Kidney and Calcium Homeostasis

Un Sil Jeon, M.D.
POSTECH Biotech Center, POSTECH, Pohang, Korea

Plasma calcium concentration is maintained within a narrow range (8.5-10.5 mg/dL) by the coordinated action of parathyroid hormone (PTH), 1,25(OH)₂D₃, calcitonin, and ionized calcium (iCa²⁺) itself. The kidney plays a key role in this process by the fine regulation of calcium excretion. More than 95% of filtered calcium is reabsorbed along the renal tubules. In the proximal tubules, 60% of filtered calcium is reabsorbed by passive mechanisms. In the thick ascending limb, 15% of calcium is reabsorbed by paracellular diffusion through paracellin-1 (claudin-16). The calcium sensing receptor (CaSR) in the basolateral membrane of the thick ascending limb senses the change in iCa²⁺ and inhibits calcium reabsorption independent to PTH and 1,25(OH)₂D₃. The fine regulation of calcium excretion occurs in the distal convoluted tubules and connecting tubules despite the fact that only 10-15% of filtered calcium is reabsorbed there. Transient receptor potential vanilloid 5 (TRPV5) and 6 (TRPV6) in the apical membrane act as the main portal of entry, calbindin-D₉K delivers Ca²⁺ in the cytoplasm, and then Na⁺/Ca²⁺ exchanger (NCX1) and plasma membrane Ca²⁺-ATPase in the basolateral membrane serve as an exit. In the cortical collecting duct, TRPV6 is expressed, but the role might be negligible. In addition to PTH and 1,25(OH)₂D₃, acid-base disturbance, diuretics, and estrogen affect on these calcium channels. Recently, klotho and fibroblast growth factor 23 (FGF23) are suggested as new players in the calcium metabolism. Klotho is exclusively expressed in the kidney and co-localized with TRPV5, NCX1, and calbindin-D₉K. Klotho increases calcium reabsorption through trafficking of TRPV5 to the plasma membrane, and also converts FGF receptor to the specific FGF23 receptor. FGF23:klotho complex bound to FGF receptor inhibits 1α-hydroxylase of vitamin D, and contributes to calcium reabsorption and phosphate excretion in the kidney.

Key Words: kidney; TRPV cation channels; klotho; fibroblast growth factor 23

Introduction

The maintenance of calcium homeostasis is very important because calcium is the main component of bony skeleton and serves as the intracellular and extracellular messenger in numerous essential cellular events such as neuronal network, immune response, muscle contraction, and hormone secretion. Total body calcium in the adult human is about 1-2 kg and 99% of total calcium exists in bone. Even though only less than 1% of body calcium is in the extracellular space, maintaining the extracellular calcium concentration within a narrow range (8.5-10.5 mg/dL) is very important for calcium homeostasis. Approximately 40% of plasma calcium is protein-bound and 10% of calcium is in a complex with anions like phosphate, citrate, and sulfate etc. Only half of plasma calcium is in its free form (ionized form, iCa²⁺) and physiologically important. The ionized calcium is tightly regulated by hormones like parathyroid hormone (PTH), 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃), calcitonin, and calcium itself. The kidney, intestine, and bone are the main target organs of these regulators, and the kidney plays a key role...
in the fine regulation of calcium excretion\textsuperscript{1}.

This review will focus on how the kidney works for calcium homeostasis on a molecular basis and discuss new players in the regulation of calcium excretion which were identified recently.

**Overview of renal Ca\textsuperscript{2+} handling**

About 50\% of plasma calcium (ionized and complexed form; ultrafilterable fraction, excluding the protein bound form) is freely filtered through the renal glomerulus, and 99\% of the filtered calcium is actually reabsorbed along renal tubules (Table 1). The excreted calcium in the final urine is about 200 mg per day in an adult person with an average diet. Several factors are involved in the regulation of calcium in renal tubules. PTH and activated vitamin D enhance calcium reabsorption in the thick ascending limb (TAL), distal convoluted tubule (DCT) and/or connecting tubule (CNT), and estrogen promotes calcium absorption in the DCT/CNT\textsuperscript{1,2}. Acidosis contributes to hypercalciuria by reducing calcium reabsorption in the proximal tubule (PT) and DCT, and alkalosis vice versa\textsuperscript{3}. Diuretics like thiazide and furosemide also alter calcium absorption in the renal tubules; thiazide promotes calcium reabsorption and furosemide inhibits it\textsuperscript{4-5}. Plasma calcium itself also controls renal calcium absorption through altered PTH secretion as well as via binding to the calcium sensing receptor (CaSR) in the TAL. To facilitate Ca\textsuperscript{2+} reabsorption along renal tubules; (i) voltage difference between the lumen and blood compartment should be favorable for Ca\textsuperscript{2+} passage, i.e., a positive voltage in the lumen; (ii) concentration difference should be favorable for Ca\textsuperscript{2+} passage with a higher Ca\textsuperscript{2+} concentration in the lumen; (iii) an active transporter should exist if the voltage or concentration difference is not favorable for Ca\textsuperscript{2+} reabsorption. Each renal tubular segment has a different Ca\textsuperscript{2+} concentration difference or voltage environment for its unique mechanism for calcium reabsorption.

