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Lecture: New light on the role of claudins in the kidney

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Lecture

New light on the role of claudins in the kidney

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The physiology of paracellular permeation of ions and solutes in the kidney is pivotally important but poorly understood. Claudins are the key components of the paracellular pathway. Defects in claudin function result in a broad range of renal diseases, including hypomagnesemia, hypercalciuria and nephrolithiasis. This review describes recent findings on the physiological function of claudins underlying paracellular transport mechanisms with a focus on renal Ca^{2+} handling. We have uncovered a molecular mechanism underlying paracellular Ca^{2+} transport in the thick ascending limb of Henle (TAL) that involves the functional interplay of three important claudin genes: claudin-14, -16 and -19, all of which are associated with human kidney diseases with hypercalciuria, nephrolithiasis and bone mineral loss. The Ca^{2+} sensing receptor (CaSR) signaling in the kidney has long been a mystery. By analyzing small non-coding RNA molecules in the kidney, we have uncovered a novel microRNA based signaling pathway downstream of CaSR that directly regulates claudin-14 gene expression and establishes the claudin-14 molecule as a key regulator for renal Ca^{2+} homeostasis. The molecular cascade of CaSR-microRNAs-claudins forms a regulatory loop to maintain proper Ca^{2+} homeostasis in the kidney.

Keywords: tight junction, calcium, claudin, paracellular channel, hypercalciuria, thick ascending limb

Abbreviations: FHHNC, hypomagnesemia with hypercalciuria and nephrocalcinosis; TAL, thick ascending limb; TER, transepithelial resistance; TJ, tight junction; GWAS, genome-wide association study; Y2H, yeast 2-hybrid assay; CaSR, Ca^{2+} sensing receptor

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Introduction

Kidneys function by initially excreting many salts and small molecules found in the blood, then selectively reabsorbing those that need to be conserved while allowing others to be excreted in the urine. The traditional view of the renal reabsorption process is that of a tandem array of ion channels and transporters located in the cell plasma membrane conducting ion transport in a coordinated manner at the expense of energy. Evidence accumulated during the last decade supports the existence of a previously unrecognized, yet pivotally important mechanism by which the kidney utilizes the cell-cell junctions to conduct ion transport. The junctional organelle is known as the tight junction (zonula occludens). It is found in vertebrate epithelia responsible for the barrier to movement of ions and molecules between apical and basal compartments.[1,2] The integral membrane proteins of the tight junction include occludin (a 65 kDa membrane protein bearing four transmembrane domains and two uncharged extracellular loops),[3] the junctional adhesion molecules (JAMs),[4] a four-member group of glycosylated proteins and the claudins.

The Claudin Family

Claudins (CLDNs) are tetraspan proteins consisting of a family with at least 26 members,[5-7] ranging in molecular mass from 20–28 kD. Claudins have four transmembrane domains, two extracellular loops, amino- and C-terminal cytoplasmic domains and a short cytoplasmic turn (**Fig. 1**). The first extracellular loop (ECL1) of claudin consists of ~50 amino acids with a common motif (-GLWCC; PROSITE ID: PS01346),[8] and intercalating negative[9,10] and positive[11,12] charges that contribute to paracellular ion selectivity. The GLWCC motif is critical as a receptor for Hepatitis C virus (HCV)

entry.[13] The charges in ECL1 regulate paracellular ion selectivity through electrostatic effects. The second extracellular loop (ECL2) consists of ~25 amino acids with a predicted helix-turn-helix motif that mediates *trans*-claudin interactions (vide infra) and claudin interactions with the *Clostridium perfringens* enterotoxin (CPE).[14] The C-terminal domain of claudin contains a PDZ (postsynaptic density 95/discs large/zonula occludens-1) binding domain (YV) that is critical for interaction with the submembrane scaffold protein ZO-1 and correct localization in the TJ.[15,16]

Claudin mutations have serious consequences, consistent with defects in epithelial ion flux. Mutations in CLDN14 cause nonsyndromic recessive deafness DFNB29,[17] ostensibly due to a failure in ion balance in the organ of Corti.[18] CLDN1-deficient mice die within one day of birth and show a loss of the water barrier function of skin.[19] Targeted deletion of CLDN5, which is known to be expressed in vascular endothelia as well as other locations,[20] results in a selective increase in brain vascular permeability to molecules $<$ 800 daltons.[21] Targeted disruption of the CLDN11 gene results in severe demyelination and male sterility, consistent with the presence of this protein at the nodes of Ranvier and in Sertoli tight junctions, leading to disrupted ionic balances.[22] Mutations in CLDN16 have been associated with human FHHNC (familial hypomagnesemia with hypercalciuria and nephrocalcinosis; OMIM 248250).[23] Transgenic RNAi depletion of CLDN16 demonstrated severe renal Mg^{2+} and Ca^{2+} losses in mice.[24]

