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The yin and yang of claudin-14 function in human diseases

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Abstract

Claudins are tight junction integral membrane proteins that are key regulators of the paracellular pathway. The paracellular pathways in the inner ear and in the kidney are predominant routes for transepithelial cation transport. Mutations in claudin-14 cause nonsyndromic recessive deafness DFNB29. A recent genome-wide association study (GWAS) has identified claudin-14 as a major risk gene of hypercalciuric nephrolithiasis. In vitro analyses show claudin-14 functions as a cation barrier in epithelial cells. The barrier function of claudin-14 is crucial for generating the K⁺ gradient between perilymph and endolymph in the inner ear. However, neither homozygous individuals with DFNB29 mutations nor claudin-14 knockout mice show any renal dysfunction. In this review, I have discussed several possible mechanisms to integrate the physiological function of claudin-14 in the inner ear and the kidney.

Keywords

tight junction; ion channel; kidney; inner ear

The **tight junction** (*zonula occludens*) is the most apical member of the junctional complex¹ found in vertebrate epithelia responsible for the barrier to movement of ions and molecules between apical and basal compartments, the paracellular pathway². The known 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. Claudins (CLDNs) 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. The cytoplasmic C-terminus of most claudins ends with a PDZ (*postsynaptic density 95/discs large/zonula occludens-1*) binding domain that is critical for interaction with the submembrane scaffold protein ZO-1 and correct localization in the TJ^{7–8}.

Claudin mutations have serious consequences, suggesting defects in epithelial ion flux. CLDN1-deficient mice die within one day of birth and show a loss of the water barrier function of skin⁹. CLDN2 knockout mice show salt wasting defects, presumably through leaky junctions in the proximal tubules of the kidney¹⁰. Targeted deletion of CLDN5, which is known to be expressed in vascular endothelia as well as other locations¹¹, results in a selective increase in brain vascular permeability to molecules <800 daltons¹². 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 balances¹³. CLDN16, also known as paracellin-1, has been

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Conflicts of interest

The authors declare no conflicts of interest.

genetically linked to the inherited disorder FHHNC (familial hypomagnesemia with hypercalciuria and nephrocalcinosis OMIM 248250)¹⁴. Many different FHHNC mutations have been identified in CLDN16 gene^{15–16}. The expression of CLDN16 is restricted to the thick ascending limb (TAL) of the nephron in the kidney¹⁴. CLDN19 mutations have also been associated with human FHHNC and renal Mg²⁺ loss¹⁷. While targeted deletion of CLDN19 in mice initially focused on its role in peripheral myelin¹⁸, promoter analysis¹⁹ and subsequent studies²⁰ have emphasized the presence of CLDN19 in the TAL of the nephron (colocalizing with CLDN16 in the kidney).

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^{21–25}, specifically to Na⁺, K⁺, H⁺ and ammonium. CLDN2 and -15 increase cation permeability^{26–28}. These properties have been attributed to charged amino acids in the first extracellular domain²⁹. These and other studies³⁰ have led to models of the claudins forming **paracellular channels**, a novel class of channels oriented perpendicular to the membrane plane and serving to join two extracellular compartments³¹. Measurement of paracellular permeability using cell membrane impermeable tracers indicate that there are 4–7 Å diameter channels in the TJ^{30;32–33}. The paracellular channels in the tight junction have properties of ion selectivity, pH dependence and anomalous mole fraction effects, similar to conventional transmembrane channels³⁰.

CLDN14 function in the inner ear

The cochlea maintains two distinct fluid compartments, the scala vestibuli/tympani and the scala media, which are filled with the perilymph and endolymph fluids. The perilymph fluid resembles the extracellular fluid, while the endolymph fluid has the characteristics of an intracellular fluid, with high K⁺ and low Na⁺ concentrations. The tight junction barrier separates perilymph from endolymph fluid and maintains the ionic composition of each fluid. Mutations in CLDN14 cause nonsyndromic recessive deafness DFNB29 in humans³⁴. CLDN14 knockout mice are also deaf, owing to rapid degeneration of the cochlear outer hair cells (OHC)²³. The OHC loss in CLDN14 KO mice starts at P8–9 and coincides with a critical developmental stage when the endocochlear potential is generated. *In vitro* experiments have demonstrated that CLDN14, when overexpressed in epithelial cells, selectively blocks the paracellular permeation of cations, including K⁺²³. The paracellular barrier provided by CLDN14 would be required for maintaining the K⁺ gradient between perilymph and endolymph. A loss of CLDN14 would result in elevated K⁺ concentration in the space of Nuel, which now becomes toxic to the OHCs.