**Renal Ca\textsuperscript{2+} handling along the tubules**

Fifty to sixty percent of filtered calcium is absorbed in parallel with sodium and water in the PT, suggesting that the passive pathway is the main route of Ca\textsuperscript{2+} absorption in this segment. Claudin-2 is especially concentrated in the tight junction and also expressed in the basolateral membrane of the PT as the candidate for paracellular Ca\textsuperscript{2+} channel in the PT\textsuperscript{6}. There is no evidence that Ca\textsuperscript{2+} reabsorption occurs in the thin descending and ascending limb. In the TAL, 15\% of filtered calcium is absorbed, and the passive absorption through paracellular space is known as the main mechanism (Fig. 1). Paracellin-1 (claudin-16) is exclusively expressed in the tight junction of TAL and has been known as the important magnesium channel in the TAL\textsuperscript{5}. Paracellin-1 mutation caused hypercalciuria and nephrocalcinosis in addition to hypomagnesemia\textsuperscript{2}. This finding supports that paracellin-1 is not only the main Mg\textsuperscript{2+}

| Renal tubule | Absorption (%) | Mechanism | Transport proteins |
|--------------|----------------|-----------|--------------------|
| Proximal tubes (S2, S3) | 50-60 | passive, paracellular | Claudin-2 (?), FGF23 (?) |
| Thin descending/ascending limb | 0 | | |
| Thick ascending limb | 15 | passive, paracellular | Paracellin-1, CaSR, TRPV5/TRPV6 |
| DCT/CNT | 10-15 | active, transcellular | Calbindin-D\textsubscript{28K}, NCX1, PMCA1b, Klotho, FGF23 (?) |
| Collecting ducts | 0 | | TRPV6 |

S2, segment 2; S3, segment 3; FGF23, fibroblast growth factor 23; CaSR, calcium sensing receptor; DCT, distal convoluted tubule; CNT, connecting tubule; TRPV, transient receptor potential vallinoid; NCX1, Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger; PMCA1b, plasma membrane Ca\textsuperscript{2+}-ATPase.
channel, but also works as the paracellular Ca\textsuperscript{2+} channel in the TAL. There are some evidences that active transport occurs in the TAL, but no specific channel has yet been identified\textsuperscript{1}. The CaSR is a member of G protein-coupled receptors and suppresses PTH secretion by sensing high plasma Ca\textsuperscript{2+} level in the parathyroid glands\textsuperscript{7}. In the kidney, the CaSR is most highly expressed in the TAL. Familial hypocalciuric hypercalcemia (FHH) is an autosomal dominant disease due to the mutation of CaSR gene, and is manifested as hypercalcemia, hypophosphatemia, parathyroid hyperplasia, and unusually low renal clearance of calcium. Hypocalciuria, despite of hyperactivity of PTH in FHH, suggests that CaSR plays a direct role in Ca\textsuperscript{2+} absorption, especially in the TAL independent to PTH action\textsuperscript{8}.

Although only 10-15% of filtered Ca\textsuperscript{2+} is absorbed in the DCT and CNT, these are the main sites in which the fine regulation of Ca\textsuperscript{2+} excretion and the major action of PTH and activated vitamin D occur. In the DCT and CNT, the luminal voltage is negative and Ca\textsuperscript{2+} concentration in the lumen is lower than that of plasma. Thus, active transport mechanism against voltage and concentration gradient should exist in these segments. Several Ca\textsuperscript{2+} transporting proteins are involved in this active transmembrane transport of Ca\textsuperscript{2+} in the DCT and CNT. Transcellular Ca\textsuperscript{2+} reabsorption can occur by three steps; (i) entry of Ca\textsuperscript{2+} through the calcium channels [transient receptor potential vanilloid (TRPV) 5, TRPV6] in the apical membrane, (ii) binding of Ca\textsuperscript{2+} with calcium-binding protein (calbindin) and diffusion in the cytoplasm (without significant change in the intracellular i[Ca\textsuperscript{2+}]), and (iii) Ca\textsuperscript{2+} extrusion via an ATP-dependent Ca\textsuperscript{2+}-ATPase (PMCA1b) or an Na\textsuperscript{2+}/Ca\textsuperscript{2+} exchanger (NCX1) in the basolateral membrane.