In renal epithelia, claudins have been shown to confer ion selectivity to the paracellular pathway resulting in differences in TER and paracellular permeabilities. Studies have shown that CLDN4, -5, -8, -11 and -14 selectively decrease the permeability of cations through tight junctions,[25-29] specifically to Na^+, K^+, H^+ and ammonium. CLDN2 and -15 increase cation permeability.[30-32] These properties have been attributed to charged amino acids in the first extracellular domain.[9] These and other studies[33] have led to models of the claudins forming the paracellular channel (**Fig. 2**), a novel class of

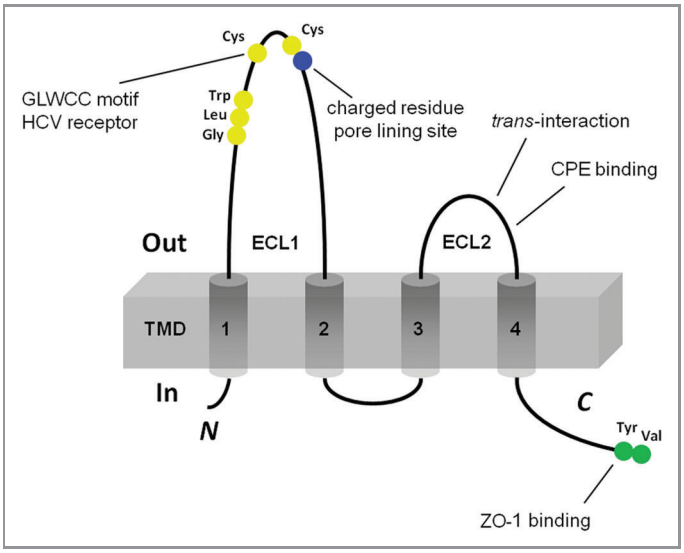

Figure 1. Schematic presentation of the topology of claudin monomer. The model depicts the conserved structural features of claudin and the known interaction sites. ECL1 and ECL2 denote the extracellular loops 1 and 2, respectively. The transmembrane domains 1 to 4 (TM1-TM4) and the regions important for hepatitis C virus (HCV) entry, paracellular ion selectivity, *Clostridium perfringens* enterotoxin (CPE) binding, and ZO-1 binding are shown.

channels oriented perpendicular to the membrane plane and serving to join two extracellular compartments.[34] Measurement of paracellular permeability using cell membrane impermeable tracers indicates that there are 4–7 Å diameter channels in the TJ.[33,35,36] The paracellular channels in the tight junction have properties of ion selectivity, pH dependence and anomalous mole fraction effects, similar to conventional transmembrane channels.[33]

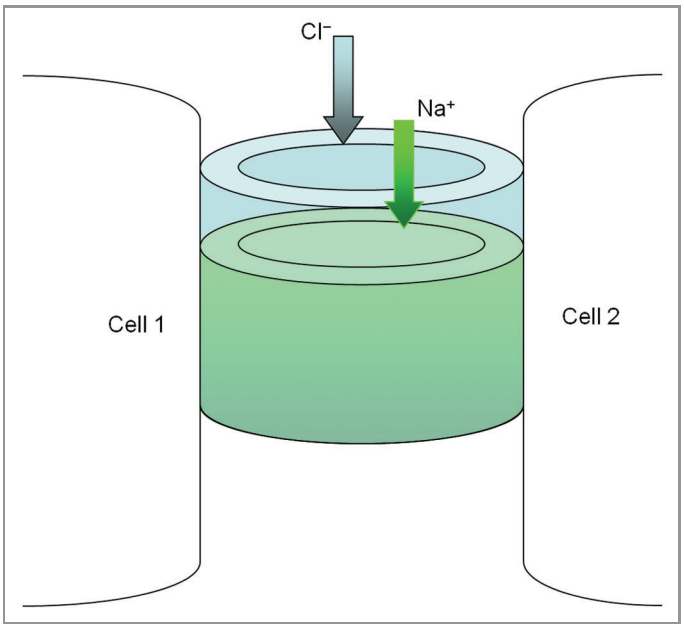

Figure 2. The structural model of paracellular channel. The paracellular channel is depicted as cylinders joining two neighboring cell membranes and allowing selective permeation of cation (Na^+) and anion (Cl^-) respectively.

