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The acid-base regulation by renal proximal tubule

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ABSTRACT

The kidney plays quite an important role in the regulation of acid-base homeostasis. The dysfunction of renal acid-base regulation causes diseases such as developmental disorder, bone malformation, calcification of eye and brain, etc. In the kidney, this regulation is performed, to a considerable part, in the proximal tubule of the nephron. In the luminal side the key player is sodium-proton exchanger type 3 (NHE3), whereas sodium-bicarbonate cotransporter (NBCe1) plays the critical role in the basolateral side. In the cytoplasm there is carbonic anhydrase type 2 (CAII) that intermediates the conversion of CO_2/ HCO_3^-. Interestingly, in human, mutations have been found in NBCe1 and CAII but not in NHE3 so far. Mutations of NBCe1 lead to severe proximal renal tubular acidosis (pRTA) and other systemic manifestations. In animal model studies, however, the relative contribution of NHE3 to proximal tubule functions remains controversial. Recently, V-ATPase with renal specific subunits is suggested to have some roles in the regulation of proximal tubule functions. In this review, we will discuss the regulation of acid-base transport in the proximal tubule and the updates.

Keywords: PRTA; NHE; NBCe1; V-ATPase

1. THE LUMINAL SIDE: NHES AND RENAL LUMINAL PROTON TRANSPORT

The Na^+/H^+ exchangers, NHEs mediate counter-transport, which translocate Na^+ and H^+ across the cell membrane [1-4]. In human there are ten NHEs, NHE1 to 9 and sperm-specific NHE [3,5].

The existence of NHEs in the kidney was first predicted by Pitts *et al*. [6,7]. Later Murer [8], Kinsella and Aronson [9] demonstrated the NHE activity by functional studies using brush border membrane vesicles. The first mammalian NHE, NHE1 was cloned by Sardet *et al*. in 1989 [10].

There are NHE3 and NHE8 in the luminal side of the proximal tubule [2,11-16]. In the mammal NHE3 exists not only in the apical side of renal proximal tubule and thick ascending limb, but also in the gastrointestinal tract, gall bladder, epididymis, and brain [2]. NHE3 consists of 834 amino acids and is thought to have twelve transmembrane domains and long C-terminal intracellular domain [17]. This is similar to other transporters, like Na^+-glucose cotransporter and Na^+, K^+-$2Cl^-$ cotransporter.

About two-thirds of filtered NaCl and water are reabsorbed via NHEs in the luminal side of the proximal tubule [2]. NHEs play an important role in the reabsorption of bicarbonate as well, because they provide proton into the lumen, which is titrated with bicarbonate by carbonic anhydrase in the lumen then comes into the proximal tubular cell in the form of CO_2. CO_2 is again transformed to bicarbonate by intracellular carbonic anhydrase and reabsorbed via the basolateral membrane by Na^+-HCO_3^- cotransporter (NBCe1).

2. STUDIES IN NHE3$^{-/-}$ MICE: WHICH IS THE MAJOR PLAYER?

NHE3 and NHE8 exist predominantly in the luminal side of the renal proximal tubule. In neonatal mice and rats NHE8 is dominant. After weaning, NHE3 dominates in the proximal tubule instead of NHE8 [16,18]. It is still controversial about how much NHE3 contributes to apical H^+ secretion, as NHE3 deficiency has not been found in human and the studies using NHE3$^{-/-}$ mice have shown the controversial results.

The first NHE3$^{-/-}$ mice was generated by Schultheis *et al*. [19]. The NHE3 null mice had only a mild metabolic acidosis of 3 to 5 mEq/l less than that of wild type mice. Wang *et al*. investigated the function of proximal convo-

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luted tubules in NHE3$^{-/-}$ mice by *in vivo* microperfusion [20]. In wild-type mice the addition of 100 μM 5-(N-Ethyl-N-isopropyl) amiloride (EIPA), which should inhibit both NHE3 and NHE8 activities [21], reduced J_{HCO_3} (rate of net HCO_3^- absorption) by approximately 50%. In NHE3 null mice, by contrast, the addition of EIPA did not reduce J_{HCO_3} and J_v (rate of net fluid absorption). On the other hand, the addition of bafilomycin, a V-ATPase inhibitor, reduced J_{HCO_3} significantly (51.2 to 21.1 pmol/ min/mm) in these mice. However it did not affect J_v in both wild-type and NHE3 null mice. From these results, they concluded that NHE3 is largely, if not completely, responsible for the EIPA-sensitive NHE activity in the luminal side of the proximal tubules. These results further indicated that V-ATPase mediates a significant component of HCO_3^- reabsorption in the proximal tubule.

