

Epigenetics of epithelial Na⁺ channel-dependent sodium uptake and blood pressure regulation

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Abstract

The epithelial Na⁺ channel (ENaC) consists of α , β , γ

subunits. Its expression and function are regulated by aldosterone at multiple levels including transcription. ENaC plays a key role in Na⁺ homeostasis and blood pressure. Mutations in ENaC subunit genes result in hypertension or hypotension, depending on the nature of the mutations. Transcription of α ENaC is considered as the rate-limiting step in the formation of functional ENaC. As an aldosterone target gene, α ENaC is activated upon aldosterone- mineralocorticoid receptor binding to the cis-elements in the α 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 α ENaC by directly binding and regulating targeted histone H3 K79 hypermethylation at the specific subregions of α 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*^{-/-} mice exhibit defects in ENaC expression, renal Na⁺ 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⁺ channel (ENaC) is a key player in sodium transport and blood pressure control. This minireview summarizes the epigenetic mechanisms governing the transcription of α 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^{-/-}* mice exhibit decreased Na⁺ 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⁺ METABOLISM

Epithelial sodium channel (ENaC) or the amiloride-sensitive sodium channel contains α , β , and γ subunits^[1]. Variations in the production of the subunits, its open probability and/or plasma membrane localization determine the rate of Na⁺ entry. ENaC-dependent Na⁺ entry occurs in the aldosterone-sensitive distal nephron (ASDN), which consists of the late distal convoluted tubule (DCT2), connecting tubule, and collecting duct^[2]. The significance of ENaC in Na⁺ 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^[3].

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^[4]. Patients with Liddle's syndrome have gain-of-function mutations in either β ENaC or γ ENaC subunit, leading to increased ENaC function that produces inappropriately large Na⁺ 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^[5,6]. Alternatively, the mutations may affect the clathrin-dependent endocytosis^[7].

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^[8].

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

Chromatin has been well established to play a critical role in transcription regulation^[9]. 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^[9], 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^[10]. 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^[11]. 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^[12]).

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^[13]. 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^[14]. Subsequent studies revealed that Dot1a represses α ENaC transcription. Chromatin immunoprecipitation (ChIP) coupled by real-time qPCR unearthed the repression associated with targeted H3 K79 hypermethylation at the specific subregions of α 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 pulldown, 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 α ENaC promoter and decreases expression of the endogenous α ENaC mRNA and α 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 α ENaC promoter^[15,16]. 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^[17].

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 α 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^+ currents in 293T cells^[18]. 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^[18]. More importantly, whole-cell patch clamping analyses revealed that the alternation in ENaC transcription was translated to the corresponding changes in benzamil-sensitive Na^+ uptake^[18]. 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^[19,20].

AF17^{-/-} MICE HAVE INCREASED Na^+ EXCRETION AND DECREASED BLOOD PRESSURE

To demonstrate the functional significance of the epigenetic mechanisms involving Dot1a-*Af9*-*Af17* in regulating Na^+ metabolism and blood pressure control, we created the first *Af17*^{-/-} 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^[21], despite increased H3 m2K79 and reduced ENaC function^[22]. 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^[22]. The abnormalities in sodium handling and blood pressure (BP) were completely corrected when *Af17*^{-/-} mice were treated with a low Na^+ diet, a high K^+ 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^[22].

REFERENCES

- 1 Canessa CM, Schild L, Buell G, Thorens B, Gautschi I, Horisberger JD, Rossier BC. Amiloride-sensitive epithelial Na^+ channel is made of three homologous subunits. *Nature* 1994; **367**: 463-467 [PMID: 8107805 DOI: 10.1038/367463a0]
- 2 Nesterov V, Dahlmann A, Krueger B, Bertog M, Loffing J, Korbmayer C. Aldosterone-dependent and -independent regulation of the epithelial sodium channel (ENaC) in mouse distal nephron. *Am J Physiol Renal Physiol* 2012; **303**: F1289-F1299 [PMID: 22933298 DOI: 10.1152/ajprenal.00247.2012]
- 3 Pradervand S, Barker PM, Wang Q, Ernst SA, Beermann F, Grubb BR, Burnier M, Schmidt A, Bindels RJ, Gatz JT, Rossier BC, Hummler E. Salt restriction induces pseudohypoaldosteronism type 1 in mice expressing low levels of the beta-subunit of the amiloride-sensitive epithelial sodium channel. *Proc Natl Acad Sci USA* 1999; **96**: 1732-1737 [PMID: 9990093 DOI: 10.1073/pnas.96.4.1732]
- 4 Rossier BC, Schild L. Epithelial sodium channel: mendelian versus essential hypertension. *Hypertension* 2008; **52**: 595-600 [PMID: 18711011 DOI: 10.1161/HYPERTENSIONAHA.107.097147]
- 5 Staub O, Dho S, Henry P, Correa J, Ishikawa T, McGlade J, Rotin D. WW domains of Nedd4 bind to the proline-rich PY motifs in the epithelial Na^+ channel deleted in Liddle's syndrome. *EMBO J* 1996; **15**: 2371-2380 [PMID: 8665844]
- 6 Abriel H, Loffing J, Rebhun JF, Pratt JH, Schild L, Horisberger JD, Rotin D, Staub O. Defective regulation of the epithelial Na^+ channel by Nedd4 in Liddle's syndrome. *J Clin Invest* 1999; **103**: 667-673 [PMID: 10074483 DOI: 10.1172/JCI5713]
- 7 Shimkets RA, Lifton RP, Canessa CM. The activity of the epithelial sodium channel is regulated by clathrin-mediated endocytosis. *J Biol Chem* 1997; **272**: 25537-25541 [PMID: 9325269 DOI: 10.1074/

