activates NF-κB in the collecting duct. J Am Soc Nephrol. 2009;20:131-44.
31. Remuzzi G, Cattaneo D, Perico N. The aggravating mechanisms of aldosterone on kidney fibrosis. J Am Soc Nephrol. 2008;19:1459-62.
32. Shibata Sh, Nagase M, Yoshida Sh, Kawachi H, Fugita T. Podocyte as the targe for aldosterone: Role of oxidative stress and SGK1. Hypertension. 2007;49:355-64.
33. Zhang W, Xia X, Reisenauer M, Hemenway Ch, Kone B. Dot1a-AF9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENac in an aldosterone-sensitive manner. Biol Chem. 2006;281:18059-68.
34. Vallon V, Wyatt A, Klingel K, et al. SGK1 dependent cardiac CTGF formation and fibrosis following DOCA treatment. J Mol Med. 2006;84:396-404.

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