Tight junction biology and kidney dysfunction

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The tight junction (TJ) forms a circumferential belt around an epithelial or endothelial cell, separating the plasma membrane into an apical and a basolateral domain. The TJ joins from one cell adjoins belts from adjacent cells, thereby forming a sheet of cellular barrier between the external environment, e.g., the luminal content of the intestine or the renal tubule, from the regulated internal environment, i.e., the interstitial fluid (155). As a “gate,” it serves as a regulatory barrier separating and maintaining biological fluid compartments of different composition. As a “fence,” it generates and maintains the apicobasal polarity of cells that form the confluent epithelium. Finally, the TJ proteins form a trafficking and signaling platform that regulates cell growth, proliferation, differentiation, and dedifferentiation. Six examples are selected that illustrate the emerging link between TJ dysfunction and kidney disease. First, the glomerular slit diaphragm (GSD) is evolved, in part, from the TJ and, on maturation, exhibits all three functions of the TJ. GSD dysfunction leads to proteinuria and, in some instances, podocyte dedifferentiation and proliferation. Second, accumulating evidence supports epithelial-mesenchymal transformation (EMT) as a major player in renal fibrosis, the final common pathway that leads to end-stage renal failure. EMT is characterized by a loss of cell-cell contact and apicobasal polarity, which are hallmarks of TJ dysfunction. Third, in autosomal dominant polycystic kidney disease, mutations of the polycystins may disrupt their known interactions with the apical junction complex, of which the TJ is a major component. This can lead to disturbances in epithelial polarity regulation with consequent abnormal tubulogenesis and cyst formation. Fourth, evidence for epithelial barrier and polarity dysregulation in the pathogenesis of ischemic acute renal failure will be summarized. Fifth, the association between mutations of paracellin-1, the first TJ channel identified, and clinical disorders of magnesium and calcium wasting and bovine renal fibrosis will be used to highlight an integral TJ protein that can serve multiple TJ functions. Finally, the role of WNK4 protein kinase in shunting chloride across the TJ of the distal nephron will be addressed.

kidney disease; zonula occludens
integrity of cell-cell contacts (72). The interrelationship between cell polarity and cell growth, differentiation, and dedifferentiation has received increasing interest (12, 54, 172) and is one focus of this discussion.

In this review, we will address selected conditions in which the biology of TJ and clinical disorders of the kidney have sufficiently interwoven to provide newer insights and allow provocative speculations.

GLOMERULAR SLIT DIAPHRAGM, PROTEINURIA, AND PODOCYTE DEDIFFERENTIATION AND PROLIFERATION

Glomerular Slit Diaphragm: A Hybrid Between TJ and AJ

The glomerular slit diaphragm (GSD) represents the junction structure that links the interdigitating foot processes from neighboring glomerular visceral epithelial cells (the podocytes) (132). Developmentally, the podocytes are evolved from primordial columnar epithelial cells linked together by an apical junction complex containing both the TJ and the AJ. As the epithelial cells develop into podocytes, each with its elaborate network of foot processes, the early apical TJ and AJ migrate basally and ultimately morph into a single GSD that links the interdigitating foot processes from adjacent podocytes (76, 128, 141). It should be appreciated that while the TJ or the AJ links one cell to another cell forming an epithelium, the GSD links one cellular process (a foot process) from one podocyte to the interdigitating cellular process of another podocyte forming a “podothelium” (Fig. 2).

The mature GSD retains features of both the TJ and the AJ. Immunostaining for the TJ-associated protein zonula occludens-1 (ZO-1) in adult rat kidney reveals the greatest intensity at the insertion sites of the GSD into the foot processes (140). Also, like the TJ, the GSD functions as a barrier (the “gate” function of the TJ) (31) that imposes selective permeability between the blood and the urinary space (117, 153), acts as a “fence” (31) that segregates the apical and the basolateral plasma membrane domains of the podocyte (104, 141), and serves as a signaling platform (11).

However, unlike the TJ, which tightly approximates the plasma membranes from adjacent cells, thereby obliterating the intervening intercellular space (35), the GSD spans an intercellular space of ~25 nm (128) to 30–45 nm (132). This separation of the plasma membrane from adjacent foot processes resembles that in the AJ, where juxtaposed cell membranes are separated by a distance of 25 nm (35). Although ZO-1 is a TJ marker in confluent epithelial sheets, in nonepithelial cells (61) and in epithelial cells before the formation of the TJ (4, 159), this protein is localized at the AJ.

Indeed, several studies have observed the presence of core AJ molecules, i.e., the cadherins, the catenins, or both in early glomerular visceral epithelium (44, 150, 158). In mature GSD, however, some investigators have observed cadherins (130, 150) or catenins (119), whereas others have failed to find either
cadherins (119, 158, 178) or catenins (44, 150, 158). Instead, Inoue and associates (60) reported the expression of FAT, another member of the cadherin superfamily, in the adult rat kidney, particularly in the podocytes, where it stains at the podocyte attachment of the GSD with its cytoplasmic domain colocalizing with ZO-1. FAT has a large extracellular domain containing 34 tandem cadherin-like repeats, five epidermal growth factor (EGF)-like repeats, and a laminin A-G domain. The authors suggested that this bulky adhesive molecule would well serve the function of bridging the relatively wide intercellular space between the foot processes.