![Fig. 1. Ca\textsuperscript{2+} absorption in the thick ascending limb of Henle (TAL). (a) The schematic view of Ca\textsuperscript{2+} reabsorption in the TAL. Paracellin-1 (claudin-16) is located in the tight junction of TAL and serves as the paracellular route for divalent cations. (b) Immunofluorescence image for paracellin-1 (claudin-16) in the mouse TAL cells. Paracellin-1 is co-localized with THP (the marker of TAL) and highly expressed in the tight junction\textsuperscript{1}. BSC1, bumetanide sensitive channel; ROMK, renal outer medullary potassium channel; ClCNKB, chloride channel Kb.](image1)

![Fig. 2. The mechanism of Ca\textsuperscript{2+} absorption in the renal epithelium. Transcellular Ca\textsuperscript{2+} reabsorption in the distal convoluted tubule (DCT) and connecting tubule (CNT) occurs by three steps: (i) entry of Ca\textsuperscript{2+} through the calcium channels [transient receptor potential vanilloid (TRPV) 5, TRPV6] in the apical membrane, (ii) binding of Ca\textsuperscript{2+} with calcium-binding protein (calbindin) and diffusion in the cytoplasm (without significant change in the intracellular i[Ca\textsuperscript{2+}]), and (iii) Ca\textsuperscript{2+} extrusion via an ATP-dependent Ca\textsuperscript{2+}-ATPase (PMCA1b) or an Na\textsuperscript{2+}/Ca\textsuperscript{2+} exchanger (NCX1) in the basolateral membrane.](image2)
Renal calcium transport proteins

The important renal calcium transport proteins are exclusively expressed in the DCT and CNT. The characteristics and regulation of calcium transporting proteins will be discussed as follows.

TRPV5 and TRPV6

Transient receptor potential (TRP) channel is a superfamily of ion channels permeable to monovalent and/or divalent cations with six-transmembrane domains. The mammalian TRP family consists of six subfamilies like TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), and TRPA (ankyrin). TRPV is one of them and consists of six members in mammalians; TRPV1 to TRPV6. TRPV5 (previously known as ECaC1) and TRPV6 (ECaC2), both cloned in 1999, have characteristics distinguished from other TRPV channels; (i) constitutively active at low intracellular Ca$^{2+}$ concentration, and (ii) exclusively selective for Ca$^{2+}$ (PCa/PNa >100). TRPV5 and TRPV6 have the highest sequence homology (~730 amino acids, amino-terminal ankyrin repeats, TM5 and TM6 each forming the pore-region composed with tetramer, on human chromosome 7q34-35) (Fig. 3a). TRPV5 is exclusively expressed in the DCT and CNT in the kidney $^{10}$ (Fig. 3b). On the contrary, TRPV6 is more ubiquitously distributed, especially in the intestine, and also found from the DCT to the CD in the kidney $^{11}$ (Fig. 3b). Both TRPV5 and TRPV6 are located in the apical plasma membrane of the tubular epithelium, and serve as the entrance of Ca$^{2+}$ from the lumen into the cytoplasm. TRPV5 knockout mice exhibited severe hypercalciuria (more than 6 times of wild type mouse) and low bone densities, but without hypocalcemia due to the compensatory elevation of activated vitamin D, clearly demonstrating that TRPV5 plays a crucial role in renal calcium reabsorption $^{12}$. TRPV6 knockout mice also showed significant hypercalciuria and bone disease $^{13}$. Even though TRPV5 and TRPV6 knockout mice showed congenital hypercalciuria, the mutation of the proteins has not been found in the human. Until now, TRPV5 is known as the main entry of Ca$^{2+}$ in renal tubular epithelial cells in the DCT and CNT, and TRPV6 is also known to contribute to renal Ca$^{2+}$ reabsorption in the distal nephron.

Several factors (PTH, 1,25(OH)$_2$D$_3$, calcitonin, estrogen, [Ca$^{2+}$], acid-base status, klotho, diuretics, and immunosuppressive drugs, etc) are involved in the regulation of both TRPV5 and TRPV6 $^{10}$ (Table 2). Alteration of TRPV5 and TRPV6 by these factors contributes in disturbance of calcium metabolism: dyscalcemia, hypo- and hypercalciuria. 1,25(OH)$_2$D$_3$-depleted rats showed decreased expression of TRPV5 and calbindin-D$_{28k}$ mRNA and protein, and repletion of the hormone restored the expression of them $^{10}$. The promoter region of TRPV5 gene

