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Epithelial sodium channel (ENaC) in GtoPdb v.2023.1

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Abstract

OverviewThe epithelial sodium channels (ENaC) are located on the apical membrane of epithelial cells in the kidney tubules, lung, respiratory tract, male and female reproductive tracts, sweat and salivary glands, placenta, colon, and some other organs [10, 48, 14, 23, 22]. In these epithelia, Na^+ ions flow from the extracellular fluid into the cytoplasm of epithelial cells *via* ENaC and are then pumped out of the cytoplasm into the interstitial fluid by the Na^+/K^+ ATPase located on the basolateral membrane [42]. As Na^+ is one of the major electrolytes in the extracellular fluid (ECF), osmolarity change initiated by the Na^+ flow is accompanied by a flow of water [7]. Thus, ENaC has a central role in regulating ECF volume and blood pressure, primarily *via* its function in the kidney [43]. The expression of ENaC subunits, hence its activity, is regulated by the renin-angiotensin-aldosterone system, and other factors involved in electrolyte homeostasis [43, 32].

The genetics of the hereditary systemic pseudohypoaldosteronism type-I revealed that the activity of ENaC is dependent on three subunits encoded by three genes [23, 12]. Within the protein superfamily that includes ENaC, the crystal structure of ASIC was determined first, revealing a trimeric structure with a large extracellular domain anchored in the membrane with a bundle of six TM helices (two TM helices/subunit) [3, 26]. The first 3D structure of human ENaC was determined by single-particle cryo-electron microscopy at a resolution of 3.7 Å [38]. A recent study improved the resolution to 3 Å [39]. These structures confirmed that ENaC has a 3D quaternary structure similar to ASIC. ENaC is assembled as a hetero-trimer with a clockwise order of α-γ-β subunit viewed from the top, as shown previously [13]. In contrast to ASIC1 which can assemble into a functional homotrimer, ENaC activity can be reconstituted fully only as a heterotrimer with an αβγ or a δβγ composition [29].

In the respiratory tract and female reproductive tract, large segments of the epithelia are composed of multi-ciliated cells. In these cells, ENaC is located along the entire length of the cilia that cover the cell surface [16]. Cilial location greatly increases ENaC density per cell surface and allows ENaC to serve as a sensitive regulator of osmolarity of the periciliary fluid throughout the whole depth of the fluid bathing the cilia [16]. In contrast to ENaC, CFTR (ion transporter defective in cystic fibrosis) is located on the non-cilial cell surface [16]. In the *vas deferens* segment of the male reproductive tract, the luminal surface is covered by microvilli and stereocilia projections with backbones composed of actin filament bundles [48]. In these cells, both ENaC and the water channel aquaporin AQP9 are localized on these projections and also in the basal and smooth muscle layers [48]. Thus, ENaC function regulates the volume of fluid lining epithelia essential for mucociliary clearance of respiratory airways, transport of germ cells, fertilization, implantation, and cell migration [37, 16, 23]. Genes and PhylogenyIn the human genome, there are four homologous genes (*SCNN1A, SCNN1B, SCNN1D*, and *SCNN1G*) that encode four proteins, α-, β-, γ-, and δ-ENaC that may be involved in the assembly of ENaC [11, 34, 47, 53]. These four subunits share 23-34% sequence identity and <20% identity with ASIC subunits [23]. The genes coding for all four ENaC subunits are present in all bony vertebrates with the exception of ray-finned fish genomes that have lost all ENaC genes. The mouse genome has lost the gene *SCNN1D* that codes for δ-ENaC [18, 23, 23]. The α-, β-, and γ-ENaC genes are also present in

jawless vertebrates (*e.g.*, lampreys) and cartilaginous fishes (*e.g.*, sharks) [23]. Examination of the methylation patterns of the 5'-flanking region of *SCNN1A, SCNN1B*, and *SCNN1G* genes in human cells showed an inverse correlation between gene expression and DNA methylation, suggesting epigenetic transcriptional control of ENaC genes [41]. Channel biogenesis, assembly and functionThe expression of ENaC subunits is regulated primarily by aldosterone and many additional extracellular and intracellular factors [43, 31, 40]. Most of the studies indicate that the expression of the three subunits is not coordinated [9]. However, the transport of the subunits to the membrane is dependent on three intact subunits. Even a missense mutation in one subunit reduces the concentration of assembled channels on the cell surface [15].

