HHS Public Access

Author manuscript

Curr Opin Nephrol Hypertens. Author manuscript; available in PMC 2017 July 01.

Published in final edited form as:

Curr Opin Nephrol Hypertens. 2016 July ; 25(4): 308–313. doi:10.1097/MNH.0000000000000239.

Claudins and mineral metabolism

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Abstract

Purpose of review—The tight junction (TJ) conductance made of the claudin based paracellular channel is important in the regulation of calcium and magnesium reabsorption in the kidney. This review describes recent findings of the structure, the function, and the physiologic regulation of claudin-14, claudin-16 and claudin-19 channels that through protein interactions confer calcium and magnesium permeability to the TJ.

Recent findings—Mutations in two TJ genes – claudin-16 and claudin-19 cause the inherited renal disorder – familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). Recent genome-wide association study (GWAS) has identified claudin-14 as a major risk gene of hypercalciuric nephrolithiasis. Claudin-14 interacts with claudin-16 and inhibits its cation permselectivity. Transgenic overexpression of claudin-14 in the kidney generated the FHHNC-like phenotypes. The crystal structure of claudin-19 has recently been resolved allowing the reconstruction of a claudin assembly model from *cis*-dimers made of claudin-16 and claudin-19 interaction. MicroRNAs have been identified as novel regulators of the claudin-14 gene. The microRNA-claudin-14 operon is directly regulated by the Ca^{++} sensing receptor (CaSR) gene in response to hypercalcemia.

Summary—The paracellular pathway in the kidney is particularly important for mineral metabolism. Three claudin proteins – claudin-14, claudin-16 and claudin-19 contribute to the structure and function of this paracellular pathway. Genetic mutations and gene expression changes in these claudins may lead to alteration of the paracellular permeability to calcium and magnesium, ultimately affecting renal mineral metabolism. Claudins can be regulated on several levels including transcription, microRNA repression, trafficking, phosphorylation, and interaction.

Keywords

claudin; tight junction; hypercalciuria; ion channel; microRNA; kidney

Introduction

The tight junction (zonula occludens) is the most apical member of the junctional complex found in vertebrate epithelia responsible for the barrier to movement of ions and molecules between apical and basal compartments, the paracellular pathway [1,2]. The known integral

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Conflict of interest

The author declares no conflict of interest.

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. Claudins are tetraspan proteins consisting of a family of at least 22 members [5,6]. They range in molecular mass from 20–28 kD with charged extracellular loops. In renal epithelia, claudins confer ion selectivity to the paracellular pathway resulting in differences in epithelial resistance and paracellular permeabilities [7]. These results led to models of the claudins forming the **paracellular channel**, a novel class of channels oriented perpendicular to the membrane plane and serving to join two extracellular compartments.

Paracellular reabsorption of Ca^{++} and Mg^{++} in the kidney

The thick ascending limb of the loop of Henle (TALH) is responsible for reabsorbing 25–40% of filtered Na^{+} [8], 50–60% of filtered Mg^{++} [9] and 30–35% of filtered Ca^{++} [10]. The dissociation of salt and water reabsorption in the TALH results in the dilution of luminal fluid and the generation of an axial corticomedullary osmolality gradient, which then drives water reabsorption in the collecting duct [11]. The dilution of luminal fluid in the TALH also establishes a basolateral-luminal transepithelial salt concentration gradient that results in lumen-positive voltages as the driving force for passive paracellular reabsorption of Ca^{++} and Mg^{++} (Figure 1) [12,13].

Human mutations in claudins causing Ca^{++} and Mg^{++} imbalance diseases

A monogenic renal disorder, autosomal recessive familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC; OMIM #248250), is caused by mutations in two claudin genes: claudin-16 [14] and claudin-19 [15]. The claudin-16 and claudin-19 proteins are exclusively localized in the TALH [16]. Hou and colleagues have established that claudin-16 and claudin-19 proteins form a paracellular cation channel through direct protein interactions, which (i) permeates Ca^{++} and Mg^{++} [17]; (ii) generates a lumen-positive voltage owing to the transepithelial salt concentration gradient to drive Ca^{++} and Mg^{++} reabsorption [18]. A recent genome-wide association study (GWAS) has identified claudin-14 as a major risk gene of hypercalciuric nephrolithiasis [19]. Four common, synonymous variants in the claudin-14 gene locus were significantly associated with kidney stones by genome wide criteria. Two of them are exonic, causing silent mutations in the claudin-14 protein: rs219779[C] (R81R) and rs219780[C] (T229T). Both exonic SNPs show significant association with decreased bone mineral density (BMD). The urinary Ca^{++} excretion is significantly higher in homozygous carriers of rs219780[C] compared to non-carriers.