---

**Fig. 3.** Ca$^{2+}$ transport proteins in the distal convoluted tubule (DCT) and connecting tubule (CNT). (a) The molecular structure of transient receptor potential vanilloid (TRPV) 5 and TRPV6. TRPV 5/6 consists of six transmembrane domains and ankyrin repeat at the N-terminal. The functional pore is composed with tetramer, on human chromosome 7q34-35. TRPV5 is exclusively expressed in the DCT and CNT in the kidney $^{10}$ (Fig. 3b). On the contrary, TRPV6 is more ubiquitously distributed, especially in the intestine, and also found from the DCT to the CD in the kidney $^{11}$ (Fig. 3b). Both TRPV5 and TRPV6 are located in the apical plasma membrane of the tubular epithelium, and serve as the entrance of Ca$^{2+}$ from the lumen into the cytoplasm. TRPV5 knockout mice exhibited severe hypercalciuria (more than 6 times of wild type mouse) and low bone densities, but without hypocalcemia due to the compensatory elevation of activated vitamin D, clearly demonstrating that TRPV5 plays a crucial role in renal calcium reabsorption $^{12}$. TRPV6 knockout mice also showed significant hypercalciuria and bone disease $^{13}$. Even though TRPV5 and TRPV6 knockout mice showed congenital hypercalciuria, the mutation of the proteins has not been found in the human. Until now, TRPV5 is known as the main entry of Ca$^{2+}$ in renal tubular epithelial cells in the DCT and CNT, and TRPV6 is also known to contribute to renal Ca$^{2+}$ reabsorption in the distal nephron.

Several factors (PTH, 1,25(OH)$_2$D$_3$, calcitonin, estrogen, [Ca$^{2+}$], acid-base status, klotho, diuretics, and immunosuppressive drugs, etc) are involved in the regulation of both TRPV5 and TRPV6 $^{10}$ (Table 2). Alteration of TRPV5 and TRPV6 by these factors contributes in disturbance of calcium metabolism: dyscalcemia, hypo- and hypercalciuria. 1,25(OH)$_2$D$_3$-depleted rats showed decreased expression of TRPV5 and calbindin-D$_{28k}$ mRNA and protein, and repletion of the hormone restored the expression of them $^{10}$. The promoter region of TRPV5 gene
Table 2. The regulation of calcium transporting proteins in the DCT and CNT

| Factors          | TRPV5 | TRPV6 | Calbindin-D28K | Mechanisms                  |
|------------------|-------|-------|----------------|-----------------------------|
| PTH              | ↑     |       | NC             | ↑ transcription             |
| Vitamin D        | ↑     | ↑     | ↑              | ↑ transcription             |
| Estrogen         | ↑     | ↑     | ↑              | ↑ transcription             |
| Low calcium diet | ↓     | ND    | NC             | ↑ transcription             |
| Acidosis         |       | ↓     | ↓              | ↑ transcription             |
| Thiazide         | C     | ND    | C              | ↑ transcription             |
| Furosemide       | ↑     | ↑     | ↑              | ↑ transcription             |
| Tacrolimus       | ↓     | ND    | ↓              | ↑ channel activity          |
| [Ca$^{2+}$]      | ↓     | ↓     | ↓              | ↑ channel activity          |
| Calbindin-D28K   | ↑     | NC    | ND             | ↑ trafficking               |
| Klotho           | ↑     | ↑     | ND             | ↑ trafficking               |

DCT, distal convoluted tubule; CNT, connecting tubule; transient receptor potential vanilloid, TRPV; PTH, parathyroid hormone; ↑, stimulation; ↓, inhibition; NC, no change; ND, not done; C, Controversial.