CLDN14 function in the kidney

There are segment-specific claudin expression profiles along the length of the nephron. Northern analysis of mouse kidneys using probes specific for CLDN1–19 reveal that only CLDN6, -9, -13 are not detectable, and CLDN5 and -15 only in endothelial cells; the rest are specifically expressed in different segments of the nephron³⁵. Using antisera available at the time to perform immunostaining on mouse kidneys^{20;35}, CLDN3, -10, -11, -16 and -19 were observed in the thick ascending limb (TAL), CLDN3 and -8 in the distal convoluted tubule, and CLDN3, -4 and -8 in the collecting duct (CLDN4 was also observed in the thick ascending limb³⁵ although absent in bovine TAL³⁶). CLDN2 is highly expressed in the “leaky” proximal nephron³⁷ consistent with its high cation permselectivity when expressed in MDCK cells^{26–27}. 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^{38–39}. Immunofluorescence analysis has shown that CLDN7 is

expressed in the thick ascending limbs of Henle's loop and collecting ducts of porcine and rat kidneys⁴⁰, although another study described CLDN7 in the distal nephron as located primarily on the basolateral membrane³⁹. In summary, while there are still some conflicting published data, CLDN2, -10, -11, -17 and -18 are expressed in proximal tubules, while CLDN3, -4, -7, -8, -10, -16 and -19 have been reported in the thick ascending limbs and the distal nephron. The renal localization of CLDN14 has been controversial. Immunofluorescence analysis showed CLDN14 gene expression in the TAL and the proximal tubules of mouse kidneys⁴¹, while another study reported no CLDN14 expression in the kidney³⁵. The thick ascending limb reabsorbs a major percentage of filtered divalent cations (30–35% Ca^{++} and 50–60% Mg^{++})⁴². At this segment, Ca^{++} and Mg^{++} are passively reabsorbed from the lumen to the interstitial space through the paracellular channel, driven by a lumen positive transepithelial voltage (V_{te}). V_{te} is generated by two mechanisms: (1) the active transport V_{te} owing to apical K^+ recycling through ROMK and basolateral Cl^- exit through ClC-Kb , coupled with NaCl reabsorption via the apical $\text{Na}^+2\text{Cl}^-\text{K}^+$ cotransporter (NKCC2); and (2) the diffusion V_{te} generated by a transepithelial NaCl concentration gradient through the cation selective paracellular channel of the TAL. A run of *in vitro*^{43–44} and *in vivo*^{45–46} studies have shown that CLDN16 and CLDN19 form a heteromeric cation channel, which (i) confers cation selectivity including Ca^{++} and Mg^{++} ; (ii) generates the diffusion V_{te} . Although mutations in CLDN16 or 19 have been linked to a severe renal phenotype—familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC), CLDN14 mutations found in recessive deafness DFNB29 cause no renal defect in affected homozygous individuals³⁴.

Analyses of CLDN14 function in kidney epithelial cells

Ben-Yosef et al²³ first determined the electrophysiological properties of CLDN14 channel in transfected kidney MDCK cells. Overexpression of CLDN14 induced a 6-fold increase in transepithelial resistance (TER), accompanied by a significant decrease in cation selectivity ($P_{\text{Na}}/P_{\text{Cl}}$)²³. The calculated Na^+ permeability (P_{Na}) through the CLDN14 channel was reduced by 72.5% compared to mock transfection while the Cl^- permeability (P_{Cl}) was not affected. *Bi*-ionic potentials found the permeability sequence to be $\text{K}^+ > \text{Na}^+ > \text{Rb}^+ > \text{Li}^+ > \text{Cs}^+$ that resembled the Eisenman selectivity sequence V – VIII²³, suggesting high field strength within the CLDN14 channel pore. In a preliminary study, I have expressed CLDN14 in MDCK cells with a retroviral infection approach and compared its electrophysiological properties with the CLDN4 channel (Figure 1). Similar to CLDN4, the first extracellular loop (ECL1) of CLDN14 is enriched with positively charged amino acids while the ECL1 of CLDN16 is abundant with negatively charged amino acids (Figure 1A). The MDCK cells express a low level of endogenous CLDN4 proteins but no CLDN14 (Figure 1B). Ectopic expression induces significant increases in expression of both claudins. The CLDN14 protein has a similar molecular weight of 21–22kDa compared to CLDN4 (Figure 1B). Both claudins show discrimination against cation (Na^+) over anion (Cl^-), reflected by a significant decrease in dilution potential (Figure 1C). CLDN14 is a more potent blocker to Na^+ permeation than CLDN4 (Figure 1C), although the protein level of CLDN14 seems lower than CLDN4 (Figure 1B).

The CLDN14 paradox

A recent genome-wide association study (GWAS) has identified CLDN14 as a major risk gene of hypercalciuric nephrolithiasis⁴⁷. Metabolic abnormalities such as hypercalciuria, metabolic acidosis and bone mineral loss, in addition to kidney stones, have been associated with common synonymous sequence variants (i.e. single-nucleotide polymorphisms, SNPs) in the CLDN14 gene⁴⁷. The previously discovered rare mutations (398delT and T254A) that associate with deafness were not identified in any kidney stone patient³⁴. The 398delT