**GSD Proteins are Responsible for its Barrier Function**

The molecular architecture of the GSD junction complex also resembles that of the TJ and AJ and consists of a collection of transmembrane-bridging proteins networking with a juxtaposed cytoplasmic platform of protein complexes, which, in turn, is linked to the actin cytoskeleton (Fig. 1). The major transmembrane and cytoplasmic plaque proteins of the GSD are listed in Table 1. Recent recognition that mutations of genes that express these proteins cause nephotic syndrome lends strong support for GSD as a selective barrier that prevents protein loss into the urinary space. One explanation is that protein flux into the urinary space is mediated through a transcellular endocytotic pathway rather than through the intercellular conduit of the urinary space. The authors suggested that this bulky adhesive molecule would well serve the function of bridging the relatively wide intercellular space between the foot processes.

**GSD May Regulate Podocyte Polarity and Proliferation**

Molecular linkage between the apical junction complex and epithelial cell growth pattern is well established. The transfection of an oncogenic Raf-1 into a rat parotid epithelial cell line leads to a complete loss of TJ function and the transformation of the monolayer into a stratified phenotype exhibiting no contact inhibition in cell growth (84). Introduction of exogenous occludin into these transformed cells results in the reappearance of TJ and restoration of the normal monolayer phenotype with a contact inhibition pattern of cell growth. The AJ has also been shown to play an important role in contact inhibition of cell growth in both the epithelium (17) and the endothelium (10). Contact inhibition (also known as density-dependent inhibition) refers to the physiological phenomenon in which growth ceases as cells reach confluent density and come into contact with each other. This process has been associated with the development of the apical junction complex (64).

Accumulating evidence supports a close linkage between the regulation of cell polarity and the control of cell proliferation (12, 172). As mentioned earlier, the GSD, like the TJ, also maintains podocyte apical-basal polarity (104, 141) and serves as a signaling platform (11). In experimental crescentic glomerulonephritis, foot process effacement and the disappearance of GSD are associated with the loss of podocyte polarity, as reflected by the apical migration of the basolateral membrane protein α3-integrin. The luminal membrane also begins secreting extracellular matrix material, normally the function of the basal membrane domain (83).

Podocyte effacement associated with massive proteinuria is reversible in some cases but not in others. Minimal-change disease and membranous nephropathy are examples of the reversible cases in which the disappearance of the foot processes and GSD is associated with actin cytoskeleton reorganization, the retention of all mature podocyte markers, and the absence of cell proliferation. In contrast, in collapsing focal segmental glomerulosclerosis (FSGS), the foot-process effacement is associated with the loss of the actin-based cytoskeleton, reduction or disappearance of markers of differentiated

### Table 1. Selected examples of proteins in the molecular architecture of the tight junction, adherens junction, and glomerular slit diaphragm

<table>
<thead>
<tr>
<th>Transmembrane-Bridging Proteins</th>
<th>Cytoplasmic Protein Plaque</th>
</tr>
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<tbody>
<tr>
<td><strong>Tight junction (42)</strong></td>
<td></td>
</tr>
<tr>
<td>Occludin</td>
<td>ZO-1</td>
</tr>
<tr>
<td>The claudins</td>
<td>ZO-2</td>
</tr>
<tr>
<td>JAM</td>
<td>ZO-3</td>
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<tr>
<td><strong>Adherens junction (106)</strong></td>
<td></td>
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<tr>
<td>Cadherins</td>
<td>α-Catenin</td>
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<tr>
<td></td>
<td>β-Catenin</td>
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<td></td>
<td>Vinculin</td>
</tr>
<tr>
<td></td>
<td>α-Actinin</td>
</tr>
<tr>
<td><strong>Glomerular slit diaphragm (see text)</strong></td>
<td>ZO-1 (α-)</td>
</tr>
<tr>
<td>Nephrin (NPHS1)††</td>
<td>CD2AP††</td>
</tr>
<tr>
<td>Podocin (NPHS2)††</td>
<td>α-Actinin-4 (ACTN4)††</td>
</tr>
<tr>
<td>NEPH1 (NEPH1)‡‡</td>
<td>α- and β-Catenins†‡‡</td>
</tr>
<tr>
<td>FAT1†</td>
<td></td>
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<tr>
<td>P-cadherin†</td>
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Reference nos. are in parentheses. ZO, zonula occludens; IAM, junctional adhesion molecule. *Both NH2 and COOH terminals are cytosolic linked by hairpin domain in plasma membrane. †Reported by some but not others (see text). ‡Gene mutations associated with phenotypic proteinuric states.
podocytes (dedifferentiation), and cell proliferation (6). The loss of GSD integrity and podocyte polarity associated with podocyte dedifferentiation and proliferation bears close resemblance to events described in epithelium associated with the loss of TJ integrity. In human immunodeficiency virus-associated nephropathy, a recognized cause of collapsing FSGS, podocyte proliferation is attributed to a loss of contact inhibition (142).

Summary

Developmentally, the GSD appears to be a product of both the TJ and AJ. Like the TJ, it functions as a selective barrier and participates in the regulation of apicobasal polarity and cell growth, differentiation, and dedifferentiation. Structural and functional disruption of the GSD gives rise to pathophysiological changes in the “podothelium” similar to those in the epithelium associated with TJ dysfunction.

EPITHELIAL-MESENCHYMAL TRANSITION AND RENAL FIBROSIS

Tubulointerstitial fibrosis, characterized by glomerulosclerosis, interstitial fibrosis, and tubular atrophy, is the final common pathway to end-stage renal disease (62, 89, 184).