Claudin-Claudin Interaction

Claudins *cis* associate within the plasma membrane of the cell into dimers, or higher oligomeric state. These associations are followed by *trans* interactions between claudins in adjacent cells, and additional *cis* interactions to assemble claudin oligomers into TJ strands. The *cis* interaction can involve a single type of claudin (homomeric interaction) or different types of claudins (heteromeric interaction); the *trans* interaction can have homotypic or heterotypic mode (**Fig. 3**).[37] There are few data available allowing an understanding of the molecular interactions between the claudins. One study has shown that heterotypic interactions of CLDN1 and CLDN3 are permitted, but that interactions between CLDN1 and CLDN2 are not observed.[37] Co-culture of HeLa cells expressing different claudin genes revealed that while CLDN1 and CLDN5 were heterotypically interacting with CLDN3, they would not heterotypically bind to CLDN4, demonstrating considerable selectivity in heterotypic claudin-claudin interactions.[38] While different claudins can assemble into the same TJ strand, current limitations of resolution do not permit a clear understanding of what heteromeric interactions (within cells) are favored. Efforts have been made to demonstrate the oligomerization properties of CLDN4 in cultured insect cells with ambiguous results.[39] FRAP studies suggest that claudin molecules assembled in tight junctions have limited mobility,[40] consistent with their known heteromeric interactions with scaffold proteins in the tight junction.

The Renal Localization of Claudins

There are segment-specific claudin expression profiles along the length of the nephron. Northern analysis of mouse kidneys using probes specific for CLDN1–16 reveal that only CLDN6, -9, -13 and -14 are not detectable. CLDN5 and -15 are present only in endothelial cells; the rest are specifically expressed in different segments of the nephron.[41] Using antisera available at the time to perform immunostaining on mouse kidneys,[41] CLDN-3, -10, -11 and -16 were observed in the thick ascending limb (TAL), CLDN-3 and -8 in the distal convoluted tubule, and CLDN-3, -4 and -8 in the collecting duct (CLDN4 was also observed in the thick ascending limb[41] although absent in bovine TAL[42]). CLDN2 is highly expressed in the "leaky" proximal nephron[43] consistent with its high cation selectivity when expressed in MDCK cells.[30,31] CLDN4 and CLDN8 are expressed primarily along the aldosterone-sensitive distal nephron, and in inner medullary segments of the thin descending limbs of juxtamedullary nephrons.[44,45] Immunofluorescence analysis has shown that CLDN7 is expressed in the TAL and collecting ducts of porcine and rat kidneys,[46] although another study described CLDN7 in the distal nephron as located primarily on the basolateral membrane.[45] In summary, while there are still some conflicting published data, CLDN-2, -10, and -11 are expressed in the proximal tubule; CLDN-3, -4, -7, -8, -10 and -16 are expressed in the thick ascending limb and the distal nephron. It is also clear that the patterns of claudin expression along the nephron changes with development, with CLDN-7 and -8 upregulated postnatally.[47]