On the other hand, Baum *et al*. used NHE3$^{-/-}$/NHE2$^{-/-}$ mice [22,23] to investigate the role of NHE3 in the proximal tubule. In *in vitro* microperfusion study, the luminal NHE activity in the proximal tubule of the NHE3$^{-/-}$ mice was about 50% of the wild-type, and most of the residual NHE activity was inhibited by 100 μM EIPA. The addition of 50 μM HOE694, which should suppress the NHE2 activity, had no effect, suggesting that NHE2 did not have a significant role in proximal tubule acidification. They speculated that there may be some residual apical EIPA-sensitive NHE activity. Another interpretation would be that EIPA, an inhibitor of NHEs, might inhibit Na^+- HCO_3^- cotransport activity.

Recently, Baum et al. investigated whether NHE8 can compensate for the absence of NHE3 in mice [24]. NHE8 is shown to exist in the intestine, mediating the salt reabsorption in the intestine of the neonatal mice [17, 25]. In NHE3$^{-/-}$ mice the expression of NHE8 protein in the renal cortex and cortical brush border membrane was higher than that of wild type mice. The luminal NHE activity in the proximal tubule of NHE3$^{+/+}$NHE8$^{-/-}$ mice was similar to that in wild-type mice. However, it was about 50% of wild-type in NHE3$^{-/-}$NHE8$^{+/+}$ mice, and about 10% of wild-type in NHE3$^{-/-}$NHE8$^{-/-}$ mice. These results indicate that while NHE3 is the predominant NHE isoform in the luminal side of the proximal tubule, NHE8 can also contribute to the luminal NHE activity, especially in the absence of NHE3.

At present, the reason why Wang *et al*. failed to detect the EIPA-sensitive component in J_{HCO_3} in NHE3$^{-/-}$ mice remains speculative, but the methodological difference may be responsible. Furthermore, the reason why NHE3$^{-/-}$NHE8$^{-/-}$ mice showed only a mild acidosis similar to that in NHE3$^{-/-}$ mice remains unknown. Probably, future studies are required to definitely determine the relative contributions of NHE3, NHE8, and V-ATPase to the net proximal bicarbonate absorption.

3. THE BASOLATERAL SIDE: NBCe1

There are a number of acid-base transporters in the basolateral side of the proximal tubule, among them NBCe1 plays a major role in the absorption of bicarbonate coupled with sodium [26]. NBCe1 was first cloned from salamander by Romero *et al*. [27]. Igarashi *et al*. found two patients who had renal proximal tubular acidosis (pRTA) with different NBCe1 mutations [28]. These patients also had band keratopathy, cataract, glaucoma, cerebral calcification, mental retardation and short stature.

NBCe1 is encoded by the gene SLC4A4 and has five splicing variants [29,30], among them NBCe1A is mainly expressed in the proximal tubule. NBCe1B is expressed widely in many tissues including the pancreas duct and corneal endothelium, while NBCe1C is almost limited in the brain [31-33]. The structures of major NBCe1 variants are shown in **Figure 1** [26].

NBCe1A and NBCe1B differ only in the N-terminus, whereas NBCe1C is identical to NBCe1B in the N-terminus but differs in C-terminus. The N-terminus of NBCe1B and NBCe1C has a binding site of IRBIT, inositol 1,4,5-triphosphate (IP3) receptor-binding protein [34].

One of the proposed models of NBCe1 topology is shown in **Figure 2** [29,35,36]. This is hypothesized based on the model of anion exchanger 1 [37]. According to

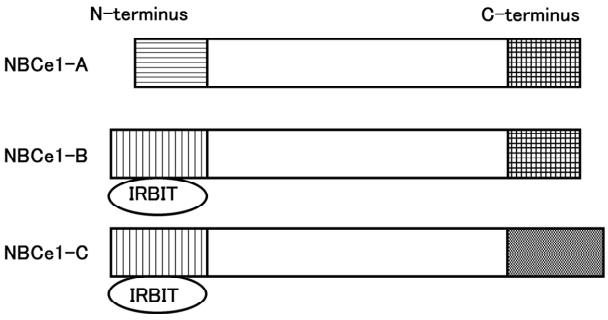

Figure 1. The structure of major NBCe1 variants.

