Review

Tight junction claudins and the kidney in sickness and in health

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ABSTRACT

The epithelial cell tight junction has several functions including the control of paracellular transport between epithelial cells. Renal paracellular transport has been long recognized to exhibit unique characteristics within different segments of the nephron, functions as an important component of normal renal physiology and has been speculated to contribute to renal related pathology if functioning abnormally. The discovery of a large family of tight junction associated 4-transmembrane spanning domain proteins named claudins has advanced our understanding on how the paracellular permeability properties of tight junctions are determined. In the kidney, claudins are expressed in a nephron-specific pattern and are major determinants of the paracellular permeability of tight junctions in different nephron segments. The combination of nephron segment claudin expression patterns, inherited renal diseases, and renal epithelial cell culture models is providing important clues about how tight junction claudin molecules function in different segments of the nephron under normal and pathological conditions. This review discusses early observations of renal tubule paracellular transport and more recent information on the discovery of the claudin family of tight junction associated membrane proteins and how they relate to normal renal function as well as diseases of the human kidney.

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Contents

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1. Introduction

The tight junction, originally described as the *zonula occludens* [1], is one of the three epithelial cell junctional complexes that are important for the development and maintenance of epithelial cell polarity and an intact barrier in all epithelial organs. These junctional complexes also include anchoring junctions and communicating junctions. The anchoring junctions are subdivided into actin filament associated complexes and intermediate filament associated complexes. The actin filament based anchoring junctions include focal adhesions (mediated in part by integrin molecules) that attach the cells to the extracellular matrix and the adherens junction (mediated in part by the transmembrane protein E-cadherin and cytosolic proteins called catenins) that is localized to the lateral membranes of adjacent cells. The intermediate filament associated anchoring junctions include desmosomes (mediated in part by cadherin molecules) that are also localized to the lateral membranes of adjacent epithelial cells and hemidesmosomes (mediated in part by integrin molecules) that also attach the cells to the extracellular matrix. The communicating junctions consist of gap junctions (mediated by connexins) that are important for cell–cell communication. The tight junction (also referred to as the occluding junction) is located at the most apical region of the lateral membrane in epithelial cell sheets. Tight junctions are composed of transmembrane and cytosolic proteins and seal the intercellular space between adjacent cells to create a primary barrier against the diffusion of fluid, electrolytes, macromolecules and pathogens via the paracellular pathway [2–4]. Two major functions of the epithelial tight junction are simply characterized as the gate and fence functions [5]. The tight junction gate function refers to the control of movement of molecules between epithelial cells via the paracellular route. The tight junction is essential for the barrier function of epithelia by restricting paracellular diffusion. Tight junctions encircle the epithelial cells at the apical end of the lateral membrane and form the boundary between the apical and basolateral membrane surfaces. The tight junction fence function refers to the maintenance of epithelial cell polarity. Tight junctions are crucial for the development and maintenance of epithelial cell surface polarity, since they form an intra-membrane diffusion fence that restricts diffusion of lipids in the exoplasmic leaflet of the plasma membrane [6,7]. Expression of a mutated tight junction associated membrane protein (occludin, see below) was found to render epithelial cells incapable of maintaining a fluorescent lipid in a specifically labeled cell surface domain [8]. The fence function of tight junctions in maintenance of protein polarity has been supported in studies demonstrating cell surface mislocalization of E-cadherin [9] and β1-intergrin [10] following modification of tight junction function. The ability of the tight junction protein complex to form a seal is dependent on the interaction of the tight junction protein complex with structurally organized actin filaments in the cytoskeleton. Assembly and disassembly of the tight junction between epithelial cells are also controlled, partially, by E-cadherin-mediated cell–cell adhesion in the adherens junction [11]. The molecular composition of tight junctions includes an array of transmembrane proteins and cytosolic plaque proteins and has been recently reviewed [3,4].