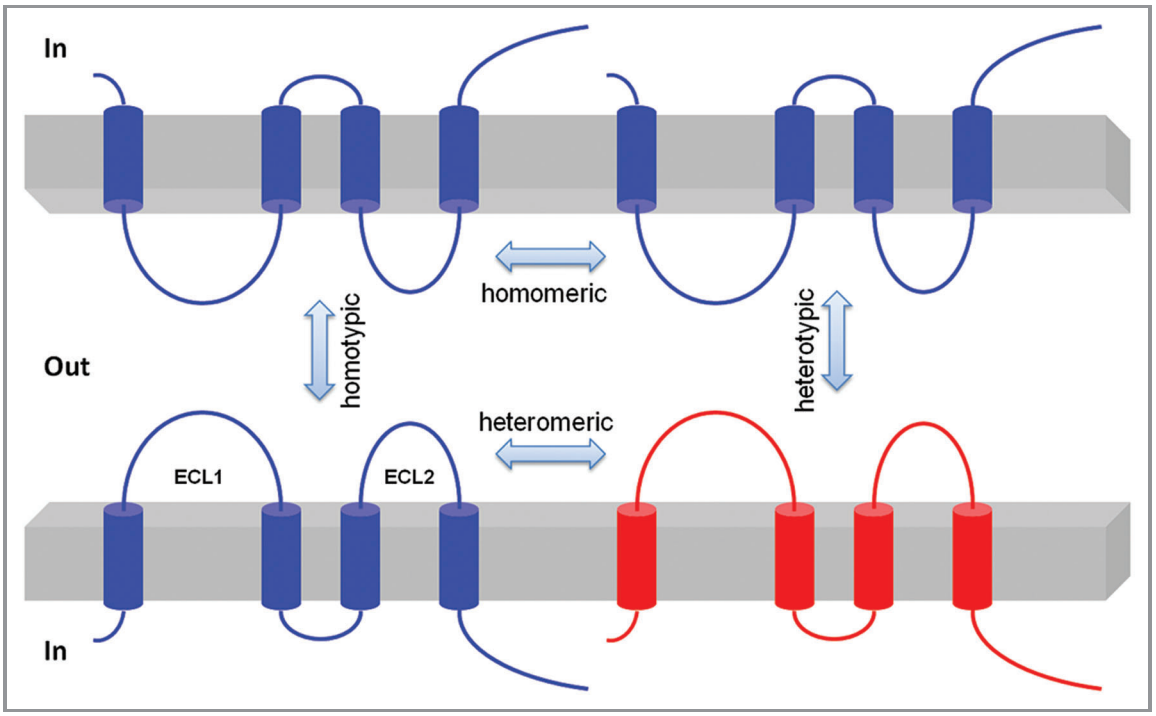

Figure 3. Schematic presentation of interaction possibilities between claudin molecules. The *cis* interaction includes homomeric or heteromeric interaction; the *trans* interaction includes homotypic or heterotypic interaction.

Claudin-16

CLDN16, also known as paracellin-1, has been identified as a renal tight junction protein that is mutated in patients with the inherited disorder FHHNC (familial hypomagnesemia with hypercalciuria and nephrocalcinosis OMIM 248250).[23] Many different FHHNC mutations have been identified in the human CLDN16 gene.[48,49] The expression of CLDN16 is restricted to the thick ascending limb (TAL) of the nephron.[23] We transfected a renal model cell line LLC-PK1 with CLDN16 and found a large increase in Na^+ permeability (P_{Na}) accompanied by an only moderately enhanced Mg^{2+} permeability (P_{Mg}).[10] The increase in P_{Na} was not affected by the Na^+/K^+-ATPase inhibitor (1 mM ouabain). However it was greatly reduced or completely disappeared in all FHHNC relevant CLDN16 mutants.[10] CLDN16 deficient knockdown (KD) mice show significantly reduced plasma Mg^{2+} levels and increased urinary excretions (approximately four-fold) of Mg^{2+} and Ca^{2+}.[50] Calcium deposits were readily observed in the basement membranes of the medullary tubules and the interstitium in CLDN16 KD mouse kidney.[50] These phenotypes of CLDN16 KD mice are very similar to the symptoms in human FHHNC patients. When TAL segments were isolated and perfused ex vivo, the paracellular ion selectivity (P_{Na}/P_{Cl}) of the TAL was significantly reduced from 3.1 ± 0.3 in WT to 1.5 ± 0.1 in CLDN16 KD.[50]

Claudin-19

CLDN19 mutations have also been associated with human FHHNC and renal Mg^{2+} loss.[51] While targeted deletion of CLDN19 in mice initially focused on its role in peripheral myelin,[52] promoter analysis[53] and subsequent studies[54] have emphasized the localization of CLDN19 in the TAL of the kidney (colocalizing with CLDN16). Using the LLC-PK1 cells, we found that CLDN19 profoundly decreased the Cl^- permeability (P_{Cl}) and functioned as a Cl^- blocker.[55] The FHHNC mutations from human patients either partially or completely abolish the CLDN19 effects on P_{Cl}.[55] Coexpression of CLDN16 and CLDN19 in LLC-PK1 cells resulted in a dramatic upregulation of P_{Na} and downregulation of P_{Cl}, generating a highly cation-selective paracellular pathway.[55] CLDN19 KD animals phenocopy CLDN16 KD and develop the FHHNC symptoms of reduced plasma Mg^{2+} levels and excessive renal wasting of Mg^{2+} and Ca^{2+}.[56]

Claudin-14

A recent genome-wide association study (GWAS) has identified CLDN14 as a major risk gene of hypercalciuric nephrolithiasis.[57] The renal localization of CLDN14 has been controversial. Immunofluorescence analysis showed CLDN14 gene expression in the TAL and the proximal tubules of mouse kidneys,[58] while another study reported no CLDN14 expression in the kidney.[41] In a CLDN14 *lacZ* reporter mouse, Gong et al. have found the promoter activity and the mRNA level of CLDN14 highly localized in the TAL segment.[59] The protein level of CLDN14, however, was extremely low in kidneys of mice fed a basal or low Ca^{2+} diet. Feeding mice a high Ca^{2+} diet profoundly upregulated the protein levels of CLDN14 in the TAL segment, suggesting a regulatory role for CLDN14 in renal function.[59] When expressed alone in LLC-PK1 cells, CLDN14 was without any significant effect on P_{Na} or P_{Cl}. Coexpression of CLDN14 with CLDN16 abolished the cation permeability of CLDN16 channel by reducing P_{Na}.[59] CLDN14 expression produced no difference in CLDN19 channel function. CLDN14 knockout (KO) mice show normal renal function under basal dietary condition.[27,58] When fed with a high Ca^{2+} diet, CLDN14 KO animals excreted significantly less Ca^{2+} and Mg^{2+} than wild-type (WT) animals.[59] The plasma Mg^{2+} level was significantly higher in CLDN14 KO than in WT.[59] Together, CLDN14 KO animals develop the renal phenotypes exactly opposite to those in CLDN16 KD, which supports the in vitro finding that CLDN14 blocks CLDN16 channel permeability.

