

## Epigenetics of epithelial Na<sup>+</sup> channel-dependent sodium uptake and blood pressure regulation

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### Abstract

The epithelial Na<sup>+</sup> channel (ENaC) consists of  $\alpha$ ,  $\beta$ ,  $\gamma$

subunits. Its expression and function are regulated by aldosterone at multiple levels including transcription. ENaC plays a key role in Na<sup>+</sup> homeostasis and blood pressure. Mutations in ENaC subunit genes result in hypertension or hypotension, depending on the nature of the mutations. Transcription of  $\alpha$ ENaC is considered as the rate-limiting step in the formation of functional ENaC. As an aldosterone target gene,  $\alpha$ ENaC is activated upon aldosterone- mineralocorticoid receptor binding to the cis-elements in the  $\alpha$ ENaC promoter, which is packed into chromatin. However, how aldosterone alters chromatin structure to induce changes in transcription is poorly understood. Studies by others and us suggest that Dot1a-Af9 complex represses  $\alpha$ ENaC by directly binding and regulating targeted histone H3 K79 hypermethylation at the specific subregions of  $\alpha$ ENaC promoter. Aldosterone decreases Dot1a-Af9 formation by impairing expression of Dot1a and Af9 and by inducing Sgk1, which, in turn, phosphorylates Af9 at S435 to weaken Dot1a-Af9 interaction. MR attenuates Dot1a-Af9 effect by competing with Dot1a for binding Af9. Af17 relieves repression by interfering with Dot1a-Af9 interaction and promoting Dot1a nuclear export. *Af17*<sup>-/-</sup> mice exhibit defects in ENaC expression, renal Na<sup>+</sup> retention, and blood pressure control. This review gives a brief summary of these novel findings.

**Key words:** Gene transcription; Chromatin; Epithelial sodium channel; Histone; Blood pressure

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**Core tip:** The epithelial Na<sup>+</sup> channel (ENaC) is a key player in sodium transport and blood pressure control. This minireview summarizes the epigenetic mechanisms governing the transcription of  $\alpha$ ENaC. The epigenetic control involves Dot1a-Af9-mediated repression through targeted hypermethylation of histone H3 K79. Aldosterone relieves the repression by decreasing Dot1a and Af9 mRNA levels and by weakening the protein-protein interaction between Dot1a and Af9 interaction *via*

Sgk1-catalyzed Af9 phosphorylation. Aldosterone-independent mechanism involves Af17 as a competitor of Af9 for binding Dot1a and stimulator of Dot1a nuclear export. *Af17*<sup>-/-</sup> mice exhibit decreased Na<sup>+</sup> reabsorption and lowered blood pressure, indicating the significance of this epigenetic control.

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## EPITHELIAL SODIUM CHANNEL IS A KEY PLAYER IN NA<sup>+</sup> METABOLISM

Epithelial sodium channel (ENaC) or the amiloride-sensitive sodium channel contains  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits<sup>[1]</sup>. Variations in the production of the subunits, its open probability and/or plasma membrane localization determine the rate of Na<sup>+</sup> entry. ENaC-dependent Na<sup>+</sup> entry occurs in the aldosterone-sensitive distal nephron (ASDN), which consists of the late distal convoluted tubule (DCT2), connecting tubule, and collecting duct<sup>[2]</sup>. The significance of ENaC in Na<sup>+</sup> metabolism and blood pressure regulation is illustrated by two human genetic diseases, Liddle's syndrome (Liddle syndrome or pseudoaldosteronism) and the autosomal recessive pseudohypoaldosteronism type 1<sup>[3]</sup>.