The first structural description of the epithelial tight junction in the kidney was provided by Farquhar and Palade in 1963 [1]. Ultrathin section electron microscopic images demonstrated that renal tight junctions form a continuous belt-like structure on the lateral membrane domain closest to the apical membrane. Hemoglobin tracer experiments provided evidence that the tight junction blocks paracellular movement across the tight junction into the intercellular space [1].

This review will focus on the role of the claudin family of tight junction associated proteins on paracellular transport in the kidney as they relate to normal and pathological physiology.

2. Early observations of renal paracellular transport

The reabsorption and secretion of various solutes across the renal tubular epithelium represents a fundamental aspect of renal physiology. Transported solutes cross the renal tubular epithelium by either the transcellular or paracellular route [12]. In the transcellular route, the solutes cross the apical membrane, traverse through the cytoplasm, and then exit the cell across the basolateral membrane. The transcellular pathway may also occur in the basolateral to apical direction as seen in the case of immunoglobulin transcytosis by the polymeric immunoglobulin receptor [13]. Specific membrane transport proteins on the apical and/or basolateral membrane frequently facilitate this process. In the case of transepithelial transport by the paracellular route, solutes cross by going through the epithelial tight junction between adjacent epithelial cells. Much less is understood about the mechanisms of paracellular transport compared to what is known about transcellular transport processes.

Approximately 40 years ago, the identification of renal paracellular transport processes originated from work by renal physiologists measuring renal proximal tubule transepithelial potentials with microelectrodes [14]. The results from these measurements showed both that the proximal tubular epithelium is an extremely leaky structure (the overall resistance is only $5–12\,\Omega\,cm^2$ [15–18] and varies with the distance from the glomerulus), and that most of the high conductance is due to sodium ions rather than chloride ions [19]. The microelectrode studies allowed for recognition and quantification of proximal tubule leakiness and determined the transport mechanism to occur via the paracellular route through the tight junction. This paracellular pathway was also referred to as a shunt by investigators. Early quantification of the proximal tubule shunt pathway was achieved by analyzing the glucose-induced potential changes in rat proximal tubules, and the results indicated that the shunt conductance was ~ 70 times higher than the conductance of the cellular transport pathway [20]. This observation suggested that virtually all passive uncoupled ion flow passes through the shunt (paracellular pathway), while all active transport proceeds through the cell membranes (transcellular pathway).

Early investigation of tight junction permeability in the cortical collecting duct was investigated using lanthanum tracer techniques [21]. These studies demonstrated that the tight junctions of the proximal and distal convoluted tubules were permeable to lanthanum while those of the cortical collecting tubules were impermeable. At the time of these early publications on renal nephron tight junction permeability, the molecular mechanisms accounting for the variable leakiness of the tight junctions in different segments of the nephron were far from clear. However, the recent discovery of a family of tight junction associated proteins called claudins provides a reasonable explanation accounting for these differences in tight junction permeabilities in the nephron [12,22].

3. Discovery of claudins

The identification of claudins was the result of an intensive search for integral transmembrane proteins localized to the tight junction of chicken liver epithelial cells by the late S. Tsukita's laboratory in the 1990s. In 1993, occludin was identified as the first integral membrane protein exclusively localized to the tight junction [23]. However, when occludin-deficient embryonic stem cells are differentiated to epithelial cells during embryogenesis, well developed tight junctions formed between adjacent epithelial cells [24]. This finding indicated that occludin is not necessary for tight junction formation. Moreover, occludin-deficient mice are viable but showed significant postnatal growth retardation [25]. At this time, the function of occludin in the tight junction is not known. Because of the vital importance of tight junctions in epithelial organ structure and function, the viable phenotype of the mice lacking occludin also suggested the presence

of other transmembrane proteins important for tight junction structure and function.