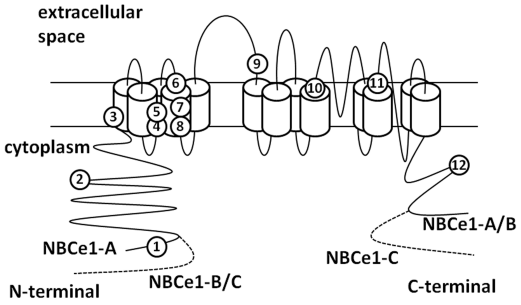

Figure 2. The topology of NBCe1. The numbers show the sites of pRTA-related mutations as follows: 1: Q29X, 2: R298S, 3: S427L, 4: T485S, 5: G486R, 6: R510H, 7: W516X, 8: L522P, 9: N721TxfsX29, 10: A799V, 11: R881C, 12: S982NfsX4.

this model, there are 13 transmembrane domains and a long extraloop with glycosylation sites between TM5 and TM6. Kurtz and colleagues propose another topology model of NBCe1A with 14 TM sites [38].

4. NBCe1 MUTATIONS AND RENAL TUBULAR ACIDOSIS

Igarashi *et al*. [39] described about a patient with systemic symptoms, including severe proximal renal tubular acidosis (pRTA), short stature, mental retardation, glaucoma, cataracts and band keratopathy. Later the mutations of NBCe1, encoded by SLC4A4, were found to be responsible for these abnormalities [28]. So far 12 symptomatic mutations of SLC4A4 have been reported; eight missense mutations, R298S, S427L, T485S, G486R, R510H, L522P, A799V and R881C [28,40-43], two nonsense mutations, Q29X and W516X [44,45], and two frameshift mutations, N721TxfsX29 and S982NfsX4 [46, 47]. Almost all of these mutations cause severe pRTA, but in S982NfsX4 the acidosis is not so severe (serum HCO_3^- around 15 to 17 mmol/l). In Xenopus oocytes expression study this mutation shows normal function, while in immunohistological study in polarized mammalian cells this shows cytoplasmic retention. The S982NfsX4 mutant is speculated to induce the clinical manifestations via cytoplasmic retention *in vivo*, but some of the mutant may reach the plasma membrane of proximal tubule, resulting in the relatively mild acidosis. Most of the other symptomatic mutations are found to be in the deep transmembrane sites [48]. The reason why there is no tight relationship between the severity of acidosis and the degree of NBCe1 inactivation remains speculative, but the trafficking defects caused by some NBCe1 mutants may be partly responsible [40,42,47].

5. ANALYSIS IN NBCe1 W516X KNOCK-IN MICE

A homozygous W516X mutation of NBCe1 was recently found in Taiwan [45]. The patient was a Chinese girl of 16 years old, presenting severe pRTA (serum HCO_3^-: 10 mmol/l), growth retardation and typical ocular abnormalities such as glaucoma, band keratopathy, and cataract.

In order to clarify the pathophysiology of this mutation, we and others created knock-in mice carrying W516X mutation. In NBCe1 W516X/W516X mice a very low amount of the truncated NBCe1 mRNA was detected, suggesting that nonsense-mediated mRNA decay (NMD) is involved in this mechanism. The homozygous mice had severe acidosis (serum HCO_3^-: 3.9 mmol/l) due to pRTA, growth retardation, hyperaldosteronism, anemia, splenomegaly, and early death before weaning. These phenotypes were very similar to those previously reported in NBCe1$^{-/-}$ mice [49]. Interestingly, pRTA has not been reported in humans carrying the heterozygous NBCe1 mutations. However, both NBCe1$^{W516X/+}$ and NBCe1$^{-/+}$ mice showed a mild acidosis, suggesting that the compensatory ability of distal tubule acidification might be higher in humans than in mice.