Table 2. The regulation of calcium transporting proteins in the DCT and CNT

recently, several studies were performed to document whether these drugs affect renal calcium transport proteins. Furosemide has been used for the treatment of hypercalcemia for many years because it was well known to reduce paracellular Ca$^{2+}$ transport via reducing lumen-positive voltage in the TAL. Recently, it was documented that furosemide also increased the mRNA levels of TRPV5, TRPV6, and calbindin-D28K in the DCT$^6$. The conflict between hypercalciuria and the increased calcium transporting proteins by furosemide might be explained by the response to the increased distal calcium delivery and compensatory adaptation of the downstream renal segments$^6$. On the contrary, the effect of thiazide on the renal calcium transport proteins is still controversial because conflicting results have been documented. Nijenhuis et al. demonstrated that chronic hydrochlorothiazide treatment significantly reduced urinary Ca$^{2+}$ excretion which was accompanied by decreased mRNA expression of TRPV5, calbindin-D28K, and NCX1 regardless of volume status$^5$. However, the same authors reported later that TRPV5 mRNA was not changed by thiazide$^{14}$. They also suggested that thiazide-induced hypocalciuria was caused by the enhanced passive Ca$^{2+}$ transport in the proximal tubule rather than by the action of Ca$^{2+}$ transport proteins in the DCT using TRPV5 knockout mice$^{43}$. On the other hand, Lee et al. reported that the effect of thiazide on calcium transport proteins depended upon the volume status; thiazide increased the expression of TRPV5 and calbindin-D28K only when there was no volume contraction$^{35}$. According to Jang et al., TRPV5 and calbindin-D28K were upregulated by thiazide in hypercalciuric rats$^{16}$. Therefore, the mechanism of hypocalciuria induced by thiazide needs to be clarified in the future. It has been demonstrated that the channel activation of TRPV5 and TRPV6 require other associated proteins like S100A10 (p11 or annexin 2 light chain)/annexin 2, Rab11b, 80K-H, and NHERF$^9$. In addition, klotho is also known to be involved in the trafficking of TRPV5, which will be discussed in detail later. The coordinated regulation of the abundance, trafficking, and channel activity of TRPV5/6 by these factors can maintain balanced renal Ca$^{2+}$ excretion.

**Calbindin-D28K**

Calbindin is a cytosolic calcium-binding protein which is involved in transcellular calcium diffusion and buffers cytosolic Ca$^{2+}$ to maintain a very low physiologic intracellular Ca$^{2+}$ concentration. Two isoforms of calbindin exist; calbindin-D28K which is exclusively expressed in the mammalian kidney and calbindin-D3K primarily in the inte-

contains putative vitamin D responsive element (VDRE) surrounded by AP-1 sites, which synergistically enhance the action of 1,25(OH)$_2$D$_3$ and 1,25(OH)$_2$D$_3$ also upregulated the renal expression of TRPV6$^{13}$. Therefore, 1,25 (OH)$_2$D$_3$ acts as one of the key regulators of renal Ca$^{2+}$ handling by alteration of the main renal Ca$^{2+}$ transport proteins; TRPV5, TRPV6 and calbindin-D28K. Acid-base status also has been known to affect the renal Ca$^{2+}$ handling. Chronic metabolic acidosis was documented to induce hypercalciuria accompanied with decreased renal expression of TRPV5 and calbindin-D28K, but not in TRPV5 knockout mice, whereas metabolic alkalosis by NaHCO$_3$ administration induced the opposite results$^9$. Nephrocalcinosis and nephrolithiasis observed in the patients of renal tubular acidosis could be explained by the decreased expression of the renal calcium channels by acidosis. Diuretics like furosemide and thiazide have been used for the therapy of hypercalcemia or hypercalciuria. Recently, several studies were performed to document whether these drugs affect renal calcium transport proteins. Furosemide has been used for the treatment of hypercalcemia for many years because it was well known to reduce paracellular Ca$^{2+}$ transport via reducing lumen-positive voltage in the TAL. Recently, it was documented that furosemide also increased the mRNA levels of TRPV5, TRPV6, and calbindin-D28K in the DCT$^6$. The conflict between hypercalciuria and the increased calcium transporting proteins by furosemide might be explained by the response to the increased distal calcium delivery and compensatory adaptation of the downstream renal segments$^6$. On the contrary, the effect of thiazide on the renal calcium transport proteins is still controversial because conflicting results have been documented. Nijenhuis et al. demonstrated that chronic hydrochlorothiazide treatment significantly reduced urinary Ca$^{2+}$ excretion which was accompanied by decreased mRNA expression of TRPV5, calbindin-D28K, and NCX1 regardless of volume status$^5$. However, the same authors reported later that TRPV5 mRNA was not changed by thiazide$^{14}$. They also suggested that thiazide-induced hypocalciuria was caused by the enhanced passive Ca$^{2+}$ transport in the proximal tubule rather than by the action of Ca$^{2+}$ transport proteins in the DCT using TRPV5 knockout mice$^{43}$. On the other hand, Lee et al. reported that the effect of thiazide on calcium transport proteins depended upon the volume status; thiazide increased the expression of TRPV5 and calbindin-D28K only when there was no volume contraction$^{35}$. According to Jang et al., TRPV5 and calbindin-D28K were upregulated by thiazide in hypercalciuric rats$^{16}$. Therefore, the mechanism of hypocalciuria induced by thiazide needs to be clarified in the future. It has been demonstrated that the channel activation of TRPV5 and TRPV6 require other associated proteins like S100A10 (p11 or annexin 2 light chain)/annexin 2, Rab11b, 80K-H, and NHERF$^9$. In addition, klotho is also known to be involved in the trafficking of TRPV5, which will be discussed in detail later. The coordinated regulation of the abundance, trafficking, and channel activity of TRPV5/6 by these factors can maintain balanced renal Ca$^{2+}$ excretion.