The first claudin isoforms were discovered in 1998 when the Tsukita laboratory reported the identification of two novel 22-kD integral membrane proteins localized to the tight junction [26]. These proteins exhibited no sequence similarity to occludin, contained four putative transmembrane domains and were named claudin-1 and -2. The name claudin was chosen from the Latin word "claudere" (to close) [26]. Expression of claudins in claudin-null fibroblasts induced the formation of tight junctions strands [26] and also demonstrated claudins to function as cell–cell adhesion molecules [27]. Epitope tagged mutants of both claudin-1 and -2 isoforms were targeted and incorporated into the tight junctions of Madin Darby canine kidney (MDCK) II epithelial cells (a widely used polarized epithelial cell culture model). Northern analysis showed expression of claudin-1 in all tissues examined (heart, brain, spleen, lung, liver, skeletal muscle, kidney and testes), while claudin-2 expression was primarily restricted to the kidney and liver [26].

A recent database search indicates the existence of 24 claudin isoforms in mammals [4]. Claudin like molecules are well conserved across a wide range of the animal kingdom including the puffer fish *Fugu*, *Drosophila*, and *C. elegans* [28]. In addition, an extensive characterization of claudins in *Fugu*, including expression profiling by RT-PCR for the kidney was done by Loh et al. [29]. Claudin like molecules are also present in the gills of tilapia and the expression of these claudin isoforms in the gills is regulated by changes in salinity [30]. In this case, claudins appear to be important in permeability changes associated with salinity acclimation and osmoregulation during changes from fresh water to seawater and vice versa.

4. Molecular mechanism of tight junction charge selectivity by claudins

Claudins appear to determine paracellular transport across tight junctions, in part, by determining ionic charge selectivity. Membrane topology of individual claudin molecules consists of four membrane spanning domains, two extracellular loops, one intracellular loop, and two cytosolic tails (NH_2 and COOH). Much of our understanding of how claudins determine paracellular permeability across the tight junctions has been obtained from experiments studying the effects of claudin overexpression in cultured renal epithelial cells. In general,

the effects of individual claudins on paracellular conductance have been characterized by overexpressing individual claudin isoforms into monolayers of Madin Darby canine kidney (MDCK) epithelial cells. A major limitation of this approach is that cultured renal epithelial cells already express tight junctions containing multiple endogenous claudins. Thus the introduction of an individual claudin isoform into the epithelial cells will document only the changes in paracellular permeability determined by the net composition of total number of claudin isoforms in the tight junction (both endogenous and exogenous) [28]. From these studies it appears that claudin isoforms -1 [31,32], -4 [33], -8 [34], and -14 [35] decrease tight junction ionic permeability. On the other hand, only claudin-2 has been shown to increase tight junction permeability in high resistance MDCK cells [36]. Expression of claudin-2 in high resistance MDCK cells increases the expression of cation-selective conductance pathways in the tight junction and increases the relative leakiness of the tight junction [37].

Site directed mutagenesis experiments have provided evidence that the paracellular permeability control by claudins appears to be determined by the amino acid residues in the first extracellular loop of individual claudin molecules (reviewed [28]). For example, replacing negatively charged amino-acids with positively charged amino-acids in the first extracellular loop of claudin-15 converts its effects on tight junction permeability from a cation-selective to anion-selective [38].