In physiological studies using isolated proximal tubules of NBCe1$^{W516X/W516X}$ mice, the NBCe1 activity was severely reduced to less than twenty percent of that in wild-type mice. Additionally, the rate of bicarbonate absorption from the proximal tubule was reduced to less than twenty percent of that in wild-type mice. These results confirmed the indispensable role of NBCe1 in HCO_3^- reabsorption in the proximal tubule. In the alkali-treated mice, the symptoms such as bone dysplasia, splenomegaly, and anemia were significantly attenuated. Administration of sodium bicarbonate elongated their life as long as seventy days. This may be a good model to try the effect of PTC124, which is expected to rescue the nonsense mutations [50,51].

6. SINGLE NUCLEOTIDE POLYMORPHISMS (SNPS) OF THE NBCe1 AND THEIR CHARACTERS

Recently SNPs of NBCe1 are reported in sequence [http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?locusId=8671]. Among them we have analyzed four missense mutations (E122G, S356Y, K558R, and N640I) [35]. In the electrophysiological study in *Xenopus* oocytes, E122G, S356Y and N640I showed almost normal function, whereas K558R had about half activity of the wild-type. The sodium affinity of K558R was similar as wild-type. In the expression study in polarized MDCK cells, these four mutants (E122G, S356Y, K558R, and N640I) showed the similar basolateral expression as wild-type, suggesting that these mutants have normal trafficking ability. In the functional analysis in HEK cells, the K558R showed significantly reduced pH recovery, while the other SNPs showed normal activity.

This suggests that K558 may play an important role in the transport function of NBCe1. It is predicted that K558 lies in the transmembrane segment 5 [37,38]. As the other SNPs do not lie in the transmembrane site (E122 and S356 in the N-terminal cytoplasmic region, N640 in the extracellular loop between TM5 and TM6), this study also confirms the importance of the transmembrane regions in the normal function of NBCe1. At present, the clinical significance of K558R SNP remains unknown.

7. VACUOLAR H^+-ATPASE IN THE KIDNEY: RENAL SPECIFIC SUBTYPES AND STUDIES OF MUTATIONS

The vacuolar H^+-ATPase (V-ATPase) is a multisubunit

complex which is ubiquitously expressed in various eukaryotic cells [52,53]. V-ATPase is committed to ATP-driven H^+ transport across membranes, including plasma membrane, lysosome, the Golgi, secretary vesicle, and endosome. As these intracellular organelles need to keep their pH in optimal range to perform the proper functions, the acidification process by the V-ATPase is crucial.

The structure of the V-ATPase is very complicated and large, constituted of two domains, V0 and V1. V0 domain is membrane-bound, including one a, five c, one d and one H domains. The a subunit has four isoforms, a1, a2, a3 and a4. a1 is expressed ubiquitously, a2 is expressed in lung, kidney and spleen. a3 is in osteoclasts, while a4 is in kidney, epididymis and inner ear [54-58]. Mutations in a4 subunit cause distal renal tubular acidosis (dRTA) with or without hearing loss in human.

V1 domain consists of eight subunits, A to H. Among them the B subunit plays an essential role. There are two subtypes in B subunit, B1 and B2 in human. The expression of B1 is restricted to renal intercalated cells, inner ear, epididymis, and ciliary body, on the other hand B2 is expressed ubiquitously [59-62]. Mutations in B1 subunit in human cause dRTA accompanied with sensorineural hearing loss. **Figure 3** shows the simplified scheme of V-ATPase.

8. RENAL SPECIFIC SUBTYPES AND ANALYSIS OF a4 SUBUNIT MUTATIONS

There are four subtypes of "a" subunits of V-ATPase, a1

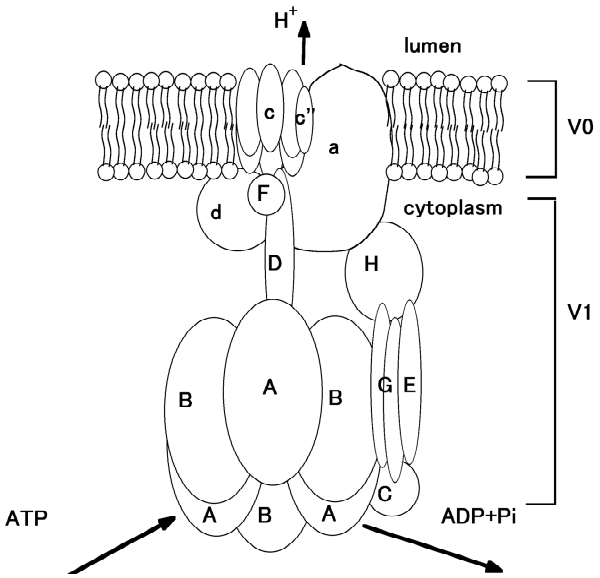

Figure 3. The simplified scheme of V-ATPase [53,63]. In V1, the cytoplasmic part, ATP is hydrolyzed to ADP and Pi, producing energy to drive proton out of the cytoplasm. Proton is exported to luminal side from V0 part.