- jbc.272.41.25537]
- 8 **Hummeler E**, Vallon V. Lessons from mouse mutants of epithelial sodium channel and its regulatory proteins. *J Am Soc Nephrol* 2005; **16**: 3160-3166 [PMID: 16192419 DOI: 10.1681/ASN.2005040450]
- 9 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]
- 10 **Jenuwein T**, Allis CD. Translating the histone code. *Science* 2001; **293**: 1074-1080 [PMID: 11498575 DOI: 10.1126/science.1063127]
- 11 **van Leeuwen F**, Gafken PR, Gottschling DE. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 2002; **109**: 745-756 [PMID: 12086673 DOI: 10.1016/S0092-8674(02)00759-6]
- 12 **Nguyen AT**, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. *Genes Dev* 2011; **25**: 1345-1358 [PMID: 21724828 DOI: 10.1101/gad.205781]
- 13 **Zhang W**, Hayashizaki Y, Kone BC. Structure and regulation of the mDot1 gene, a mouse histone H3 methyltransferase. *Biochem J* 2004; **377**: 641-651 [PMID: 14572310 DOI: 10.1042/BJ20030839]
- 14 **Zhang W**, Xia X, Jalal DI, Kunciewicz T, Xu W, Lesage GD, Kone BC. Aldosterone-sensitive repression of ENaC α transcription by a histone H3 lysine-79 methyltransferase. *Am J Physiol Cell Physiol* 2006; **290**: C936-C946 [PMID: 16236820 DOI: 10.1152/ajpcell.00431.2005]
- 15 **Zhang W**, Xia X, Reissenauer MR, Rieg T, Lang F, Kuhl D, Vallon V, Kone BC. Aldosterone-induced Sgk1 relieves Dot1 α -Af9-mediated transcriptional repression of epithelial Na⁺ channel α . *J Clin Invest* 2007; **117**: 773-783 [PMID: 17332896 DOI: 10.1172/JCI29850]
- 16 **Zhang W**, Xia X, Reissenauer MR, Hemenway CS, Kone BC. Dot1 α -Af9 complex mediates histone H3 Lys-79 hypermethylation and repression of ENaC α in an aldosterone-sensitive manner. *J Biol Chem* 2006; **281**: 18059-18068 [PMID: 16636056 DOI: 10.1074/jbc.M601903200]
- 17 **Zhang W**, Yu Z, Wu H, Chen L, Kong Q, Kone BC. An Af9 cis-element directly targets Dot1 α to mediate transcriptional repression of the α ENaC gene. *Am J Physiol Renal Physiol* 2013; **304**: F367-F375 [PMID: 23152297 DOI: 10.1152/ajprenal.00537.2011]
- 18 **Reissenauer MR**, Anderson M, Huang L, Zhang Z, Zhou Q, Kone BC, Morris AP, Lesage GD, Dryer SE, Zhang W. Af17 competes with AF9 for binding to Dot1 α to up-regulate transcription of epithelial Na⁺ channel α . *J Biol Chem* 2009; **284**: 35659-35669 [PMID: 19864429 DOI: 10.1074/jbc.M109.038448]
- 19 **Wu H**, Chen L, Zhou Q, Zhang W. Af17 facilitates Dot1 α nuclear export and upregulates ENaC-mediated Na⁺ transport in renal collecting duct cells. *PLoS One* 2011; **6**: e27429 [PMID: 22087315 DOI: 10.1371/journal.pone.0027429]
- 20 **Reissenauer MR**, Wang SW, Xia Y, Zhang W. Dot1 α contains three nuclear localization signals and regulates the epithelial Na⁺ channel (ENaC) at multiple levels. *Am J Physiol Renal Physiol* 2010; **299**: F63-F76 [PMID: 20427473 DOI: 10.1152/ajprenal.00105.2010]
- 21 **Zhang Z**, Huang L, Reissenauer MR, Wu H, Chen L, Zhang Y, Xia Y, Zhang W. Widely expressed Af17 is likely not required for embryogenesis, hematopoiesis, and animal survival. *Genesis* 2010; **48**: 693-706 [PMID: 21170927 DOI: 10.1002/dvg.20679]
- 22 **Chen L**, Wu H, Pochynyuk OM, Reissenauer MR, Zhang Z, Huang L, Zaika OL, Mamenko M, Zhang W, Zhou Q, Liu M, Xia Y, Zhang W. Af17 deficiency increases sodium excretion and decreases blood pressure. *J Am Soc Nephrol* 2011; **22**: 1076-1086 [PMID: 21546577 DOI: 10.1681/ASN.2010121270]

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