The Dynamic Trio in Renal Disease

The phenotypic similarities of CLDN19 KD with CLDN16 KD can be explained by the *cis* interaction between the two claudins. Using a split-ubiquitin yeast 2-hybrid (Y2H) assay, we found strong CLDN16 and CLDN19 heteromeric interaction.[55] In mammalian cells such as human embryonic kidney (HEK) 293 cells, CLDN16 can be coimmunoprecipitated with CLDN19.[55] Freeze-fracture replicas revealed the assembly of tight junction strands in L cells coexpressing CLDN16 and CLDN19, supporting their heteromeric interaction. The point mutations in CLDN16 (L145P, L151F, G191R, A209T and F232C) or CLDN19 (L90P and G123R) that are known to cause human FHHNC disrupt the CLDN16 and CLDN19 heteromeric interaction.[55] The same mutations also abolish the cation selectivity generated by CLDN16 and CLDN19 in LLC-PK1 cells, suggesting a mechanism for the role of claudin mutations in the development of FHHNC.[55] In vivo, knockdown of CLDN19 causes a loss of CLDN16 from tight junctions in the TAL without a decrease in CLDN16 expression level, whereas knockdown of CLDN16 produces a similar effect on CLDN19.[56] CLDN14 was observed to interact with CLDN16 but not with CLDN19 using several criteria.[59] Y2H analysis showed strong CLDN14–16 interaction the strength of which was similar to the CLDN16–19 interaction.[59] In doubly transfected HEK293 cells, CLDN14 coprecipitated with CLDN16 but not with CLDN19. Intriguingly, in triply transfected HEK293 cells, CLDN14 coprecipitated not only with CLDN16 but also with CLDN19.[59] There are two explanations for these findings relating to the CLDN14 assembly mechanism: (1) CLDN14 integrates into the CLDN16–19 channel to form a higher oligomeric complex with novel physiological signature; (2) CLDN14 replaces CLDN19 to form an independent channel with CLDN16 that coexists with the CLDN16–19 channel. The available biochemical data clearly favor the first hypothesis.

MicroRNA as Guardian Molecule for Claudin

Although the promoter activity and the mRNA level of CLDN14 are very high in

the kidney, its protein level is surprisingly low, suggesting post-transcriptional regulation. MicroRNAs are key regulatory molecules that regulate gene expression on the post-transcriptional level by inducing target mRNA decay or translational repression.[60] Gong et al. have identified two microRNA molecules—miR-9 and miR-374 from TAL cells, both of which recognize partially complementary binding sites located in 3'-UTRs of CLDN14 mRNA.[59] MiR-9 and miR-374 suppress CLDN14 translation and induce its mRNA decay in a synergistic manner.[59] Under normal dietary conditions, miR-9 and miR-374 tightly regulate the gene expression level of CLDN14 and protect CLDN16–19 channel function. The observed association between CLDN14 and hypercalciuric nephrolithiasis[57] can be explained by CLDN14 deregulation that escapes microRNA suppression, inhibits CLDN16–19 channel permeabilites and phenocopies FHHNC to variable degrees. High Ca^{2+} intake significantly downregulates the expression levels of miR-9 and miR-374 in TAL cells,[59] which in turn causes an increase in CLDN14 expression level discussed elsewhere in this review. The microRNA-CLDN14 cascade is under direct regulation of the Ca^{2+} sensing receptor (CaSR) in the TAL, a site at which the CaSR monitors the circulating Ca^{2+} levels and adjusts excretion rates accordingly.[59] The dietary regulation of microRNA suggests a physiological role for microRNA based signaling in the kidney. A physiological role of microRNA signaling provides a rationale for studying pathological changes such as nephrolithiasis, because any pathological abnormality must have a physiological origin.