The biochemical basis of the paracellular channel made of claudins

The structure and function claudin-16

In low-resistance Madin-Darby canine kidney type II (MDCK-II) cells, Ikari and colleagues have found that transfected claudin-16 increased paracellular Ca^{++} but not Mg^{++} flux rates [20]. Using a high-resistance MDCK-C7 cell line, Kausalya and colleagues have revealed a modest effect of claudin-16 increasing paracellular Mg^{++} permeability [21]. Nevertheless, the effects of claudin-16 on divalent cation permeabilities measured by both studies are so

small (<50%) that the theory of claudin-16 forming a divalent channel cannot explain the dramatic phenotype caused by loss of claudin-16 function in FHHNC patients or animal models [14,22,23]. Serendipitously, Hou and colleagues have found that claudin-16 induced a profound increase in paracellular Na^+ permeability compared to the modest increase in Mg^{++} permeability with the low-resistance LLC-PK1 cells [17]. The permeabilities of claudin-16 to other alkali metal cations were also significant and resembled the Eisenman selectivity sequence V-VIII: $K^+ > Rb^+ > Na^+ \approx Li^+$ [17]. The Eisenman selectivity sequence reflects the charge density within an ion channel pore that will stabilize the permeating ions through electrostatic interaction [24]. Notably, the Eisenman selectivity sequence of claudin-16 is similar to that of claudin-2, a classic cation channel [25]. Mutagenesis studies have identified a locus of negatively charged amino acid residues in the 1st extracellular loop domain of claudin-16 (D104, D105, E119, D126 and E140) that are all required for its cation permeability.

The structure and function of claudin-19

In the cultured LLC-PK1 cells, transfected claudin-19 significantly decreased the paracellular permeability to Cl^- without affecting the Na^+ or Mg^{++} permeability [18]. Another study using the MDCK-II cells has found some conflicting results that claudin-19 rather decreased the permeability to Mg^{++} [26]. Collectively, both studies argue against the hypothetic theory that FHHNC is caused by kidney's inability to reabsorb Ca^{++} and Mg^{++} through a divalent cation channel in the tight junction [14]. Until recently, the 3D crystal structure of claudin-19 has been resolved at 3.7Å resolution [27]. The claudin-19 monomer adopts characteristic β-sheet folds in the extracellular loop domains, which are anchored to four transmembrane α-helical bundles (Figure 2A). A locus of charged amino acids in the 1st extracellular loop domain are purported to interact with the permeating ions by electrostatic field (Figure 2A). The electrostatic potential surface of the 1st extracellular loop domain reveals the location of residues (D38 and E48) potentially important for charge selectivity (Figure 2B).

The claudin-16 and claudin-19 interaction

When expressed separately in LLC-PK1 cells, claudin-16 increased paracellular permeability to Na^+ while claudin-19 decreased paracellular permeability to Cl^- [17,18]. Co-expression of claudin-16 and claudin-19 resulted in more pronounced changes in both Na^+ and Cl^- permeabilities compared to their each individual levels, therefore suggesting a functional synergy between these two claudins [18]. Using sucrose gradient sedimentation and chemical crosslinking, Gong and colleagues were able to isolate a stable dimer made of claudin-16 and claudin-19 from transfected HEK293 cells and Sf9 cells [28]. The dimerization appeared to be mediated through the *cis*-association of the transmembrane domain #3 and #4 in both claudin-16 and claudin-19 [28]. When mutations that selectively disrupted the claudin-16 and claudin-19 dimerization were introduced to either claudin, the resultant tight junction dramatically lost its cation permeability, suggesting a mechanism for the role of claudin interaction in the development of FHHNC [28]. Compatible with this hypothesis, *in vivo* in transgenic animal models, deletion of claudin-16 rendered claudin-19 delocalization from the tight junction and *vice versa* [16].