5. Distribution of claudin isoforms in the kidney

Most data on the nephron-specific distribution of claudins have been determined in adult murine kidney. Of the 24 known mammalian claudin isoforms many are expressed in specific segments of the mouse nephron (see Fig. 1 for summary of available data). Claudins 6, 9 and 13 are expressed in the neonatal murine kidney [39]. Claudin 9 and 13 are not expressed in the adult murine kidney while claudin-6 can be detected only at a low levels using RT-PCR (but not by immunohistochemistry) [39]. Claudins 6, 9 and 13 may play a role in developmental changes in renal paracellular permeability. Recently, claudin-6 has been detected in the glomerular podocytes of the adult rat kidney [40]. This observation suggests that claudin-6 may determine, in part, the permeability characteristics of the glomerular filtration barrier. The nephron-specific expression of these different claudin isoforms is complex and currently recognized to be an important determinant of the paracellular transport properties of the
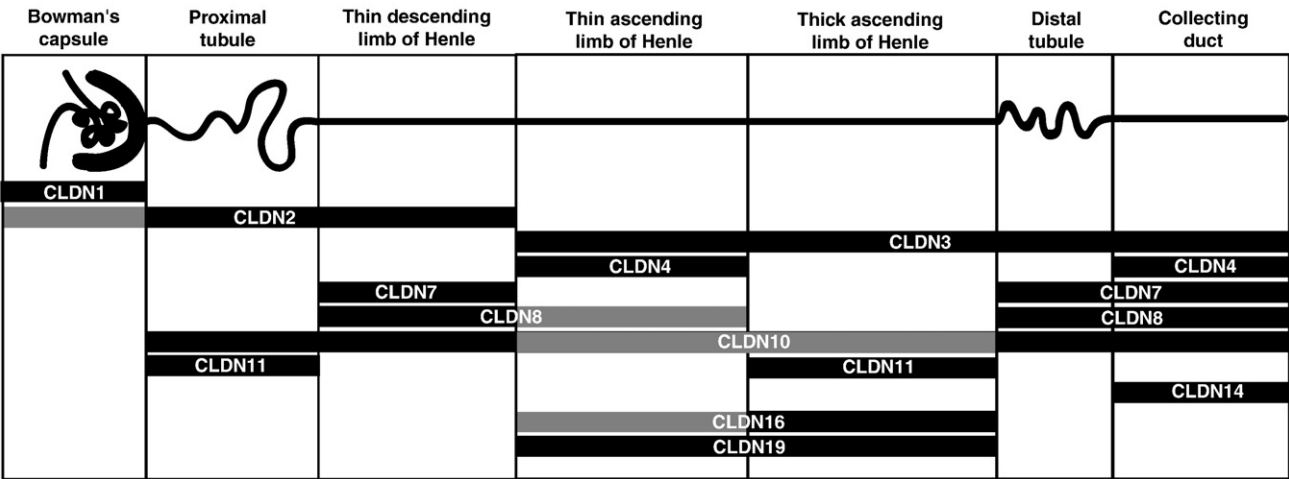

Fig. 1. Summary of reported nephron segment distribution of claudin isoforms in the adult murine kidney. This figure was adapted from [22] with modifications. The following are references for localization of individual claudin isoforms to specific nephron segments. *Bowman's capsule*: claudin-1 [49], claudin-2 [49] not observed by [74] (grey); *Proximal tubule*: claudin-2 [49,74], claudin-10 [75] not observed by [49] (grey), claudin-11 [49]; *Thin descending limb*: claudin-2 [49,74], claudin-7 [66], claudin-8 [66] not observed by [49] (grey), claudin-10 [75] not observed by [49]; *Thin ascending limb*: claudin-3 [49], claudin-4 [49], claudin-8 [49] not observed by [66] (grey), claudin-10 [75] not observed by [49] (grey), claudin-16 [45,47,48] not observed by [49] (grey), claudin-19 [47,48]; *Thick ascending limb*: claudin-3 [49], claudin-10 [49,75], claudin-11 [49], claudin-16 [45,47–49], claudin-19 [47,48]; *Distal tubule*: claudin-3 [49], claudin-7 [66], claudin-8 [49,66], claudin-10 [75] not observed by [49] (grey); *Collecting duct*: claudin-3 [49], claudin-4 [49], claudin-7 [66], claudin-8 [49,66], claudin-10 [75] not observed by [49] (grey), claudin-14 [35].

tight junctions in different nephron segments [12,22]. Clearly, more work will be required to precisely define the localization of claudins within the renal nephron. Specifically, the nephron-specific distribution of claudin isoforms in human nephron segments is not well known. The availability of reagents to investigate the expression and function of claudins in the kidney is increasingly available and makes the above studies more feasible [41].