An Integrated Signaling Pathway

The thick ascending limb (TAL) is a predominant renal tubular segment responsible for Mg^{2+} and Ca^{2+} reabsorption.[61,62] The epithelial cells in the TAL form a water-impermeable barrier, actively transport Na^+ and Cl^- via the transcellular route, and provide a paracellular pathway for the selective reabsorption of Mg^{2+} and Ca^{2+}.[63–64] The paracellular reabsorption of Mg^{2+} and Ca^{2+} is driven by a lumen-positive transepithelial voltage (V_{te}). The generation of V_{te} can be attributed to: (1) the active transport V_{te} due to apical K^+ secretion through ROMK and basolateral Cl^- exit through ClC-Kb/barttin channels; (2) the diffusion V_{te} generated because of the transepithelial NaCl concentration gradient and the cation selectivity of the tight junction.[65] In the early TAL segment, it is the first mechanism that provides a voltage around +8 mV. There is minimal contribution of diffusion potential at this early segment since the concentration gradient has not yet been built up (**Fig. 4A**). With continuous NaCl reabsorption along the axis of the TAL, the lumen fluid is diluted and a large NaCl gradient is generated in the late TAL segment. Because the paracellular permeability of the TAL is cation-selective, the diffusion V_{te} is superimposed onto the active transport V_{te} and becomes the major source of the lumen-positive V_{te},

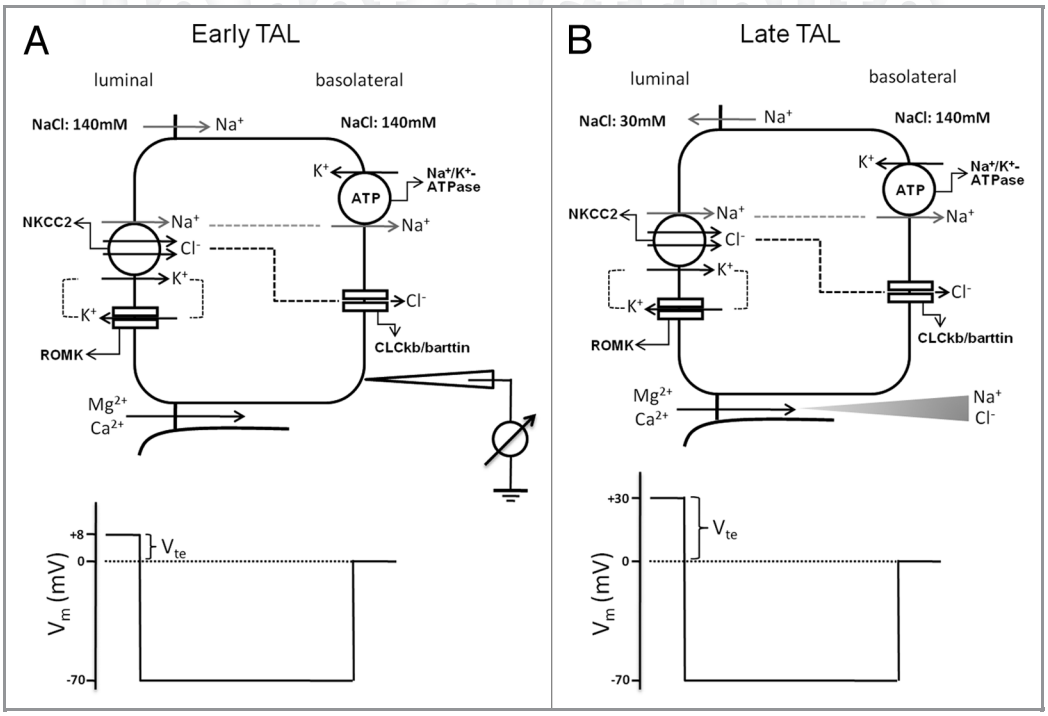

Figure 4. Transepithelial ion transport in the thick ascending limb segment. (A) When similar salt concentrations are present at the luminal and basolateral sides, the luminal spontaneous potential V_{te} of +8mV is generated by the concerted action of luminal K^+ channels, basolateral Cl^- channels, the $Na^+2Cl^-K^+$ cotransporter, and the Na^+K^+-ATPase. V_{te} drives Na^+ absorption through the paracellular pathway. (B) When a dilute luminal fluid is present after NaCl absorption along the water-tight TAL, the luminal potential V_{te} of +30 mV is now generated as a diffusion voltage by the 'backleak' of Na^+. The diffusion voltage depends on the permselectivity of the tight junction. The membrane voltage (V_m) trace depicts the virtual measurement by an electrode that is pushed from the basolateral side through the cell to the luminal side.

which now increases substantially—up to +30 mV (**Fig. 4B**).