The claudin-14 and claudin-16 interaction

A recent GWAS study has identified claudin-14 as a major risk gene for hypercalciuric nephrolithiasis [19]. Gong and colleagues have found that claudin-14 protein is predominantly localized in the TALH, thus implicating claudin-14 to be anther candidate with potential roles in paracellular Ca^{++} and Mg^{++} transport [29]. In transfected LLC-PK1 cells, claudin-14 reduces the cation permeability of the claudin-16 channel, but not that of the claudin-19 channel, which is further supported by the finding that claudin-14 physically interacts with claudin-16 but not with claudin-19 [29]. Consistent with the hypothesis that claudin-14 acts as a negative regulator of the divalent cation permeation in the TALH, the TALH-specific overexpression of claudin-14 in transgenic animals have generated the FHHNC resembling phenotypes, including hypomagnesemia, hypermagnesiuria and hypercalciuria [30].

The regulatory mechanism of the paracellular channel made of claudins

Regulation of claudin localization in the tight junction

The carboxy-terminal domain of claudin-16 contains a PDZ (postsynaptic density 95/discs large/zonula occludens-1)-binding motif (TRV) that interacts with the PDZ domain of the schaffolding proteins such as ZO-1 [20]. Muller and colleagues identified a mutation in the PDZ-binding motif of claudin-16 (TRV to RRV) from patients with childhood FHHNC, which disrupted the claudin-16 binding to ZO-1 and caused claudin-16 delocalization from the tight junction [31]. Claudin phosphorylation status seemed to affect its interaction with ZO-1 and localization in the tight junction. The Serine 217 in the carboxyl terminus of claudin-16 is phosphorylated by protein kinase A (PKA) and dephosphorylation with PKA inhibitors reduced its interaction with ZO-1 and caused its mislocalization to the lysosome [32]. Extracellular Mg^{++} levels appear to regulate the tight junction localization of claudin-16 by phosphorylation dependent mechanism. In MDCK cells, low extracellular Mg^{++} levels reduced the localization of claudin-16 to the tight junction, which can be abolished by the extracellular signal-regulated kinase (ERK) inhibitor – U0126 [33]. With yeast two-hybrid screening approach, Ikari and colleagues have demonstrated direction interaction between claudin-16 and syntaxin-8, an important protein trafficking regulator [34]. Knockdown of syntaxin-8 in renal cells decreased the cell surface localization of claudin-16. On the other hand, the claudin-19 localization to tight junction is determined by its interaction with claudin-16. Deletion of claudin-16 in the mouse kidney caused claudin-19 delocalization from tight junction without affecting its mRNA or protein expression levels [16].

Transcriptional regulation of claudin gene expression

Changes in extracellular Mg^{++} levels may regulate claudin-16 gene expression as a feedback mechanism to regulate renal Mg^{++} reabsorption rates. Mice fed with low Mg^{++} diet had significantly higher expression levels of claudin-16 in the kidney compared to those fed with normal or high Mg^{++} diet [35]. The 2.5kb proximal promoter of the claudin-16 gene seems to contain sequences that can directly respond to extracellular Mg^{++} alterations [35]. The promoter activity of claudin-16 can also be regulated by 1,25-$(OH)_2$ vitamin D_3. In mice treated with 1,25-$(OH)_2$ vitamin D_3, the renal expression levels of claudin-16 were

significantly reduced [36]. Luk and colleagues have systematically analyzed the promoter binding sites in the claudin-19 gene and found a strong specific protein 1 (SP1) binding region located from −39 to −108 upstream of the transcription initiation site [37]. SP1 is a zinc finger transcription factor involved in many cellular processes, including chromatin remodeling, DNA damage control and apoptosis. It is not known what kind of extracellular signal regulates SP1 binding to the claudin-19 promoter and its activity. One study has revealed decreased claudin-19 expression levels in human polycystic kidneys [38].