6. Role of claudins in renal related disease conditions

6.1. Familial hypomagnesemia with hypercalcuria and nephrocalcinosis a human disease of the tight junction proteins claudin-16 and claudin-19

The human renal disorder familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) is characterized by progressive renal Mg^{+2} and Ca^{+2} wasting leading to impaired renal function and chronic renal disease. The renal reabsorption of Mg^{+2} occurs predominantly by paracellular route in the thick ascending limb of Henle by a process driven by a positive transepithelial membrane potential [42,43]. While having high Mg^{+2} transport, this segment of the nephron is highly impermeable to water [44]. Ca^{+2} reabsorption also occurs in this segment of the nephron by a paracellular process also dependent on a positive transepithelial membrane potential. However, Mg^{+2} and Ca^{+2} do not directly compete with each other for reabsorption in the thick ascending limb of Henle, indicating the presence of distinct transport mechanisms [43]. Because of the role of paracellular transport on Mg^{+2} and Ca^{+2} reabsorption, FHHNC was thought to represent a human disease caused by abnormal epithelial tight junction function.

In 1999, Simon et al. reported the identification of a human gene called paracellin-1 by positional cloning in families with typical features of FHHNC [45]. Paracellin is a member of the claudin family of tight junction proteins and is also known as claudin-16. In some families with FHHNC, claudin-16 mutations affect the trafficking of claudin-16. For example, mutation of the C-terminal cytosolic tail of claudin-16 reduces cell surface expression of claudin-16 and inhibition of endocytosis can increase the cell surface expression of this mutant claudin-16 isoform [46]. Other families with FHHNC lacked mutations in the claudin-16 gene, but were found to have mutations in claudin-19 [47]. In the mouse kidney, claudin-19 was shown to co-localize with claudin-16 in the thick and thin ascending limb of Henle [47–49]. Thus, claudin-16 and -19 were logically thought to form divalent cation pores in the tight junction to facilitate paracellular transport of Mg^{+2} and Ca^{+2}. However, this did not prove to be entirely clear. Epithelial cell culture models in which claudin-16 is over expressed demonstrate increased paracellular transport of Na^+ with only a small increase in Mg^{+2} paracellular transport [50,51]. One study reported an increase in Mg^{+2} transport in cells overexpressing claudin-16 [52]. There is also evidence for regulation of paracellular Mg^{+2} transport in cultured renal epithelial cells. Activation of the polyvalent cation-sensing receptor (also known as calcium sensing receptor (CaSR)) inhibits protein kinase A activity, resulting in a decrease in phosphorylated claudin-16, translocation of claudin-16 to lysosomes and a decrease in magnesium reabsorption [53].

Another functional analysis demonstrated that claudin-19 acts as blocker of paracellular Cl^- transport [54]. In addition, claudin-16 and claudin-19 have been shown to interact with each other by co-trafficking/co-localization in epithelial cells, co-immunoprecipitation in epithelial cells, yeast 2-hybrid, and functional synergistic effects on increasing the cation selectivity of the tight junction [54]. Collectively, these data show that claudin-16 interacts with claudin-19 and that this association confers a tight junction with cation selectivity. A model for this mechanism of paracellular transport of Mg^{+2} and Ca^{+2} in the thick ascending limb is well described by Hou et al. [54] and illustrated in Fig. 2. In the thick ascending limb, Na^+ and Cl^- are actively reabsorbed from the lumen by transcellular mechanisms to

create a transcellular concentration gradient for Na^+ and Cl^- with the tubular lumen having low concentrations of Na^+ and Cl^-. Claudin-16 and -19 then confer tight junction permeability to Na^+, but not Cl^-, which in turn, creates a luminal positive transepithelial potential to serve as the driving force for paracellular transport of Mg^{+2} and Ca^{+2}. Moreover, mutations to either claudin-16 or -19 would abrogate the development of this luminal positive transepithelial potential and secondarily inhibit paracellular transport of Mg^{+2} and Ca^{+2}. Presently, the tight junction proteins that directly mediate paracellular transport of Mg^{+2} and Ca^{+2} in the thick ascending limb are not clearly defined.