**Calbindin-D28K**

Calbindin is a cytosolic calcium-binding protein which is involved in transcellular calcium diffusion and buffers cytosolic Ca$^{2+}$ to maintain a very low physiologic intracellular Ca$^{2+}$ concentration. Two isoforms of calbindin exist; calbindin-D28K which is exclusively expressed in the mammalian kidney and calbindin-D3K primarily in the inte-
Calbindin-D<sub>28K</sub> is expressed in the cytoplasm of DCT, CNT cells, and principal cells of CD, and co-localized with TRPV5 and NCX1 in the same cells (Fig. 3b). It is regulated by the same factors for TRPV5 such as 1,25(OH)<sub>2</sub>D<sub>3</sub>, acid-base status, and furosemide. Thus, calbindin-D<sub>28K</sub> is one of the essential components of transepithelial Ca<sup>2+</sup> transport, not only as the cytoplasmic delivery vehicle of Ca<sup>2+</sup>, but also as the dynamic regulatory factor of TRPV5 channel activity.<sup>17</sup>

**NCX1 and PMCA1b**

NCX1 and PMCA1b are expressed in the basolateral membrane of the DCT and CNT, and act as the main exit of absorbed Ca<sup>2+</sup> to the blood compartment (Fig. 3b). Since the luminal voltage and transcellular Ca<sup>2+</sup> concentration difference in the DCT and CNT are not favorable for Ca<sup>2+</sup> reabsorption, an active transport mechanism is necessary in these segments. NCX1 is a secondary active transporter generated by the action Na<sup>+</sup>,K<sup>+</sup>-ATPase for calcium countertransport<sup>1</sup>. PMCA1b is a primary active calcium pump and most of its activity is seen in the DCT<sup>1</sup>. Both NCX1 and PMCA1b are regulated by PTH, calcitonin, and 1,25(OH)<sub>2</sub>D<sub>3</sub> in the distal nephron (Fig. 4).

**New players of renal calcium handling**

During the past decade, two new proteins; klotho and fibroblast growth factor 23 (FGF23) emerged as the new players of calcium, magnesium, and phosphate metabolism. Klotho and FGF23 are closely linked and play a role in calcium metabolism by their action on parathyroid glands, bone and kidney.

**Klotho**

Klotho [Clotho] was the youngest of the Moirae of Greek mythology (three sisters who determined the destiny and life of humans; Clotho, Lackesis and Atropos) and the "spinner" who controlled the thread of life. In 1997, the Klotho gene was first discovered by Kuro-o et al. and named it as it is now, because it was originally known as an aging-suppressor gene.<sup>18</sup> Klotho mutant mice resembled human aging; a short lifespan, infertility, arteriosclerosis, skin atrophy, uncoordinated movement, osteoporosis, emphysema, etc.<sup>18</sup> Klotho protein belongs to type 1 membrane protein (single transmembrane domain, 1,014 amino acids, ~130 kDa) and has an extracellular domain with similarity to β-glycosidase, the enzyme involved in the digestion of sugar moieties of substrates. Although klotho is a membrane protein, it is also abundant in the cytoplasm and the cleaved extracellular domain is secreted into blood, cerebrospinal fluid and urine.<sup>19</sup>
Klotho has been suggested to play an important role in the kidney because it is highly expressed in the kidney and klotho knockout mice showed severe hyperphosphatemia associated with increased 1,25(OH)2D₃ levels. α-Klotho (α-Kl) is highly co-expressed with TRPV5, NCX1, and calbindin-D₂₈K in the kidney (Fig. 5a), and also abundant in the organs involved in calcium homeostasis such as the parathyroid glands and the choroid plexus. The data strongly indicate that klotho is a regulator of calcium metabolism both in the parathyroid glands and the kidney. The mechanism of calcium regulation by klotho is not clear yet, but there are several suggestions about it. Imura et al. documented that α-Kl made complexes with the α and β subunit of Na⁺,K⁺-ATPase. α-KI was required for the rapid recruitment of Na⁺,K⁺-ATPase to the cell surface in response to high extracellular Ca²⁺ concentration. This activation of Na⁺,K⁺-ATPase by α-Kl enhanced calcium reabsorption in the kidney and secretion of PTH in the parathyroid glands (Fig. 5b). Chang et al. documented that α-Kl was secreted in the urine and hydrolyzed the extracellular sugar residue on TRPV5 by β-glucuronidase activity. This hydrolyzed TRPV5 was entrapped in the plasma membranes and then caused increase in Ca²⁺ reabsorption (Fig. 5b). In addition, klotho has interactions with FGF23 and contributes to renal Ca²⁺ handling. Klotho binds to canonical GFG receptor (FGFR) and converts it into a specific receptor for FGF23, and then α-Kl in combination with FGF23 negatively regulates vitamin D production in the kidney. Therefore, klotho acts as a new player in renal Ca²⁺ handling by the regulation of multiple steps of calcium metabolism.