MicroRNA based post-transcriptional regulation of claudin

MicroRNAs are key regulatory molecules that regulate gene expression on the post-transcriptional level by inducing target mRNA decay or translational repression [39]. Gong and colleagues have identified two microRNA molecules from the kidney – miR-9 and miR-374, both of which recognize complimentary binding sites located in the 3'-UTR of the claudin-14 mRNA [29]. Experiments using antisense oligonucleotide against miR-9 or miR-374 revealed that both microRNAs suppressed claudin-14 translation and induced its mRNA decay in a synergistic manner [29]. In vivo in the mouse kidney, anti-miR-374 oligonucleotide injection caused pronounced renal wasting of Ca^{++} and Mg^{++} due to unopposed expression of claudin-14 [40]. High Ca^{++} intake increases while low Ca^{++} intake decreases the expression levels of miR-9 and miR-374 in the kidney, which in turn causes a reciprocal increase in the claudin-14 expression level [29]. Toka and colleagues found that kidney specific deletion of the Ca^{++} sensing receptor (CaSR) caused pronounced reduction in the claudin-14 gene expression levels but the authors did not detect any change in the kidney microRNA level [41]. Using CaSR specific agonist (calcimimetic) or antagonist (calcilytic), Gong and colleagues further demonstrated that CaSR activation caused decreases in miR-9 and miR-374 expression levels but increases in claudin-14 expression levels, while CaSR inhibition exerted the opposite effects on microRNA and claudin-14 via histone deacetylation dependent epigenetic mechanisms [30,40]. These results suggest that the microRNA-claudin-14 molecular pathway is under direct regulation of CaSR and forms an essential part of the CaSR signaling cascade in the kidney (Figure 3).

Open questions

The previous macroscopic recordings of claudin-14, -16 and -19 conductance reflect the aggregates of thousands, or even millions of channel conductive events, with current density reaching $\mu A/cm^2$. Clearly, these measurements lack the resolution *on the molecular level*. Chen and colleagues developed a novel tool based on the Scanning Ion Conductance Microscopy (SICM) [42] to record the claudin-2 channel in MDCK cells with the conductance resolution reaching pA and the spatial resolution reaching nanometer [43]. Gong and colleagues applied this tool to resolve the TJ conductance difference made of the *cis*- and *trans*-interactions between claudin-16 and -19 [28]. However, single-channel conductance for claudin based paracellular channel has yet been captured using this approach. The major difficulty is how to separate the TJ conductance from nearby membrane conductance. Zhou and colleagues have proposed a novel route to independent measurements of TJ conductance by simultaneously neutralizing the apical membrane currents [44]. PTH and CaSR are two primary signaling mechanisms for extracellular Ca^{++}

homeostasis. Both signals have been shown to directly regulate the renal reabsorption of Ca^{++} through the tight junction [45,46]. While claudin-14 has been found as a downstream effector of CaSR, it remains unknown how PTH regulates the tight junction permeability to Ca^{++} and what the underlying molecules are. In addition to microRNA-based mechanism, phosphorylation, palmitoylation, and trafficking, may also exist for claudin-14, -16, and -19 that may explain the changes in paracellular Ca^{++} transport induced by PTH [45,46]. Finally, how claudins assemble into functional TJ architecture remains to be a major mystery. Based upon the spatial arrangement of claudin-15 within its crystal lattice, Suzuki and colleagues proposed a *cis*-dimeric assembly model through interactions of the β4 domain of the extracellular loop 1 (ECL1) of claudin-15 [47]. Using a systematic mutagenesis approach, Gong and colleagues suggested a similar *cis*-dimeric assembly model for claudin-16 and -19 that featured anti-parallel association between the 3rd and 4th transmembrane domains of the two claudins [28]. To resolve the real TJ architecture from its native lipid environment, nevertheless, may need methodological breakthroughs in protein structural biology.

Conclusion

The concept of paracellular ionic pathway has been known for decades. Its importance in renal mineral metabolism has received growing attention because of the recent genetic, biochemical and biophysical analyses of the claudins that caused mineral imbalance diseases. Newly discovered regulatory mechanisms acting upon the claudins may reveal novel therapeutic directions to correct these mineral imbalance diseases.

Acknowledgments

None

Financial support and sponsorship

This work was supported by the National Institutes of Health Grants RO1DK084059 and P30 DK079333, and American Heart Association Grant 0930050N.

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Bullet points

- Paracellular reabsorption of Ca^{++} and Mg^{++} is driven by a lumen positive potential through a cation selective paracellular pathway.
- Three claudin proteins – claudin-14, claudin-16 and claudin-19 make the paracellular pathway for Ca^{++} and Mg^{++} permeation.
- Genetic mutations or gene expression changes in all three claudins cause mineral imbalance diseases.
- The paracellular pathway made of claudins can be regulated by extracellular Ca^{++} levels via the Ca^{++} sensing receptor (CaSR).
- MicroRNAs represent a novel regulatory mechanism for claudins.