ENaC is a constitutively active channel, *i.e.*, the flow of Na^+ ions is not dependent on an activating factor. Hence, heterologous cells expressing ENaC (*e.g.*, *Xenopus* oocytes), must be maintained in a solution that contains amiloride to keep ENaC inhibited. To measure ENaC activity, the bath solution is switched to a solution without amiloride. ENaC has two major states: 1) Open, and 2) Closed. The probability of ENaC being in the open state is called ENaC open probability (Po). ENaC activity is regulated by a diverse array of factors that exert their effects by modifying, directly or indirectly, two major parameters: 1) The density of ENaC in the membrane; and 2) The channel open probability [27, 29]. The Po of ENaC is greatly decreased by external Na^+ and this response is called Na^+ self-inhibition [49, 4, 25].

An important aspect of ENaC regulation is that the α and the γ subunits have conserved serine protease cleavage sites in the extracellular segment [23]. Cleavage of these subunits by proteases such as furin and plasmin leads to the activation of ENaC [44, 30, 1].Diseases associated with ENaC mutationsMutations in any of the three genes (*SCNN1A*, *SCNN1B*, and *SCNN1G*) may cause partial or complete loss of ENaC activity, depending on the mutation [12, 20]. Such loss-of-function mutations are associated with a syndrome named "systemic" or "multi-system" autosomal recessive pseudohypoaldosteronism type I (PHA1B) [19, 12, 23, 16, 55, 46]. So far, no mutation has been found in the *SCNN1D* gene that causes PHA. PHA patients suffer from severe salt loss from all aldosterone target organs expressing ENaC, including kidney, sweat and salivary glands and respiratory tract. During infancy and early childhood, the severe electrolyte disturbances, dehydration and acidosis may require recurrent hospitalizations. The severity and frequency of salt-wasting episodes improve with age [21]. PHA1B is also associated with a dysfunctional female reproductive system [16, 6].

The carboxy-terminal of ENaC includes a short consensus sequence called the PY motif. Mutations in this motif in *SCNN1B* and *SCNN1G* are associated with Liddle syndrome, which is characterized by early-onset hypertension [5, 50]. The PY motif is recognized by Nedd4-2 that is a ubiquitin ligase. Thus, mutations in the PY motif reduce ubiquitylation of ENaC leading to the accumulation of ENaC in the membrane, consequently enhance the activity of ENaC [45].ENaC expression in tumorsThe observation that [Na^+] is higher in many cancerous cells as compared to non-cancerous cells has led to the suggestion that enhanced expression of ENaC may be responsible for increased metastasis [33]. However, analysis of RNA sequencing data of ENaC-encoding genes, and clinical data of cervical cancer patients from The Cancer Genome Atlas showed a negative correlation with histologic grades of tumor [51]. Similarly, studies on breast cancer cells that altered α-ENaC levels by over-expression or siRNA-mediated knockdown showed that increased α-ENaC expression was associated with decreased breast cancer cell proliferation [54]. In contrast, analysis of RNA sequencing data from The Cancer Genome Atlas showed that high expression of *SCNN1A* was correlated with poor prognosis in patients with ovarian cancer [35]. These findings indicate that the association of ENaC levels with tumorigenesis varies depending on the tissue.COVID-19The surface of SARS-CoV-2 virions that cause COVID-19 is covered by many glycosylated S (spike) proteins. These S proteins bind to the membrane-bound angiotensin-converting enzyme 2 (ACE2) as a first step in the entry of the virion into the host cell. Viral entry into the cell is dependent on the cleavage of the S protein (at Arg-667/Ser-668) by a serine-protease. Anand *et al.* showed that this cleavage site has a sequence motif that is homologous to the furin cleavage site in α-ENaC [2]. A comprehensive review on the pathological consequences of COVID-19 suggests a role for ENaC in the early phases of COVID-19 infection in the respiratory tract epithelia [17].

Contents

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