**FGF23**

FGF23, a member of the FGF family (type I transmembrane phosphotyrosine kinase receptors), is a 30 kDa secreted protein and inactivated by cleavage into two smaller fragments (N-terminal 18 kDa fragment and C-terminal 12 kDa fragment) by a pro-convertase enzyme, furin. It was first cloned as the candidate gene for autosomal dominant hypophosphatemic rickets (ADHR). FGF23 is primarily expressed in the osteoblasts and osteocytes. Because Fgf23 knockout mice showed very similar phenotype to Klotho knockout mice including severe hyperphosphatemia and osteoporosis, and gain of function mutation of Fgf23 gene was observed in ADHR patients. The main studies about the role of FGF23 in the kidney have focused on phosphate metabolism rather than calcium metabolism.
For phosphate metabolism, FGF23 acts mainly on the kidney and increases renal phosphate excretion by the inhibition of type IIa Na\(^{+}\)/Pi cotransporter expression in the PT and 1α-hydroxylase of vitamin D\(^{24, 25}\). Klotho converts canonical FGFR to specific FGF23 receptor to make it possible for the FGF23:klotho complex to bind to the receptor, and then this complex regulates the activation of vitamin D (Fig. 6b). However, the exact mechanism how FGF23:klotho regulates renal phosphate excretion is still unknown. It is still unknown how the FGF23:klotho complex from the DCT acts in the PT because the main action site of FGF23 in the kidney is the PT, whereas the FGF23:klotho complex is most abundant in the DCT. Both overexpression and deficiency of FGF23 cause several clinical diseases including ADHR and HFTC (hyperphosphatemic familial tumoral calcinosis). Recently, FGF23 was suggested as a potential biomarker for management of phosphate balance in chronic kidney disease (CKD) patients because the circulating FGF23 level was higher in CKD patients than healthy controls and the increased FGF23 level was an independent risk factor for higher mortality among dialysis patients\(^{26}\). FGF23 also plays some roles in the parathyroid glands and other organs like the choroid plexus, pituitary gland, and bone. However, further studies are needed to clarify the roles and the mechanisms.

Conclusion

The kidney has been known as the central organ for calcium homeostasis through fine regulation of renal calcium excretion. For the past decade, there has been big progress in the understanding of the roles of the kidney in calcium homeostasis. The identification of calcium transport proteins and the molecular approach to the regulatory mechanisms achieved a major contribution to this progress. TRPV5, TRPV6, calbindin-D\(_{28K}\), NCX1, and
PMCA1b have been identified as the main calcium transport proteins in the distal nephron. PTH, vitamin D, \([\text{Ca}^{2+}]\), CaSR, and other various conditions control renal calcium excretion through the regulation of these transport proteins. Klotho and FGF23 emerged as new players in calcium metabolism in the kidney. Thus, the role of the klotho-FGF23 axis in the regulatory mechanisms of calcium transport needs to be addressed.

**References**

1) Mount DB, Yu AS: Transport of Inorganic Solutes: Sodium, Chloride, Potassium, Magnesium, Calcium, and Phosphate. In: Brenner & Rector's the kidney, 8th ed., edited by Brenner BM, Philadelphia, 2008, p.185-192

2) Hoenderop JG, Bindels RJ: Epithelial Ca\(^{2+}\) and Mg\(^{2+}\) channels in health and disease. J Am Soc Nephrol 16:15-26, 2005

3) Nijenhuis T, Renkema KY, Hoenderop JG, Bindels RJ: Acid-base status determines the renal expression of Ca\(^{2+}\) and Mg\(^{2+}\) transport proteins. J Am Soc Nephrol 17:617-626, 2006

4) Lee CT, Chen HC, Lai LW, Yong KC, Lien YH: Effects of furosemide on renal calcium handling. Am J Physiol Renal Physiol 293:F1231-1237, 2007

5) Nijenhuis T, Hoenderop JG, Loffing J, van der Kemp AW, Loffing J: Thiazide-induced hypocalciuria is accompanied by a decreased expression of Ca\(^{2+}\) transport proteins in kidney. Kidney Int 64:555-564, 2003

6) Kiuchi-Saishin Y, Gotoh S, Furuse M, Takasuga A, Tano Y, Tsukita S: Differential expression patterns of claudins, tight junction membrane proteins, in mouse nephron segments. J Am Soc Nephrol 13:875-886, 2002