Epithelial-Mesenchymal Transition and its Role in Renal Fibrosis

Resident fibroblasts and the infiltrated mononuclear cells have been considered the major players in renal fibrosis. However, accumulating evidence suggests that new fibroblasts are also derived from tubular epithelial cells through a process termed epithelial-mesenchymal transformation (EMT) and that the infiltrating monocytes/macrophages may, in fact, play a beneficial role through their phagocytic activity on extracellular (ECM) matrix and apoptotic cells (62, 89, 184).

EMT in epithelium is characterized by the disruption of epithelial junction complexes and the loss of cell polarity, transforming stationary epithelial cells into migratory mesenchymal fibroblast-like cells. Normal epithelial cell interacts with ECM through anchorage between its basal plasma membrane domain and the basement membrane. The transformed cell, on the other hand, loses this domain-specific anchorage and acquires the ability to invade the ECM (137). In embryonic development, EMT allows the migration of epithelial cells to distant sites and forms tissues such as the mesoderm and the neural crest (33, 49). In mature tissue, it is responsible for the progression of noninvasive tumor cells into malignant, metastatic cancers (160).

Evidence for epithelial-derived fibroblasts in renal fibrosis has been demonstrated in transgenic mice genetically engineered to express the LacZ marker in renal tubular epithelia (63). Unilateral ureteral obstruction in R26R mice produces progressive interstitial fibrosis and complete destruction of the kidney in 3–4 wk. The normal contralateral kidney prevents the development of azotemia. Following an obstructive injury, LacZ-expressing (tubular) cells lose their epithelial morphology and organization and migrate into the interstitium in large numbers. These epithelial-derived cells expand by cell division and express fibroblast-specific protein-1 (FSP1). These transformed cells synthesize collagen, as reflected by the expression of heat shock protein 47, which is known to increase to chaperon type I collagen in cells activated to synthesize collagen (107).

Role of TJ and AJ in EMT

The association between EMT and structural and functional alterations in TJ and AJ is well established. Transforming growth factor-β1 (TGF-β1)-induced EMT is associated with reduced expression of ZO-1 (114, 174, 184) and E-cadherin (177). In a mouse hepatic cell line, oncogenic Ral-1-induced EMT is associated with a marked downregulation of occludin and claudin-2 expression at both the transcriptional and translational levels (82).

Snail, a zinc-finger transcriptional factor, is another important player implicated in the activation of EMT (109). It binds to three E-boxes in the promoter of the human E-cadherin gene, blocking its transcription (9). Madin-Darby canine kidney (MDCK) epithelial cells transfected with Snail downregulate E-cadherin expression, increase expression of mesenchymal markers (vimentin and fibronectin), transform to a fibroblastoid phenotype, and exhibit tumorigenic invasive properties (23). Snail protein is also present endogenously and is associated with a loss of E-cadherin expression in invasive mouse and human tumors and in epithelial tumor cell lines (9, 23).

However, Snail-mediated phenotypic changes cannot be accounted for completely by a loss of E-cadherin. Blockage of E-cadherin expression by stable antisense transfection leads to the acquisition of invasive and metastatic properties but is insufficient to induce full-scale EMT (23). A more recent study demonstrated that EMT induced in cultured mouse epithelial cells is associated with concomitant repression in the transcription and translation of claudins-3, -4, -7, and occludin (58). Snail binds to the E-boxes of the promoters of the genes of these TJ proteins, with consequent complete repression of their promoter activity. Snail inhibition of occludin transcription is confirmed in MDCK cells. However, the transcription of claudin-1 (unlike the mouse claudins-3, -4, -7 mentioned above) and ZO-1 are unaffected, and their downregulation is attributed to posttranscriptional events (113). Thus Snail-induced EMT is associated with downregulation of TJ and AJ proteins at both the transcriptional and posttranscriptional levels.

Spatiotemporal studies indicate that loss of cell-cell adhesion and cell polarity precedes full-scale EMT (62, 89, 184). In rat lung carcinogenesis, losses of ZO-1 and E-cadherin expression are early events that precede EMT, raising the possibility of a cause-and-effect relationship (14). In primary culture of porcine thyroid epithelium treated with both TGF-β and epidermal growth factor (EGF), reduced expression of claudin-1 and occludin occurred in the first 24 h, accompanied by a loss of transepithelial resistance (TER) and a marked increase in the paracellular flux of [3H]inulin. Both the expression and localization of E-cadherin are not altered at 24 h, although the clear loss of its expression did occur by 48 h and was associated with fibroblastic transformation of the epithelial cells (46). These data suggest that alterations in TJ precede those in AJ and the evolution of EMT.

A coordinated role of TJ and AJ in EMT is demonstrated using MDCK-1 monolayers and COOH-terminal deletion mutants of ZO-1. Wild-type ZO-1 contains five major domains, PDZ-PDZ-PDZ-SH3-GUK. A deletion mutant (ZO-1-PDZ) constructed by the removal of the GUK and the SH3 domains...
Invited Review

TIGHT JUNCTION AND RENAL DYSFUNCTION

Polycystins

Almost all cases of ADPKD are the consequence of mutations in either the PRKDK1 (type I ADPKD) or the PRKDK2 (type II ADPKD) gene that encodes the proteins polycystin-1 (PKD1) and polycystin-2 (PKD2), respectively. The two proteins function as a receptor-ion channel complex, with PKD1 transducing mechanical cues that gate a Ca²⁺-permeant PKD2 channel, thereby initiating a cascade of Ca²⁺ signals (108). Thus loss-of-function mutations in either protein predictably give rise to qualitatively similar phenotypic manifestations (30, 81, 169).