The manifestations of Liddle's syndrome are similar to those caused by mineralocorticoid excess, including hypertension and, in some patients, hypokalemia and metabolic alkalosis. Moreover, plasma and urinary aldosterone levels are reduced, not increased as in primary aldosteronism. Presentation in most patients takes place at a young age, suggesting the possibility of a genetic disorder rather than an adrenal adenoma. Subsequent studies defined it as an autosomal dominant disorder in which excess loss of potassium and reabsorption of sodium take place in the ASDN. The therapy of the disease consists of a low sodium diet in conjunction with potassium-sparing diuretic medicines such as amiloride. The disorder is extremely rare. Less than 30 families or isolated occurrence have been described in the world as of 2008<sup>[4]</sup>. Patients with Liddle's syndrome have gain-of-function mutations in either  $\beta$ ENaC or  $\gamma$ ENaC subunit, leading to increased ENaC function that produces inappropriately large Na<sup>+</sup> absorption by ASDN. All of these mutations impact a highly-conserved PPxY domain. Further analyses resulted in identification of the PPxY domain as the ENaC regulatory region. The mutations cause hyperactivity of the channel. Such hyperactivity probably results from changes in protein-protein interactions regulating the channel degradation through Nedd4 ubiquitin ligase<sup>[5,6]</sup>. Alternatively, the mutations may affect the clathrin-dependent endocytosis<sup>[7]</sup>.

Autosomal recessive pseudohypoaldosteronism type 1 results from reduced ENaC function. The clinical features including aldosterone resistance, sodium wasting, hypovolemia, and hyperkalemia are presented in affected individuals in infancy. These features are similar to those in other forms of hypoaldosteronism in children, with exception of elevated, not reduced plasma aldosterone levels. The disorder results from loss-of-function mutations in any of the three genes encoding ENaC subunits.

Deletion of all three ENaC subunit genes induces perinatal lethality, associated with failure in lung fluid clearance, and/or an acute pseudohypoaldosteronism type 1 featured by metabolic acidosis and severe hyperkalemia<sup>[8]</sup>.

## EPIGENETIC CONTROL OF ENaC TRANSCRIPTION BY ALDOSTERONE-SENSITIVE DOT1A-AF9 COMPLEX

Chromatin has been well established to play a critical role in transcription regulation<sup>[9]</sup>. One of the mechanisms controlling chromatin structure is the post-translational modification of histone N-terminal tails such as acetylation and methylation. According to the histone code hypothesis<sup>[9]</sup>, these histone tails are exposed, unstructured and accessible to various regulatory proteins that recognize a variety of modifications of specific amino acids in the histones or their combinations, giving rise to altered chromatin structures that control particular cellular processes.

Histone methylation can have distinct effects on gene activation, depending on its chromosomal location, the specifically targeted lysines, argines and combinations undergoing posttranslational modifications, and the enzyme (or protein complex) involved in the particular modifications<sup>[10]</sup>. Members of histone methyltransferase Dot1 family methylate histone H3 K79, which resides in the globular domain. They can modify H3 K79 with one, two, and three methyl groups, leading to mono-, di-, and trimethylated K79<sup>[11]</sup>. These methylation events are referred as H3 m1, m2, and m3K79. Such complicity of the modifications may contribute to the functional diversity. In fact, Dot1 proteins have various functions, ranging from telomeric and HM silencing, cell cycle regulation, cell proliferation, meiotic checkpoint, DNA replication, apoptosis, leukemogenesis, to blood pressure control (reviewed in<sup>[12]</sup>).

Our previous work led to cloning of mouse Dot1-like (*Dot1l*) gene, which is featured by at least five isoforms (Dot1a-e). These isoforms are generated by alternative splicing. Among them, Dot1a is the most characterized variant<sup>[13]</sup>. The first clue of functional significance of Dot1a in renal physiology came from the observation that aldosterone downregulates Dot1a mRNA level in IMCD3 cells derived from mouse inner medullary collecting duct. Aldosterone regulates Dot1a