7) Ward DT, Riccardi D: Renal physiology of the extracellular calcium-sensing receptor. Pflugers Arch 445:169-176, 2002

8) Attie MF, Gill JR, Jr., Stock JL, Spiegel AM, Downs RW, Jr., Levine MA, et al.: Urinary calcium excretion in familial hypocalciuric hypercalcaemia. Persistence of relative hypocalciuria after induction of hypoparathyroidism. J Clin Invest 72:667-676, 1983

9) van de Graaf SF, Hoenderop JG, Bindels RJ: Regulation of TRPV5 and TRPV6 by associated proteins. Am J Physiol Renal Physiol 290:F1295-1302, 2006

10) Hoenderop JG, Muller D, Van Der Kemp AW, Hartog A, Suzuki M, Ishibashi K, et al.: Calcitriol controls the epithelial calcium channel in kidney. J Am Soc Nephrol 12:1342-1349, 2001

11) Nijenhuis T, Hoenderop JG, van der Kemp AW, Bindels RJ: Localization and regulation of the epithelial Ca\(^{2+}\) channel TRPV6 in the kidney. J Am Soc Nephrol 14:2731-2740, 2003

12) Hoenderop JG, van Leeuwen JP, van der Eerden BC, Kersten FF, van der Kemp AW, Merillat AM, et al.: Renal Ca\(^{2+}\) wasting, hyperabsorption, and reduced bone thickness in mice lacking TRPV5. J Clin Invest 112:1906-1914, 2003

13) Hoenderop JG, Bindels RJ: Calciotropic and magnesiotropic TRP channels. Physiology (Bethesda) 23:32-40, 2008

14) Nijenhuis T, Vallon V, van der Kemp AW, Loffing J, Hoenderop JG, Bindels RJ: Enhanced passive Ca\(^{2+}\) reabsorption and reduced Mg\(^{2+}\) channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. J Clin Invest 115:1651-1658, 2005

15) Lee CT, Shang S, Lai LW, Yong KC, Lien YH: Effect of thiazide on renal gene expression of apical calcium channels and calbindins. Am J Physiol Renal Physiol 287:F1164-1170, 2004

16) Jang HR, Lee JW, Heo NJ, Lee JH, Oh YK, Na KY, et al.: Effects of thiazide on the expression of transient receptor potential vanilloid 5 and calbindin-D\(_{28K}\) in a hypercalciuria rat model [Abstract]. J Am Soc Nephrol 17:355A, 2006

17) Lambers TT, Mahieu F, Oancea E, Hoofd L, de Lange F, Mensenkamp AR, et al.: Calbindin-D\(_{28K}\) dynamically controls TRPV5-mediated Ca\(^{2+}\) transport. EMBO J 25:2978-2988, 2006

18) Kuro-o M, Matsumura Y, Aizawa H, Kawaguchi H, Suga T, Utsumi T, et al.: Mutation of the mouse klotho gene leads to a syndrome resembling ageing. Nature 390:45-51, 1997

19) Imura A, Tsuji Y, Murata M, Maeda R, Kubota K, Iwano A, et al.: alpha-Klotho as a regulator of calcium homeostasis. Science 316:1615-1618, 2007

20) Chang Q, Hoefs S, van der Kemp AW, Topula CN, Bindels RJ, Hoenderop JG: The beta-glucuronidase klotho hydrolyzes and activates the TRPV5 channel. Science 310:490-493, 2005

21) Nabeshima Y, Imura H: alpha-Klotho: a regulator that integrates calcium homeostasis. Am J Nephrol 28:455-464, 2008

22) Urakawa I, Yamazaki Y, Shimada T, Iijima K, Hasegawa H, Okawa K, et al.: Klotho converts canonical FGF receptor into a specific receptor for FGF23. Nature 444:770-774, 2006

23) Yamashita T, Yoshioka M, Itoh N: Identification of a novel fibroblast growth factor, FGF-23, preferentially expressed in the ventrolateral thalamic nucleus of the brain. Biochem Biophys Res Commun 277:494-498, 2000

24) Razzoque MS, Lanske B: The emerging role of the fibroblast growth factor-23-klotho axis in renal regulation of phosphate homeostasis. J Endocrinol 194:1-10, 2007

25) Liu S, Quarles LD: How fibroblast growth factor 23 works. J Am Soc Nephrol 18:1637-1647, 2007

26) Gutierrez OM, Mannstadt M, Isakova T, Rauh-Hain JA, Tamez H, Shah A, et al.: Fibroblast growth factor 23 and mortality among patients undergoing hemodialysis. N Engl J Med 359:584-592, 2008