The PKD protein complex has been localized to all three membrane domains of renal tubular cells: the apical cilium, the lateral cell-cell junction complex, and the basal cell membrane-matrix anchorage (169). Thus it links all components of the extracellular environment surrounding the cell membrane to the intracellular actin-tubulin cytoskeleton and the protein signaling networks, rendering it an ideal candidate for generating and maintaining cell polarity and cell orientation. Direct association between polycystin-2 and the actin microfilament (85) and centrosome protein pericentrin (65) has been demonstrated.

Cell Polarity, Polycystins, Tubulogenesis, and Cystogenesis

A large body of evidence suggests tubulogenesis is coupled closely to an orchestrated modification and regulation of cell-cell contact and polarity (90, 166, 183). In this context, the polycystins have been shown to induce tubule formation (15). MDCK cells form cysts in three-dimensional collagen gel matrix and differentiate into tubules in the presence of hepatocyte growth factor (HGF) (101). Interestingly, overexpression of human PKD1 in these cells is sufficient to induce tubulogenesis in the absence of HGF. Clones that lose the expression of recombinant PKD1 lose the ability to form tubules and revert back to forming cysts (16). With use of the same model, it has been shown that in HGF-induced tubulogenesis the development of tubular cell polarity and the formation of tubular lumen coincide with the expression of endogenous (canine) PKD-1 at intercellular contacts (19). Compared with the tubules, PKD1 mRNA is markedly down-regulated in cysts and the PKD1 protein is mislocated from the plasma membrane to cytoplasmic pools (19). Finally, growth factors, including HGF, induce cell polarity generation and branching tubulogenesis in normal human renal epithelial cells but not in epithelial cells from cysts of ADPKD (which express mutated proteins) (24), again suggesting the importance of PKD proteins in normal tubulogenesis.

Polycystins and the Apical Junction Complex

There is evidence supporting the interplay between each of the three components of the apical junction complex [TJ, AJ, and the desmosomes or the desmosomal junction (DJ)] with the polycystins and tubulogenesis. The exocyst, an eight-protein complex, is involved in the biogenesis of cell polarity from yeast to mammals (179). In the early stages of cell-cell contact formation, the exocyst is associated with the AJ, but as the apical junction complex matures, the bulk of the exocyst is sorted to the TJ (179). In the three-dimensional MDCK II cell culture model, the exocyst has been shown to play a central role in tubulogenesis. Its expression in sprouting tubules syn-
chronizes with the orchestrated changes in cell polarity and lumen formation (88). The observation that in ADPKD cells the exocyst is depleted from the plasma membrane (26) is consistent with the thesis that this polarity-regulating complex at the TJ is important in normal tubulogenesis. More recently, the presence of exocyst has also been demonstrated in the primary cilium of MDCK II cells (133).

There is also a molecular and functional association between the AJ and the polycystins. Endogenous PKD1 is found in a complex with E-cadherin and the catenins, the major components of AJ (52). In ADPKD cells, cellular E-cadherin expression is lower than that in normal kidney cells and is translocated from the plasma membrane into the cytoplasm (26). These observations raise the possibility that PKD1 mutations disrupt both the normal AJ cytoarchitecture and the integrity of the apical junction complex, resulting in a loss of cell polarity regulation that is necessary for normal tubulogenesis.

Finally, the polycystins have also been localized to the third component of the apical junction complex, the DJ (19, 139). Like the TJ and AJ, the DJ is an intercellular adhesive junction (35). However, the two major transmembrane desmosomal glycoproteins, desmogleins and desmocollins, exhibit little or no adhesive activities (28, 75). PKD1, on the other hand, displays strong homophilic interaction between its multiple extracellular Ig-like domains (56) and has been proposed to mediate DJ adhesion (19). In HGF-induced tubulogenesis, AJ and DJ play prominent roles, particularly during the phase of modified polarity (166).

The presence in the apical membrane of some proteins normally localized to the basolateral membrane, e.g., Na\(^+\)-K\(^+\)-ATPase and EGF receptors, provides further evidence supporting the presence of a polarity defect in ADPKD (169). This can be attributed to a defect in the “fence function” of the TJ resulting in protein mistargeting. Another possibility is a dysfunctional TJ, and its partner components of the apical junction complex, can lead to epithelial cell dedifferentiation. The “abnormal” polarization may represent a reversion of tissue to a developmentally more primitive state. Na\(^+\)-K\(^+\)-ATPase in the normal fetus is made up of \(\alpha_1\beta_2\)-subunits and is localized apically, whereas in the adult kidney it is complexed as \(\alpha_1\beta_1\)-subunits and is localized basolaterally. In ADPKD kidneys, Na\(^+\)-K\(^+\)-ATPase is expressed as \(\alpha_1\beta_2\) in the apical plasma membrane (170), suggesting dedifferentiation into fetal epithelium.

In a similar fashion, fetal epidermal growth factor receptor (EGFR) is expressed apically as a heterodimer (one copy each of EGFR and erb-b2), whereas the adult EGFR is basal in location and expressed as a homodimer (two copies of EGFR). In ADPKD, EGFR expression and localization duplicate the fetal pattern (169). This abnormal apical localization of EGFR, coupled with the finding that ADPKD patients secrete mitogenic concentrations of EGF into the lumen of the cysts (131), “creates a sustained cycle of autocrine-paracrine stimulation of proliferation in the cysts” (169).