6.2. Claudins and the WNK (with no K (lysine) protein kinase) family of protein kinases — a genetic form of human hypertension

Alteration in renal claudin function has been implicated in a genetic form of human hypertension. Mutations in the genes encoding WNK1 and WNK4 have been identified families with an inherited hypertension and hyperkalemia disorder called pseudohypoaldosteronism type II (PHAII, also know as Gordon's syndrome) [55]. This same study demonstrated localization of WNK1 and 4 in the distal nephron with WNK1 in the cytoplasm and WNK4 in the tight junctions. Overexpression of WNK4 in Madin Darby canine kidney (MDCK) epithelial cells increased paracellular ion flux through tight junctions, by an effect that was postulated to result from phosphorylation of claudins [56]. Mutations in WNK4 were associated with increased
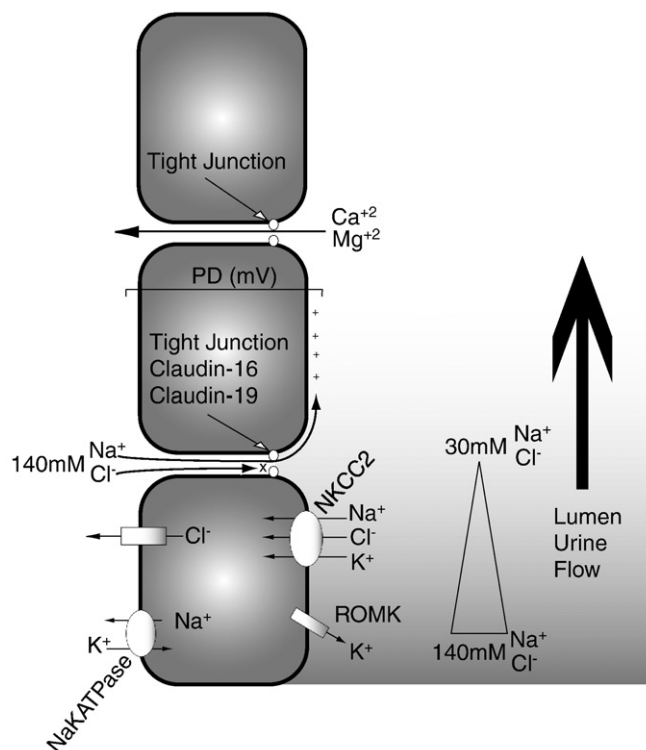

Fig. 2. Role of tight junction claudins-16 and -19 in Mg^{+2}/Ca^{+2} reabsorption. In the thick ascending limb (TAL) of the kidney, epithelial cells reabsorb NaCl through the $Na^+K^+Cl^-$ cotransporter type 2 (NKCC2) in the apical membrane. Basolateral NaKATPase allows for Na^+ exit from the cell in exchange for K^+ entry. K^+ is secreted back into the lumen by the renal outer medullary K^+ channel (ROMK) and Cl^- exits the cell through the basolateral Cl^- channel. Collectively, these transport processes allow for the reduction of lumen NaCl concentration from approximately 140 mM to 30 mM. This, in turn, creates a transepithelial NaCl concentration gradient. This NaCl concentration gradient results in a lumen-positive potential (PD) through selective tight junction permeability for Na^+, but not Cl^- ions. Claudin-16 and claudin-19 in the tight junction are responsible for this tight junction cation selectivity. The lumen-positive PD drives paracellular reabsorption of Mg^{+2} and Ca^{+2} across the tight junction.

phosphorylation of claudins 1–4 and increased Cl^- flux across the tight junction. An increase in paracellular Cl^- reabsorption would lead to secondary increase in Na^+ reabsorption. Thus, hypertension in patients with WNK4 mutations may be caused by increased NaCl reabsorption, in part, through the paracellular pathway [57].