mutation causes a frameshift within the codon of Met133, which substitutes 23 incorrect amino acids and prematurely terminates translation 69 nucleotides later³⁴. The T254A mutation substitutes aspartic acid for valine (V85D)³⁴. Valine 85 is a conserved site within the second transmembrane domain (TM2) of CLDN14 protein. Aspartic acid at position 85 is predicted to affect hydrophobicity and disrupt the secondary structure of TM2. Therefore, both 398delT and T254A are loss-of-function mutations. Nevertheless, none of the deaf individuals homozygous for these mutations show signs of renal dysfunction. CLDN14 KO mice show normal renal functions, ruling out any direct renal requirement of CLDN14. If the physiological function of CLDN14 is to provide a cation barrier against K⁺ permeation between perilymph and endolymph in the inner ear, this similar function will not be required for maintaining normal kidney physiology. On the other hand, the mechanism provided by CLDN14 in kidney stone diseases must not affect the physiological function of CLDN14 in the inner ear, i.e. the cation barrier function. Because kidney stone is a common condition while deafness is a rare disease, the functional role played by CLDN14 in two diseases must be different. There are two hypotheses. (1) The function of CLDN14 depends on its binding partner. It is well known that different claudins interact to form an oligomer. Because the binding partners are different in the inner ear from the kidney, the CLDN14 functions vary accordingly. (2) The function of CLDN14 is the same in two organs but its physiological roles are different. The CLDN14 channel is required for the barrier function in the inner ear. The same barrier function plays a negative regulatory role in the kidney. CLDN14 may actually block Ca⁺⁺ permeation in the kidney, unlike CLDN16 and CLDN19 that permeate Ca⁺⁺. Knocking out CLDN14 will not damage normal Ca⁺⁺ reabsorption but instead protect against hypercalciuria. The mechanism underlying kidney stone pathogenesis is provided by a gain-of-function effect of CLDN14 rather than a loss-of-function effect. These two hypotheses are now being tested in my lab. We will ask: (1) What are the binding partners for CLDN14? (2) What is the function of CLDN14 in the kidney? (3) Is CLDN14 required for renal Ca⁺⁺ homeostasis?

In conclusion, CLDN14 is an important tight junction molecule for a broad range of epithelial physiologies. Mutations in CLDN14 have been found in both rare (monogenic) and common (polygenic) forms of human genetic diseases. Although preliminary studies have suggested a role for CLDN14 as cation blocker in the inner ear, its precise role in the kidney is still elusive. I have hypothesized two mechanisms to unite the seemingly different functions of CLDN14 in the physiology of the inner ear and the kidney.

Materials and methods

The following antibodies were used in this study: rabbit polyclonal anti-CLDN14 antibody (Zymed Laboratories); mouse monoclonal anti-CLDN4 (Zymed Laboratories). MDCK-II cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% FBS, penicillin/streptomycin, and 1mM sodium pyruvate. The claudin expressing MDCK-II cells were lysed in 50 mM Tris (pH 8.0) by 25–30 repeated passages through a 25-gauge needle, followed by centrifugation at 5,000g. The membranes of lysed cells were extracted using CSK buffer (150 mM NaCl; 1% Triton X-100; 50 mM Tris, pH 8.0; and protease inhibitors). Lysates (containing 20 µg total membrane proteins) were subjected to SDS-PAGE and immunoblotting. Electrophysiological studies were performed on cell monolayers grown on porous filters (Transwell). Voltage and current clamps were performed using the EVC4000 Precision V/I Clamp (World Precision Instruments) with Ag/AgCl electrodes and an Agarose bridge containing 3 M KCl. Transepithelial resistance (TER) was measured using the Millicell-ERS and chopstick electrodes (Millipore). All experiments were conducted at 37°C. TER of the confluent monolayer of cells was determined in buffer A (145 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES, pH 7.4) and the TER of blank filters was subtracted. Dilution potentials were measured when buffer B (80 mM NaCl, 130 mM

mannitol, 1 mM CaCl₂, 1 mM MgCl₂, and 10 mM HEPES, pH 7.4) replaced buffer A on the apical side or basal side of filters. Electrical potentials obtained from blank inserts were subtracted from those obtained from inserts with confluent growth of cells. The ion permeability ratio (P_{Na}/P_{Cl}) for the monolayer was calculated from the dilution potential using the Goldman-Hodgkin-Katz equation. The absolute permeabilities of Na⁺ (P_{Na}) and Cl⁻ (P_{Cl}) were calculated by using the Kimizuka-Koketsu equation.

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Figure 1. Influences of claudin-4 and claudin-14 on Na⁺ permeation through tight junction. (A) Alignment of amino acid sequences of the first extracellular loop of claudin-4, claudin-14 and claudin-16 (paracellin-1). Note the relative abundance of positively charged amino acids in the first extracellular loop of claudin-4 and claudin-14 (labeled in blue); and abundance of negatively charged amino acids in claudin-16 (labeled in red). (B) Expression of claudin-4 and claudin-14 in MDCK cell membranes. Claudin-4 and claudin-14 migrates as a 21–22 kDa band. Note that MDCK cells express endogenous claudin-4 but no claudin-14. (C) Both claudin-4 and claudin-14 suppress the permeability of Na⁺ and show discrimination against cation (Na⁺) over anion (Cl⁻). *: *p*<0.01, *n*=4.