論文の内容の要旨

論文題目：Epigenetic modulation of the renal ß-adrenergic-WNK4 pathway in salt-sensitive hypertension

和訳：食塩感受性高血圧の発症機序：腎ß受容体－WNK4系のEpigenetic調節

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平成１９年４月入学

医学博士課程

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Hypertension continues to be a major public health problem and contributes to deaths from stroke, myocardial infarction, and kidney failure. A high prevalence of hypertension is seen in populations with high dietary salt intake. For several decades' studies, the activation of the renal sympathetic nervous system has long been suspected to play a key role in the development of salt-induced hypertension through sodium retention, for example, a recent clinical study showed that renal denervation with catheter-induced electrical ablation markedly decreased blood pressure in patients with resistant hypertension *(Krum H, et al. Lancet 373:1275-1281, 2009).* However, the precise molecular mechanism for sympathetic activity induced sodium retention still

remains unclear. It is especially unknown what factors mediate the inter-individual variation in salt sensitivity of blood pressure. Recent studies have shown that the serine-threonine kinases with-no-lysine (K) (WNK) play an important role in renal tubular sodium re-absorption. Among WNKs, WNK4 which is expressed in the distal nephron, have the ability to inhibit renal sodium channels, the Na^+-Cl^- co-transporter (NCC) and the epithelial Na^+ channel (ENaC), to express in cell membrane, thus prevent NCC and ENaC mediated sodium re-absorption in the distal nephron in kidney. On the other hand, an inactive mutation of WNK4 had been proved as the cause of pseudohypoaldosteronism type II (also known as Gordon's syndrome), due to sodium over-re-absorption through the hyper-activated sodium channels.

This study, for the first time, has demonstrated that epigenetic modulation is essentially involved in the development of salt-sensitive hypertension, which is attributable to aberrant ß-adrenergic receptor-WNK4 pathway in the kidney. By using *in-vivo* mice model, the current study I clarified that $ß_2$-adrenergic receptor ($ß_2$-AR) stimulation inhibited renal WNK4 expression, thus up-regulated sodium channel NCC and ENaC and finally caused salt-sensitive hypertension via sodium retention. Moreover, *in-vitro* experiments by using mouse (m) DCT cells, demonstrated glucocorticoid receptor (GR) activation is essential in $ß_2$-AR induced WNK4 inhibition. That GR binding to the negative GR element (nGRE) in the WNK4 promoter region mediate the inhibition of WNK4 transcription, and this binding was enhanced by $ß_2$-AR stimulation. Furthermore, the mechanism of ß-stimulation induced enhancement of GR and nGRE binding in WNK4 promoter was clarified. ß-AR stimulation through the cAMP-PKA pathway phosphorylated Histone deacetylase (HDAC) 8. This

phosphorylation reduced HDAC8 activity, removed it from histones, and induced to hyper-acetylation of histones embedded in WNK4 promoter. Resultantly, the acetylated histone provided relaxed chromatin allowed more GR binding to the nGRE to further suppressed WNK4 transcription. I proved the above process in two rodent models of salt-sensitive hypertension: in an acquired model, DOCA-salt rats and a classic genetic model Dahl salt-sensitive rats. In DOCA-salt model, the increased renal sympathetic activity down-regulated WNK4 expression and resulted in salt-induced hypertension. Renal denervation could recover the renal WNK4 expression thus return the blood pressure to normal. In Dahl salt-sensitive rats, using renal denervation and mineralocorticoid receptor (MR) blocker together also returned elevated blood pressure to normal. Which suggesting sympathetic activation and MR activation are two separated systems that both contribute to salt-induced hypertension in this model.

Taken together, this study suggested that salt loading increases renal sympathetic activity in salt-sensitive animals, and as a result, $ß_2$AR-stimulation induces histone modulation of the WNK4 promoter containing nGRE. The increased GR-nGRE binding results in decrease in WNK4 expression, activates NCC activity, leading to sodium retention and the development of salt-sensitive hypertension. Thus, the $ß_2$AR-WNK4 pathway in the kidneys can serve as an alternative target for the treatment of salt-sensitive hypertension. And this work provides useful information that can lead to a new therapeutic strategy of salt-sensitive hypertension based on its pathogenesis.