It is clear that two basic prerequisites are required for the paracellular Mg^{2+} and Ca^{2+} reabsorption in the TAL: the lumen-positive V_{te} as the driving force and the paracellular cation permeability. The CLDN16 channel provides cation permeability to the tight junction. The CLDN19 channel increases the cation selectivity of the tight junction and the diffusional V_{te} by: (1) selectively blocking anion permeation; (2) interacting with CLDN16 to increase the overall cation selectivity of the tight junction. Removal of either claudin would cause the tight junction to lose its cation selectivity and generate renal defects in Mg^{2+} and Ca^{2+} reabsorption. The phenotypic similarities between CLDN16 KD and CLDN19 KD animals can be explained by the molecular interaction between CLDN16 and CLDN19. This molecular interaction provides a mechanistic basis for the role of claudin mutations in the development of FHHNC. The discovery of CLDN14 as a regulatory molecule for renal Ca^{2+} homeostasis is particularly important. First, accumulating data have demonstrated that paracellular Ca^{2+} reabsorption in the TAL can be directly regulated by CaSR activation during hypercalcemia.[66,67] CLDN14 is the "underlying factor" sought for many years. Through physical interactions, CLDN14 blocks the paracellular cation channel made of CLDN16 and CLDN19, suggesting a mechanism for its role in nephrolithiasis. Second, tight junction proteins were previously considered as constitutive and structural molecules. CLDN14 is the first TJ molecule the expression level of which can be regulated in response to pathophysiological changes. The tight junction is not as 'lethargic' as previously thought. The renal regulation of Ca^{2+} excretion involves the functional interplay of three important claudin genes: CLDN14, 16 and 19, all of which are associated with human kidney diseases. The claudin channel molecules are part of the CaSR signaling cascade that employs two microRNA molecules to transduce signals. The molecular axis of CaSR-microRNA-CLDN14-CLDN16/19 provides a feedback mechanism to counterbalance extracellular Ca^{2+} variations. Increases in extracellular Ca^{2+} levels activate CaSR → CaSR activation downregulates miR-9 and miR-374 expression → decreases in microRNA levels relieve their suppression of CLDN14 → increases in CLDN14 levels suppress CLDN16–19 permeabilities, promoting Ca^{2+} excretion by the kidney; and vice versa (**Fig. 5**).

The Journey Ahead

The GWAS study established a genetic link between CLDN14 sequence variants and nephrolithiasis.[57] However, none of the identified sequence variants in CLDN14 appear to be causative. Because two of the four identified variants are located in the last exon of CLDN14 gene, this exon may contain rare causative variations related to microRNA regulation, mRNA stability and translational efficiency. Gong et al. have established a physiological role for CLDN14 in renal Ca^{2+} handling.[59] It will be essential to understand the pathogenic role of CLDN14 in nephrolithiasis. There are two interrelating hypotheses: (1) CLDN14 overexpression in the kidney leads to hypercalciuria and nephrolithiasis. Because CLDN14 is a negative regulator of the CLDN16–19 channel, overexpression of CLDN14 will lead to renal Ca^{2+} wasting. This hypothesis can be tested by generating a CLDN14 overexpressing ($CLDN14^{OX}$) mouse model. (2) The kidney will restore normal Ca^{2+} homeostasis through other nephron segments when the TAL is deregulated. Because the distal convoluted tubule (DCT) expresses a Ca^{2+} channel—TRPV5, Ca^{2+} excretion changes in the TAL will be compensated for in the DCT. This hypothesis can be tested by crossing $CLDN14^{OX}$ with $CLDN14^{-/-}$ to generate a TAL deregulated animal model—$CLDN14^{OX-/-}$. The microRNAs identified by Gong et al. are of critical importance.[59] Owing to the short seed sequence of microRNA, a cognate microRNA regulates multiple target genes. Although miR-9 and miR-374 converge onto CLDN14, they could extend extracellular Ca^{2+} signaling to cellular functions beyond the paracellular channel in the TAL. What remain largely unknown are the microRNA targeted genes in the kidney. From a therapeutic point of view, small RNA molecules such as antagomirs that repress microRNA function in vivo in the kidney may represent a novel tool for clinical intervention in renal Ca^{2+} excretion. Nevertheless, manipulation of microRNAs in vivo may induce unwanted side effects if their downstream target genes have not been thoroughly studied.

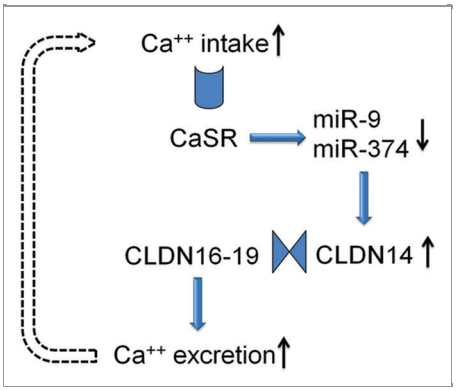

Figure 5. The feedback loop of CaSR signaling in the thick ascending limb cell. The microRNAs and CLDN14 are intermediate molecules that transduce CaSR signals to the Ca^{2+} effector in the plasma membrane. The claudin interactions underlie the function change in paracellular permeability.