Summary

Evidence for interactions among components of the apical junction complex, the polycystins, and polarity and growth regulation in ADPKD is beginning to emerge. The role of TJ, a component of the apical junction complex and a major player in epithelial polarity regulation, in ADPKD is relatively unexplored and deserves further study.

BARRIER AND POLARITY DYSREGULATION IN ISCHEMIC ACUTE RENAL FAILURE

About 50% of acute renal failure (ARF) encountered in hospital practice is attributable to hypoxic or ischemic injuries (151). The pathogenetic mechanism of ischemic ARF is complex and remains poorly defined.

Sublethal, Reversible Ischemic Experimental Model

The experimental model of sublethal and reversible ischemic injury provides an important conceptual advance over the original notion that ARF is predominantly the result of tubular necrosis (“acute tubular necrosis”) (87). This sublethal model is characterized by the reversible loss of epithelial barrier function and polarity (20, 144) and is the central theme of this review. The examination of ischemia-induced epithelial structural and functional changes is greatly facilitated by the use of cultured epithelium subjected to ATP depletion, a model that mimics molecular and cellular effects of renal ischemia, which is also characterized by a rapid depletion of cellular ATP (20, 87).

ARF, TJ, and AJ

In general, studies in ARF suggest the involvement of both TJ and AJ, reiterating the importance of both junction structures in the maintenance and regulation of epithelial permeability and polarity. Both the TJ and the AJ are intimately associated with the perijunctional actin-myosin ring (3, 157). The apical actin cytoskeleton, particularly that of the proximal tubular cells, is particularly sensitive to ischemia, exhibiting changes and loss of the majority of F-actin within 5 min (70). Among the early consequences of this perturbation are the alteration in junction complexes and cell polarity (97). Rho GTPases regulate actin cytoskeleton and cell adhesion (37, 48) and play a central role in cell polarization (38). Activation and inhibition of Rho ATPase signaling, respectively increases and decreases protection of MDCK II monolayer TJ from damage during ATP depletion (43).

The association of ischemia or ATP-depletion with TJ alterations is documented in clinical (78), animal (99), and in vitro cell culture studies (22, 91). Altered cellular distribution of the TJ proteins occludin (43), ZO-1 (5, 43), and ZO-1, ZO-2, and cingulin in combination (154) has been observed with ATP depletion. ATP depletion also causes rapid internalization of E-cadherin, suggesting disruption of the AJ (92). Degradation of E-cadherin and disruption of its interaction with the catenins have been demonstrated in ischemic whole kidney and in ATP-depleted cultured MDCK monolayers (21). Reduced expression and redistribution of ZO-1 and AJ proteins (\(\alpha\)-, \(\beta\)-, and \(\gamma\)-catenins) have also been documented in renal allografts with postischemic injury (78).

One mechanism for the reduction in GFR in ARF is the “backleak” of the filtrate across the damaged tubular epithelium and intratubular obstruction (Fig. 3). Evidence for backleak of glomerular filtrate is derived from both animal (32) and human studies (78, 102, 105). Recent demonstration of TJ and AJ abnormalities discussed above provides the molecular and
Fig. 3. Acute renal failure (ARF): the "back-leak and intratubular obstruction hypothesis. A: normal tubular cells linked by TJ into an epithelial sheet that forms a selective barrier (the "gate" function) between the tubular fluid compartment and the interstitium. The "fence" function of the TJ regulates the apicobasal polarity, segregating molecules such as the integrins to the basal plasma membrane domain forming the adhesive anchorage of the cell-basement membrane junction. B: ischemia-induced TJ dysfunction leads to a loss of barrier function, allowing the leakage of glomerular filtrate (tubular fluid) back to the interstitium and a loss of polarity function, allowing the apical migration of basal molecules such as the integrins. C: loss of basal anchorage molecules allows cell or cell fragments to exfoliate from the basement membrane and adhere and aggregate with other exfoliated cells or fragments within the tubular lumen or with other anchored cells with apical adhesive molecules. The obstruction-induced increase in pressure proximal to the obstruction can both oppose glomerular filtration as well as accelerate backleak of tubular fluid across the denuded basement membrane.

The disruption of polarity is also manifested by the apical localization of basolateral membrane proteins, such as the integrins (Fig. 3), which normally anchor tubular cells to the basement membrane. ATP depletion results in the redistribution of β1-integrin subunits to the apical membrane, and the loss of anchorage allows the exfoliation of viable cells into the tubular lumen (86, 100). Cell detachment would be expected to expose a greater area for glomerular filtrate backleak. The integrins, which are major cell adhesion receptors, promote cell-cell aggregation of exfoliated cells with one another or with normally anchored cells expressing apical integrins (Fig. 3). This constitutes a second cause for obstruction (see discussion of membrane bleb aggregation in the previous paragraph) of tubular fluid flow, with a consequent increase in tubular pressure proximal to the obstruction, thereby magnifying the backleak of glomerular filtrate. Tubular cell detachment (45, 115, 123) and obstruction (32, 149) have been reported in both clinical and experimental studies.

Ischemic damage also causes ectopic localization of Na⁺-K⁺-ATPase to either the cytoplasmic compartment (77, 162) or the apical membrane (98). In addition, a reduction in its expression has also been reported (79). It has been proposed that Na⁺-K⁺-ATPase plays a direct role in TJ assembly, and an ischemic-induced alteration in its expression and localization may disturb TJ assembly with consequent loss of permeability and polarity regulation (124–126).