6.3. Altered claudin expression in acute kidney injury

While much is known about the distribution of nephron distribution of claudins under "normal" physiological conditions, much less is known about nephron claudin expression during pathological conditions. For example, renal ischemic injury alters the expression pattern of renal claudins. Claudin-1, -3, and -7 mRNA levels are increased in response to experimental murine renal ischemia-reperfusion injury [58]. The significance of these changes in claudin expression during ischemic acute kidney injury requires further investigation.

This same study also demonstrated a 1.9-fold reduction in claudin-2 (a proximal tubule claudin) mRNA expression following ischemic injury. This observation is interesting in the context of the role of hepatocyte growth factor (HGF) in acute kidney injury. HGF is known to be an important growth factor for the kidney following injury [59]. HGF levels have been shown to increase markedly in human patients with acute kidney injury [60] as well as in rodent models of kidney disease, including ischemic renal disease [61], nephrotoxic acute tubular necrosis [62], and acute rejection following renal transplantation [63]. Interestingly, HGF also dramatically inhibits the expression of claudin-2 in cultured renal epithelial cells [64,65]. These observations raise the possibility of a role for claudins (especially claudin-2) in the development of or recovery from ischemic acute kidney injury.

6.4. Potential roles of claudins in potassium and acid/base homeostasis

Both claudin-4 and -8 are expressed in the distal nephron (Fig. 1) [49,66] and have been shown to negatively regulate paracellular transport of cations in MDCK cells [34,67]. In addition, claudin-8 appears to function specifically as a paracellular transport brake for H^+, bicarbonate and ammonium ions in distal nephron [68]. Based on this information, claudin-8 and -4 appear to be important for renal acid/base and K^+ balance. Therefore, alterations in their function in the tight junction may lead to forms of hyperkalemic acidosis [34,67]. However one study could not find a strong relationship between claudin-8 mutations and patients with pseudohypoaldosteronism type I (PHAI) which is characterized, in part, by hyperkalemia and acidosis [69].

In a preliminary study [70], Amlal reported that development of metabolic acidosis in rats inhibited the expression of claudin-2 and claudin-10 in the proximal tubule. This observation was based on renal cortex levels of claudin-2 and claudin-10 mRNA. While claudin-10 is present in all nephron segments, claudin-2 expression is restricted to the proximal tubule and upper segments of the thin descending limbs of loop of Henle; segments known to be highly permeable to Na^+. These data provide evidence that paracellular transport processes are important for acid/base homeostasis and that regulation of claudin expression in the kidney may be an important adaptive mechanism during alterations in acid/base balance. Knockout of claudin-2 gene expression in mice resulted in significant reduction in net absorption of Na^+, Cl^-, and water in the proximal tubule [71]. However, these knockout animals were not subjected to metabolic acid/base disturbances. Because claudin-2 is important of for proximal tubule paracellular Na^+ reabsorption, an inhibition of claudin-2 expression under metabolic acidosis conditions may reduce net paracellular Na^+ reabsorption and increase the availability of Na^+ for transcellular reabsorption by Na^+/H^+ exchanger isoform 3 in the proximal tubule. This isoform of Na^+/H^+ exchanger is critical for proximal tubule bicarbonate reabsorption and acid/base balance [72] NHE-3 expression is upregulated in metabolic acidosis [73].

7. Conclusions

The recent discovery of the claudin family of tight junction associated membrane proteins represents a major break through in our understanding of the structure and function of tight junctions in epithelial organs such as the kidney. We are only in the infancy of understanding how claudins function in the mammalian nephron tight junction. Important clues into the function of a few of the claudin family members are emerging from genetic human diseases of the tight junction, animal knockout models, and epithelial cell culture models. It is becoming increasingly apparent that the family of claudin molecules is of critical importance in renal function during normal and diseased physiological conditions. However, much more work will be required to fully understand how these claudins function normally within the tight junction as well as how abnormal claudin function in the kidney relates to human disease processes.

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