to a4. Among them a4 subunit is kidney-specific [64]. At first mutations in *ATP6V0A4*, the gene coding a4 subunit, were shown to cause recessive distal renal tubular acidosis (dRTA) with preserved hearing [65], however later it was found that there are patients carrying homozygous mutations in *ATP6V0A4* with both dRTA and hearing loss [66]. The reason why only a subset of a4 deficient patients develops hearing disorder has not been clarified yet.

As previously mentioned, V-ATPase was thought to have some roles in H^+ secretion in the proximal tubule [20]. Recently some groups investigated the details of a4 subunit deficiency using targeted gene knockout mice. Norgett *et al*. [67] made *Atp6v0a4* KO mice and clarified that a4 null mice had severe metabolic acidosis, hypokalemia, hyperchloridemia, and early nephrocalcinosis. These mice also lost the sense of smell and had severe hearing impairment, showing elevated threshold of auditory brainstem response and loss of endocochlear potential. a4 null mice died without alkali treatment, and even if they survived the weaning period they had a mild acidemia and alkali urine. Heterozygous mice did not show academia. When they were challenged with acid, however, they became more acidic than the wild-type mice.

On the other hand, a4 knockout mice produced by Hennings *et al*. [68] had profound deafness and enlarged endolymphatic fluid compartments, like that in pendrin knockout mice [69]. It was also clarified that in the inner ear a4 colocalizes with pendrin. Pendrin is one of the anion exchangers, coded by *SLC26A4* [70-72]. Disruption of pendrin causes Pendred syndrome, which shows various phenotypes like sensorineural deafness and thyroid dysfunction [70]. They speculate that pendrin and V-ATPase co-operate in the regulation of endolymph homeostasis.

Interestingly, Hennings *et al*. also proposed that a4 deficiency causes proximal tubule dysfunction, as these mice showed trafficking disorder of NaPi-IIa (sodium-phosphate transporter), proteinuria, phosphaturia and accumulation of lysosomal material in the proximal tubule. They also reanalyzed the clinical data with recessive dRTA and found that the patients with a4 mutations had severer acidosis, lower serum bicarbonate, and lower blood pH than the patients with B1 mutations. The patients with a4 mutations were diagnosed earlier than the patients with B1 mutations. It remains unknown, however, whether a4 deficiency affects proximal functions also in human.

The reason why these a4 null mice (created by Norgett *et al*. and Hennings *et al*.) show the different phenotypes is not entirely clear. Both mice show metabolic acidosis, hypokalemia, and hyperchloridemia. While Norgett's mice do not show hypercalcemia and hypercalciuria seen

in human dRTA, Hennings' mice show hypercalcemia and hyperphosphateueria (urine calcium not measured). Different background of the mice may be at least partially responsible for these different phenotypes.

9. CONCLUSIONS

Renal proximal tubule plays an important role in the regulation of acid-base homeostasis. Recently there have been some remarkable progresses in the investigation of proximal tubule acid-base transporters such as NHEs and NBCe1. As proximal tubule reabsorbs most of the bicarbonate filtered in the glomeruli, it is quite important to clarify the detailed functions of these transporters and their regulations. Moreover, V-ATPase, a main acid-base regulator of the distal tubule, is now suggested to have some regulatory roles in the proximal tubule.

The animal model studies, especially using transgenic mice, have considerably contributed to the detailed analysis of these transporters. Some models mimic the human phenotypes of these deficient diseases to some extent. In some cases, however, the phenotypes of human and mice are not identical, showing the limitation of these approaches.

Clinically, acid-base balance is quite important in the development of neonatal period, keeping fluid homeostasis, bone metabolism, and even the survival of the individual. We hope that the further understanding of acid/base transporters will lead to the development of more effective treatment of acid/base disorders.

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