Basally located Na⁺-K⁺-ATPase plays a major role in the tubular reabsorption of sodium. The ectopic localization of Na⁺-K⁺-ATPase away from the basolateral membrane, particularly in tubular segments proximal to the macula densa, is expected to enhance distal sodium delivery in renal ischemic injuries (77, 78). Reduction in proximal sodium reabsorption is compounded by the decrease in apical surface area for reabsorption, as discussed earlier. It is proposed that this increase in sodium delivery to the macula densa activates tubuloglomerular feedback and results in a reduction in GFR. This constitutes an important alternative mechanism linking polarity dysregulation to ischemia-induced reduction in GFR because evidence for tubular obstruction is not a consistent finding in animal (93) and human (78) studies. Indeed, a prior study implicated an afferent vasoconstriction (consequence of tubuloglomerular feedback) rather than tubular obstruction as the cause for the depression in transcapillary hydraulic pressure difference and loss of GFR in posts ischemic renal allografts (2).
Endothelial Junction Complex in ARF

Alterations in the structure and function of the endothelial cell junction complex may also contribute to ischemic damage by causing microvascular dysfunction (97). In a rodent model of acute ischemic renal failure, early disruption of the actin cytoskeleton is followed by the loss of staining of endothelial cell-cell junctions and an increase in endothelial permeability (147).

Summary

Experimental and clinical studies support important associations between epithelial barrier and polarity dysregulation and ARF. Emerging evidence suggests similar association between the endothelial apical junction complex and ARF.

PARACELLIN-1, Mg2+, AND Ca2+ WASTING AND RENAL FIBROSIS

Paracellin-1/Claudin-16 and Mg2+ Wasting

Renal Mg2+ reabsorption occurs mostly through the paracellular pathway in the thick ascending limb of Henle (TAL) (122). In the TAL, electrically neutral lumen-to-cell cotransport of Na+-K+-2Cl- is followed by the recycling of K+ through renal outer medullary K+ (ROMK) channel back into the lumen, thereby generating a lumen-positive potential (Fig. 4). This transepithelial voltage drives the positively charged Mg2+ and Ca2+ through the paracellular conduit in an absorptive direction. The high Mg2+ and Ca2+ conductance through this intercellular pathway contrasts with its water impermeability (74), and the molecular mechanism for this divalent ion conductance has been elucidated recently.

Positional cloning has identified a human gene, paracellin-1 (PCLN-1), that encodes the protein, paracellin-1 (PCLN-1) (145). This protein is colocalized with occludin at the TJ of the TAL of the loop of Henle and, when mutated, causes renal Mg2+ wasting in the syndrome of familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC). Of the 10 mutations identified in 1 series, 8 were homozygous, and 2 compound heterozygous (145). In another series of 33 patients, 19 homozygous and 13 compound heterozygous mutations were documented, with 1 patient demonstrating no mutation within the coding sequence (164).

PCLN-1 exhibits structural and sequence homology to the claudin family of tetraspan transmembrane TJ proteins with two extracellular loops (156, 181) and is now classified as claudin-16. Its relationship to paracellular Mg2+ handling has led to the concept that the TJ is populated with channels that mediate selective and regulated transport of solutes across the paracellular conduit (173, 181). Among the many other phenotypic manifestations of this disorder is the frequent association with deterioration in renal function, terminating, in many cases, in end-stage renal failure (120, 164).

Paracellin-1/Claudin-16 and Hypercalciuria

Of interest is the report of frequent, isolated hypercalciuria with or without kidney stones (HC) in otherwise healthy family members with no additional manifestations of FHHNC (120, 164). It was proposed that these subjects represent heterozygous mutation carriers of the claudin-16 gene (164). This raises the interesting question of whether some patients with HC may in fact represent heterozygous mutations in the claudin-16 gene and manifest a dominant inheritance pattern.

However, screening in a cohort of families with idiopathic hypercalciuria came up with an unexpectedly novel finding. A single homozygous substitution of a cytosome for a guanine at position 697 of the open reading frame was identified in four patients from two families (103). Unlike the FHHNC, in which all homozygous or compound heterozygous mutations affect amino acids in either the extracellular or the transmembrane segments of the claudin-16 molecule, this new homozygous mutation results in the substitution of threonine (T) for an arginine (R) (T233R) in the last amino acid triplet (threonine-arginine-valine at positions 233–235) of the COOH terminal of the claudin-16 molecule (Fig. 5). Patients present with childhood hypercalciuria, which progressively resolves, with urine Ca2+ excretion normalizing in adulthood. Another difference from the classic FHHNC is the absence of deterioration of GFR in all four patients. Urine Mg2+ excretion, measured in three patients, is slightly elevated compared with the unaffected members, but frank hypomagnesemia is detected only in one patient. This suggests that the mild renal wastage is compensated for, probably through an increase intestinal absorption. Whether renal Mg2+ handling changes with maturation is not mentioned.