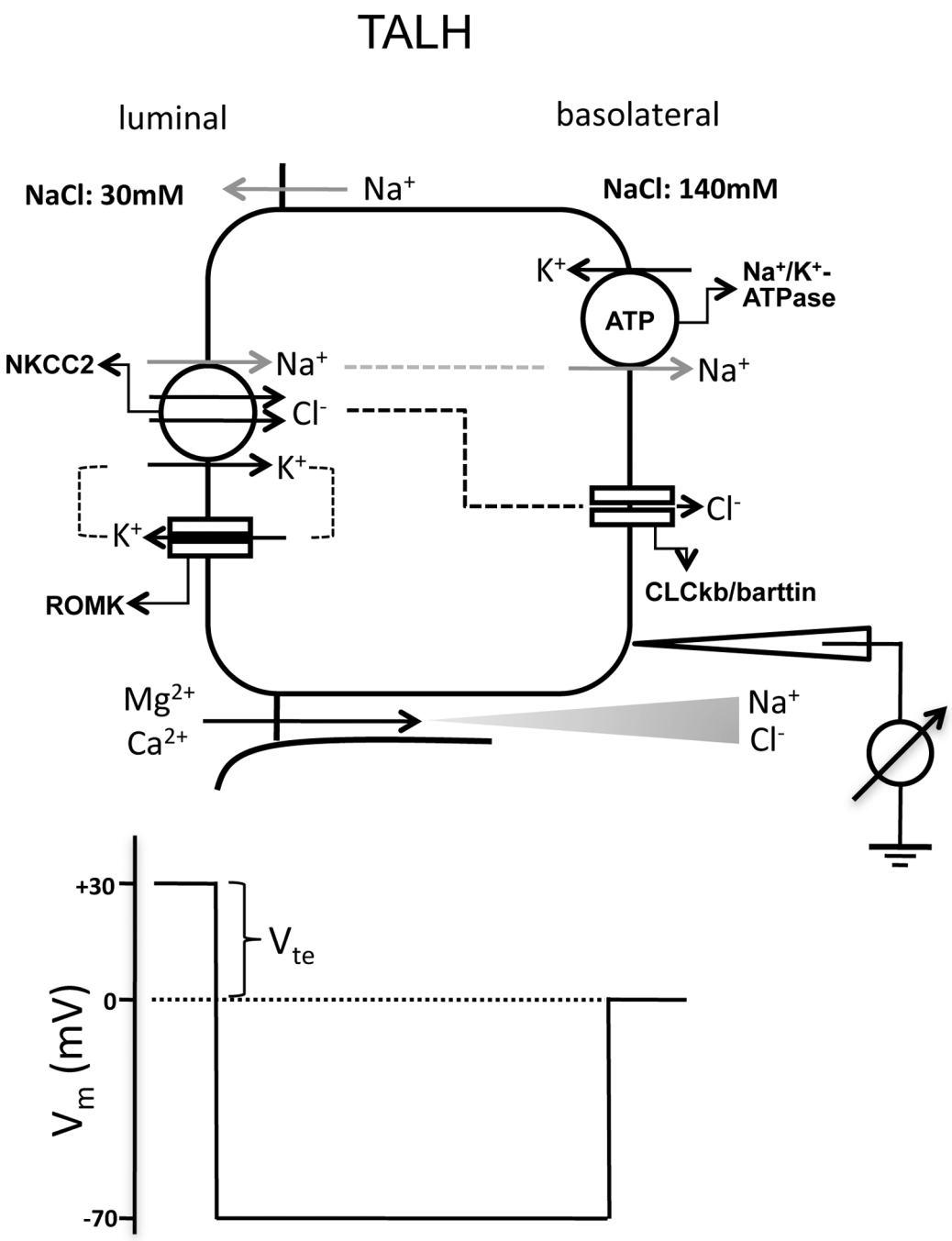

Figure 1.

Ion transport mechanism in the thick ascending limb of Henle's loop (TALH). 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. In this example, the basolateral membrane voltage is −70mV and the luminal membrane voltage is −100mV, resulting in a transepithelial voltage (V_{te}) of +30mV with respect to the basolateral side. V_{te} drives Ca^{++} and Mg^{++} transport through the tight junction. NKCC2: $Na^+/K^+/Cl^-$-cotransporter type 2; ROMK: renal outer medullary K^+ channel; CLCkb: Cl^- channel type kb.