Questions and Answers

Dr Eduardo Slatopolsky, Joseph Friedman Professor of Medicine: You showed very nicely the importance of the thick ascending limb of Henle (TAL) and claudin on magnesium reabsorption. But still there is a significant amount of magnesium that escapes the reabsorption and goes into the distal tubule where the epithelial magnesium channel, Transient Receptor Potential Melastatin subtype 6 (TRPM6)

participates in its reabsorption. Do defects in magnesium reabsorption in more distal nephron segments result in stone disease?

Dr Jianghui Hou, Assistant Professor of Medicine: Indeed, the TRPM6 channel plays an important role in the kidney, in addition to its role in the colon. Mutations in the TRPM6 channel are linked to HSH syndrome: hypomagnesemia with secondary hypocalcemia. I am not aware of a TRPM6 knockout mouse model that is not an embryonic lethal.[68] The human patient data suggest hypocalcemia and possibly hypercalciuria are caused by parathyroid gland failure induced by hypomagnesemia. When there is hypercalciuria, the risk of developing kidney stones will be higher. The mystery is where calcium loss occurs. In contrast to the TAL where calcium and magnesium handling is coupled, the distal convoluted tubule employs a different mechanism to handle calcium, through another channel: TRPV5.

Dr Slatopolsky: So your point is that they get hypermagnesuria but they do not get kidney stones.

Dr Hou: They may develop kidney stones or have higher risks. The underlying mechanism may be an indirect effect through claudin-14 or TRPV5 or other molecules.

Dr Aubrey Morrison, Professor of Medicine: How do you think the calcium sensing receptor (CaSR) functions in the presence of the lower magnesium? Are you implying that the calcium sensory receptor is able to discriminate between the calcium and magnesium?

Dr Hou: That is a very good question. Unlike in the parathyroid gland where CaSR shows higher affinity toward calcium than magnesium (approximately 5:1), kidney tubular cells seem to show a different affinity pattern. Our in vitro measurements found a rather equal affinity of CaSR toward calcium and magnesium. Actually, there is an independent study published a few years ago that supports our data and shows slightly higher affinity of CaSR toward magnesium than calcium in kidney tubular cells.[69]

Dr Slatopolsky: Alex, are you aware of two different types of calcium receptors?

Dr Alex Brown, Associate Professor of Medicine: No. The calcium receptor turns on different signaling pathway in different cell types.

Dr Slatopolsky: We joke and we call the calcium receptor a promiscuous receptor because it can detect not only calcium and magnesium, but also many other agonists. Have you tried other CaSR agonists?

Dr Hou: Yes, we have tried another CaSR agonist, Gadolinium, and found a similar effect on claudin-14 and microRNA expression. In addition, we tested the effects of PTH on claudin expression but found none, suggesting the observed effects are mediated through the CaSR but not PTH.

Dr Jeffrey Miner, Professor of Medicine: Have you tried expressing the claudin-14 either in vitro or in vivo without the 3' untranslated region?

Dr Hou: I haven't done it. We are generating mutations in the microRNA binding sites in claudin-14 3'UTR in order to determine whether microRNA effects are mediated through the 3'UTR of claudin-14.

Dr Maggie Chen, Assistant Professor of Medicine: I think the current concept is that after activation of the CaSR in the basal lateral membrane of the TAL there is inhibition of the ROMK channel in the apical membrane that can cause hypercalciuria and hypermagnesuria. Which part plays the bigger role?

Dr Hou: I will answer your question from two perspectives. The traditional view of CaSR function in the kidney is that it will transduce signals germane to the transcellular pathway, e.g., ROMK channel. The microRNAs we have identified may also regulate the transcellular pathways and serve as a converging point for signal transduction. My colleague Markus Bleich has measured the effects of CaSR activation on both the transcellular and paracellular ion conductance in perfused TAL. He was able to capture changes in the paracellular pathway but not in the transcellular pathway, further supporting our view of paracellular channels playing a major role in the CaSR signaling pathway.[70]

Dr Maggie Chen: You said there are two types of CaSR: in the basolateral membranes of the TAL and also in the apical site of the proximal tubule and the collecting duct. What is the role of the apical CaSR?

Dr Hou: That is a major mystery. Some suspect the apical CaSR senses changes of calcium in the tubular filtrate and transduces signals to cells. For example, CaSR in the proximal tubule could transduce signals to regulate calcitriol synthesis, which in turn affects calcium reabsorption in the distal nephron. The CaSR in the collecting duct has also been implicated in the regulation of water transport. However, I think the primary role of CaSR is still its classic role in the TAL—sensing the changes in circulating calcium levels.

Note

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