Using polarized MDCK epithelium, the authors demonstrated that the wild-type claudin-16 binds ZO-1, the prototypic TJ scaffold protein in polarized epithelium, and the two proteins colocalize at the TJ. They reasoned that claudin-16/ZO-1 binding is most likely mediated through claudin-16’s COOH-
terminal threonine-arginine-valine (TRV) sequence (which resembles the type 1 PDZ-binding motif (55, 135) with the PDZ domain of ZO-1 (41). Indeed, binding between ZO-1 and the T233R mutant is markedly impaired, and the bulk of the mutated claudin-16 is mislocalized from the plasma membrane into the lysosomes (103). The authors postulated that the mutated claudin-16 is targeted to the TJ but its residency there is markedly shortened because of its inability to tether to ZO-1, resulting in a marked reduction of channel-conducting activities at the TJ. A higher claudin-16 expression coupled with the anticipated lower Ca\textsuperscript{2+} turnover rate in adults may explain the resolution of hypercalciuria with maturation. In microperfusion of mouse cortical TAL, paracellular absorption of Ca\textsuperscript{2+} and Mg\textsuperscript{2+} is higher in tubules from adults (8 wk) than from young (4 wk) mice (171).

Another study demonstrated that the threonine or the valine residue of the COOH-terminal TRV sequence of PCLN-1 is necessary for binding to ZO-1. Mutants with an alanine substitution of threonine (ARV) or valine (TRA), or with deletion of TRV (delta TRV), inhibit this PCLN-1/ZO-1 association, and PCLN-1 becomes widely distributed along the lateral membrane rather than selectively at the TJ. Mutants with an alanine substitution of arginine (TAV) behave and function as the wild-type and both exhibit apical-to-basal \textsuperscript{45}Ca\textsuperscript{2+} transport activity at levels much higher than those in the other three mutations. Ca\textsuperscript{2+} transport is enhanced by a positive electrical potential gradient and inhibited by increasing Mg\textsuperscript{2+} concentrations (57).

Paracellin-1/Claudin-16, Renal Fibrosis, and Renal Failure

Null mutation of the claudin-16 gene in Japanese Black cattle (Wagyu) is associated with autosomal recessive chronic interstitial nephritis with diffuse zonal fibrosis (CINF) (51, 73, 110). Clinical manifestations include growth retardation with hoof elongation, proteinuria, and death with renal failure (111). The initial mutation (type 1) identified is characterized by a 37-kb deletion containing the first four exons of the claudin-16 gene, resulting in the absence of a claudin-16 transcript (51). This was followed by the identification of a second mutation (type 2), consisting of a 56-kb deletion containing the first four exons and 21-bp of the fifth exon (50). Compared with the normal cattle, the affected cattle exhibit a reduction in creatinine and Mg\textsuperscript{2+} clearance, an elevation in fractional excretion of Mg\textsuperscript{2+}, and normal serum Mg\textsuperscript{2+} concentration (112).

Earlier, we mentioned that patients with FHHNC frequently progressed to end-stage renal failure. Praga and associates (120) reported that six of eight patients required maintenance dialysis from within 1–10 yr following presentation. The two remaining patients showed mild renal insufficiency after 11 and 9 yr of follow-up, respectively (120). Twelve of 33 patients from 12 European pediatric departments developed end-stage renal failure at a median age of 14.5 yr, with a range of 5.5–37.5 yr. A less aggressive progression to renal failure is reported in a series of seven Arab patients. However, the oldest of the seven patients at the last follow-up is 16 yr, and the age range in the remaining six patients is 0.9–8.5 yr (69), raising the possibility that the clinical course may change with a longer follow-up.

While the deterioration in renal function associated with progressive tubulointerstitial nephropathy in FHHNC has traditionally been attributed to the concomitant hypercalciuria and nephrocalcinosis, long-term correlative clinical experience has not consistently supported this association (120, 164). These authors observed that the correlation between renal failure and hypercalciuria and nephrocalcinosis is also not borne out in conditions such as distal renal tubular acidosis or antenatal Bartter syndrome.

It appears that the site and extent of claudin-16 gene mutation determine the phenotypic manifestation, which can span the spectrum from a variation in channel conductance to an alteration in cell proliferation and differentiation (Fig. 5). We raise the possibility that gene mutations in some cases of FHHNC may affect claudin-16 structure and function, important not only in Mg\textsuperscript{2+} and Ca\textsuperscript{2+} reabsorption but also in cell growth regulation. The latter dysfunction can lead to renal fibrosis, which is qualitatively similar but quantitatively less aggressive than those of the null mutants in cattle. Emerging evidence supports a role for the claudins in epithelial cell growth regulation. The association between the claudins and EMT resulting in renal fibrosis has already been discussed. In
bovine CINF, tubular epithelial cells were reported as “immature” with loss of polarization and attachment to the basement membrane. A close association between fibrosis and abnormal tubules was noted, and the term “renal tubular dysplasia” was used to underline the lesion initiates in the epithelial cells of the renal tubules (136). These features are consistent with EMT as discussed earlier. A number of reports have also documented an association between changes in specific claudin expression and human cancers (1, 96, 127).

**Physiological Regulation of Paracellin-1**

In vitro transcriptional analysis of human paracellin-1, as reflected by its promoter activities, has been published recently (34). Human paracellin-1 reporter activities (using luciferase reporter vectors), mRNA, and protein are detected in renal but not in nonrenal cell lines. High ambient Mg2+ concentration [Mg2+] increases, whereas low [Mg2+] reduces the promoter activities relative to that measured in normal [Mg2+]. 1,25-Dihydroxyvitamin D decreases human paracellin-1 promoter activities, and this action appears to be mediated through the single peroxisome-proliferator-response element (PPRE) within the promoter region.