mRNA abundance in a time- and dose-dependent manner, resulting in a decrease in overall H3 K79 methylation<sup>[14]</sup>. Subsequent studies revealed that Dot1a represses  $\alpha$ ENaC transcription. Chromatin immunoprecipitation (ChIP) coupled by real-time qPCR unearthed the repression associated with targeted H3 K79 hypermethylation at the specific subregions of  $\alpha$ ENaC promoter. Dot1a is recruited to these subregions, most likely through Dot1a-binding partner ALL1-fused gene from chromosome 9 (*Af9*), a putative transcription factor. There are multiple independent lines of evidence in favor of this hypothesis. First, Dot1a interacts with Af9 in a variety of assays including yeast two-hybrid assays, mammalian two-hybrid assays, GST pull-down, co-immunoprecipitation, colocalization, and re-ChIP. Secondly, aldosterone reduces the levels of Af9 mRNA and protein; thirdly, Af9 overexpression induces hypermethylation of histone H3 K79 at particular subregions of the  $\alpha$ ENaC promoter and decreases expression of the endogenous  $\alpha$ ENaC mRNA and  $\alpha$ ENaC promoter-luciferase reporters. In contrast, depletion of Af9 by specific RNAi causes the opposite results. Fourthly, ChIP assays unearth the association of Dot1a-Af9 protein complex in the corresponding subregions of  $\alpha$ ENaC promoter<sup>[15,16]</sup>. Finally, we identified the first Af9 cis-element (+78/+92) in the primary site for Dot1a-Af9 interaction and demonstrated Af9 binding to this element in electrophoretic mobility shift assay<sup>[17]</sup>.

Dot1a-Af9-mediated repression can be relieved in an aldosterone-dependent and -independent manner through multiple mechanisms. Aldosterone impairs the formation of Dot1a-Af9 protein complex associated with the  $\alpha$ ENaC promoter by (1) decreasing abundance of Dot1a and Af9; (2) attenuating the interaction between Dot1a and Af9 via Sgk1-catalyzed phosphorylation of Af9 at Ser 435; and (3) counterbalancing the repression through binding to mineralocorticoid receptor (MR) and facilitating its localization in the cell nucleus, where MR and Dot1a compete for binding Af9. Aldosterone-independent de-repression is achieved through the action of ALL1 fused gene from chromosome 17 (*Af17*). We first demonstrated that Af17 upregulates ENaC transcription and benzamil-sensitive Na<sup>+</sup> currents in 293T cells<sup>[18]</sup>. We showed that the same domain of Dot1a serves as the target for competitive binding by Af17 and Af9. Such competitive binding was mutually verified in a variety of assays. Functionally, Af17 and Af9 had antagonistic effects on expression and activity of ENaC. Af17 promoted decreased Dot1a nuclear expression, at least in part by facilitating its nuclear export, leading to a relief in repression of ENaC mediated by Dot1a-Af9 protein complex<sup>[18]</sup>. More importantly, whole-cell patch clamping analyses revealed that the alternation in ENaC transcription was translated to the corresponding changes in benzamil-sensitive Na<sup>+</sup> uptake<sup>[18]</sup>. In more physiologically relevant systems such as M1 and

IMCD3 cells, we used equivalent short-circuit current and single-cell fluorescence imaging to examine ENaC activity. We confirmed similar mechanisms by which Dot1a and Af17 regulate ENaC expression and activity<sup>[19,20]</sup>.

## AF17<sup>-/-</sup> MICE HAVE INCREASED NA<sup>+</sup> EXCRETION AND DECREASED BLOOD PRESSURE

To demonstrate the functional significance of the epigenetic mechanisms involving Dot1a-Af9-Af17 in regulating Na<sup>+</sup> metabolism and blood pressure control, we created the first *Af17*<sup>-/-</sup> mice, characterized *Af17* expression pattern during development, and found that *Af17* is not required for hematopoiesis and embryogenesis. Deletion of *Af17* has little effect on long-term survival<sup>[21]</sup>, despite increased H3 m2K79 and reduced ENaC function<sup>[22]</sup>. The impaired ENaC function is a result of downregulated ENaC mRNA and protein levels, lowered channel open probability, decreased active channel numbers, and attenuated effective activity<sup>[22]</sup>. The abnormalities in sodium handling and blood pressure (BP) were completely corrected when *Af17*<sup>-/-</sup> mice were treated with a low Na<sup>+</sup> diet, a high K<sup>+</sup> diet, or aldosterone infusion, all of which bolster plasma aldosterone to high levels. These studies establish *Af17* as a potential player for tight regulation of sodium and BP and a potential target for developing new therapeutic strategies in fighting abnormal BP<sup>[22]</sup>.

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