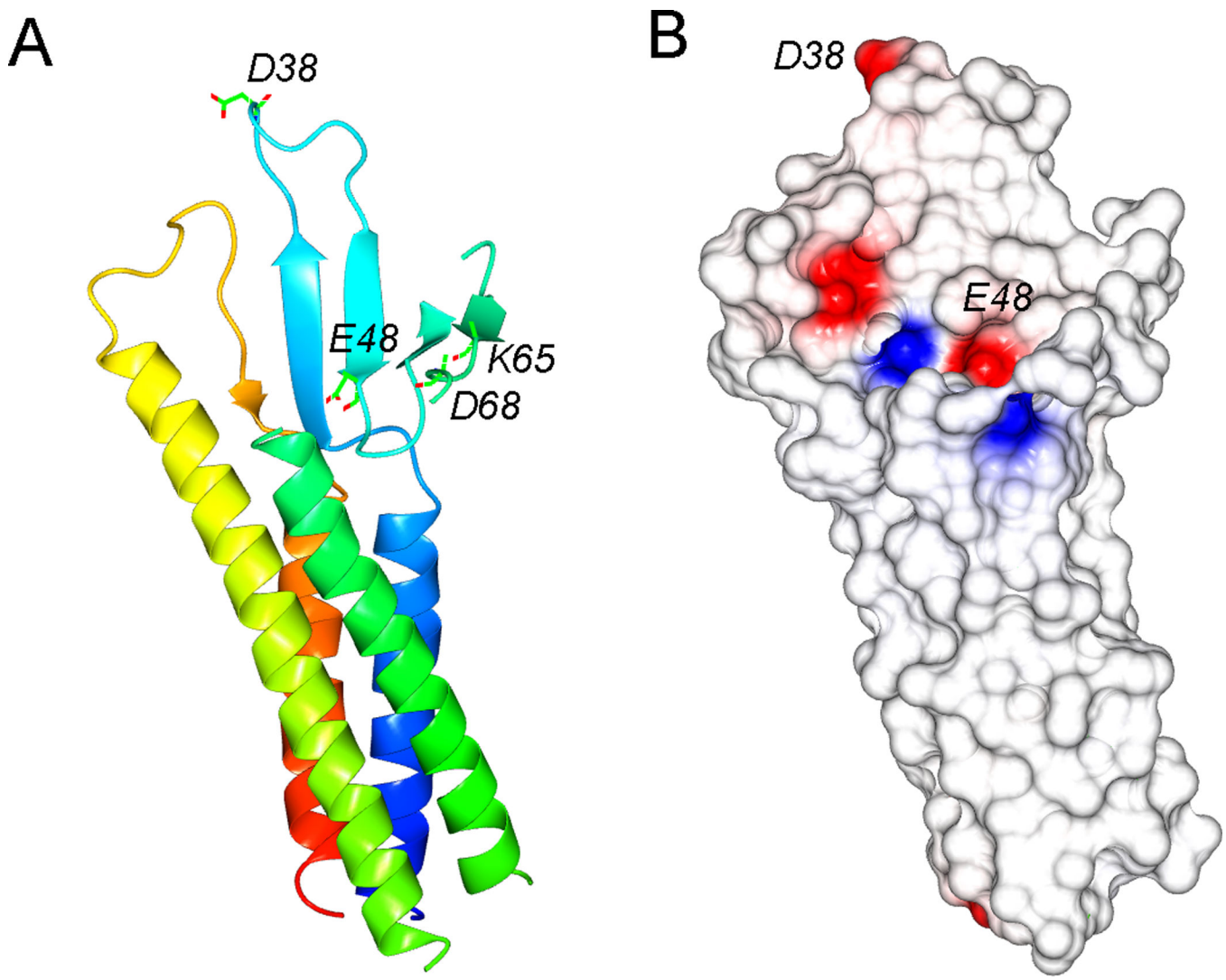

Figure 2.

Structural insights of claudin-19 protein. (A) Structure of monomeric claudin-19 in ribbon representation. The color changes gradually from the N terminus (blue) to the C terminus (red). The charged residues in the ECL1 are highlighted. (B) Electrostatic potential surface of the extracellular domains viewed in (A), contoured from −2kT/e (red) to +2kT/e (blue). Residues potentially important for charge selectivity are located.