**Summary**

Mutations of paracellin-1/ Claudin-16 affecting amino acids at the extracellular and transmembrane segments cause FH-HNC and are associated with frequent progression to end-stage renal failure. The high density of negative charges in the first extracellular loop of this TJ protein channel may account, in part, for its cationic selectivity (145). Mutations affecting the extracellular loop of this TJ protein channel may account, in part, for its cationic selectivity. Null mutations leading to the absence of the claudin-16 transcript cause bovine chronic interstitial nephritis and death with renal failure. Claudin-16 may provide much insight into the many structural and functional facets of the TJ.

**WNK4 PROTEIN KINASE AND PARACELLULAR “CHLORIDE SHUNT”**

Pseudohypoaldosteronism type II (PHAIIT), also known as Gordon syndrome or familial hypertension with hyperkalemia, is an autosomal dominant disorder characterized by hypertension, hyperkalemia, and hyperchloremic metabolic acidosis (39, 68). Hypertension is attributed to enhanced renal salt reabsorption with consequent volume overload that is reflected by hyporeninemia. Plasma and urine aldosterone levels have been reported as normal or high and have been attributed to a K+-induced stimulation of aldosterone secretion. Both hyperkalemia and metabolic acidosis are ascribed to decreases in renal excretion of these ions in the face of a normal GFR.

Elegant clearance studies predicted a distal chloride shunt as the basis for this constellation of findings (138, 148). In the cortical collecting duct, Na+ enters from the tubular lumen into the principal cell through the epithelial Na+ channel, ENaC (Fig. 6). This electrogenic transport step creates a favorable electrical driving force for apical K+ and H+ secretion from the principal cell and the α-intercalated cell, respectively, and paracellular Cl− reabsorption. Abnormal increases in Cl− reabsorption across the TJ (chloride shunt) can increase salt reabsorption and intravascular volume, while at the same time dissipate the electrical gradient for K+ and H+ secretion with consequent hyperkalemia and metabolic acidosis.

Positional cloning linked mutations in two homologous protein kinase genes, WNK1 and WNK4 (for with no lysine K), i.e., lacking a lysine normally present in the catalytic domain of this kinase family), to PHAIIT (167). Both WNK1 and WNK4 proteins are localized to the distal convoluted tubule and the cortical collecting duct. WNK1 is also present in the medullary collecting duct. In contrast to the intracellular distribution of WNK1, WNK4 is present at the intercellular junctions, colocalizing with the TJ proteins ZO-1 (167) and occludin (175). MDCK II monolayers transfected with wild-type WNK4 increased paracellular Cl− permeability in one study (66) but not in another (175). However, in both studies PHAIIT-mutants WNK4 D564A (175) and Q562E and E559K (66) increased paracellular Cl− conductance. The study of Kahle et al. (66) demonstrates that the increase in Cl− conductance induced by the mutant proteins is greater than that induced by the wild-type, while the study of Yamauchi et al. (175) demonstrates that WNK4 binds and phosphorylates claudins 1–4 with the mutant protein, exhibiting a greater effect than the wild-type protein. Both the expression and localization of TJ proteins and

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Fig. 6. K+ and H+ excretion in cortical collecting duct. Luminal Na+ entry into the principal cell through the epithelial Na+ channel (ENaC) creates a favorable electrical driving force for apical K+ and H+ secretion from the principal cell and the α-intercalated cell, respectively, and Cl− reabsorption across the TJ, through the paracellular conduit. An abnormal increase in Cl− reabsorption (“chloride shunt”) would be expected to reduce the normal lumen-negative potential and, thereby, reduce K+ and H+ secretion.
ultrastructure of the TJ are not altered by WNK4 or its mutants (66). Thus the original chloride shunt may represent a gain-of-function mutation of WNK4 that mediates its action through functional regulation of the paracellular Cl⁻ channel(s) at the TJ.

It is important to point out that an increase in the activity of the thiazide-sensitive sodium-dependent chloride cotransporter (NCC, NCCT, or TSC) can also account for all the phenotypic features of PHAII. The reported resolution of phenotypic features of PHAII with thiazide diuretics lends support to this possibility (95). The notion is further supported by the observation that WNK4 inhibits NCC activity in *Xenopus laevis* oocytes through reducing its expression at the plasma membrane (168, 176). Some (176) or all (168) PHAII-causing mutants studied reduce or eliminate this inhibition of NCC, thereby permitting increases in salt resorption. The consequent reduction in salt delivery to the more distal cortical collecting duct decreases electrogenic Na⁺ resorption, resulting in the dissipation of the electrical gradient for K⁺ and H⁺ secretion.

Additional factors that can cause hyperkalemia are attributed to the inhibitory action of WNK4 on ROMK channel activity through enhancing clathrin-dependent endocytosis of this channel. Interestingly, WNK4 mutations, which lead to a loss-of-function of NCC, cause a gain-of-function in ROMK, i.e., a further decrement in K⁺ conductance surface expression of ROMK (39, 68).

**Summary**

WNK4 protein kinase modulates both paracellular Cl⁻ conductance and transepithelial sodium chloride transport. Adding to the complexity is the finding that it also inhibits ROMK channel activity. WNK4 mutations decrease (loss-of-function) inhibition of NCC but increase (gain-of-function) inhibition of ROMK activity and paracellular Cl⁻ conductance. Attempts at integrating these mechanisms into a unified conceptual framework have already begun to provide new insights into the pathophysiology of fluid and electrolyte balance and its interaction with volume and blood pressure regulation (13, 67, 134).

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