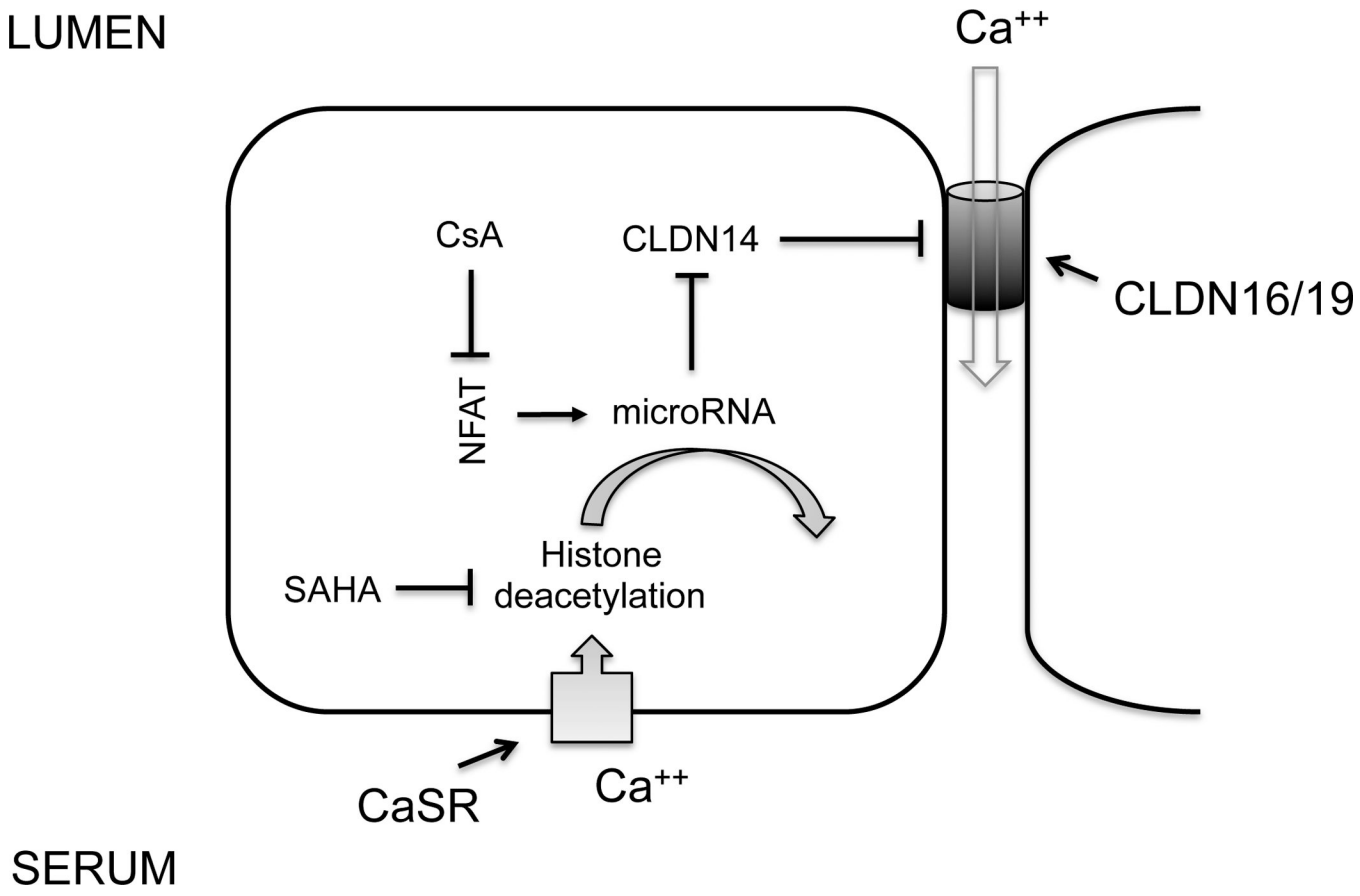

Figure 3.

CaSR signaling cascade in the kidney. A feedback control loop of CaSR regulation in the TALH includes the following key components: microRNAs (miR-9 and miR-374) and claudin-14. Claudin-14 negatively regulates claudin-16 and -19 permeability via direct interaction. MicroRNAs regulate claudin-14 mRNA stability and translational efficacy. The gene transcription of microRNAs themselves is regulated by the transcriptional factor – NFAT and via deacetylation of nearby histone molecules. NFAT: nuclear factor of activated T cells; CsA: cyclosporine A; SAHA: suberanilohydroxamic acid.