Review Article

Possible Involvement of Tight Junctions, Extracellular Matrix and Nuclear Receptors in Epithelial Differentiation

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Tight junctions are intercellular junctions localized at the most apical end of the lateral plasma membrane. They consist of four kinds of transmembrane proteins (occludin, claudins, junctional adhesion molecules, and tricellulin) and huge numbers of scaffolding proteins and contribute to the paracellular barrier and fence function. The mutation and deletion of these proteins impair the functions of tight junctions and cause various human diseases. In this paper, we provide an overview of recent studies on transmembrane proteins of tight junctions and highlight the functional significance of tight junctions, extracellular matrix, and nuclear receptors in epithelial differentiation.

1. Introduction

The epithelial tissue in various organs (e.g., lungs, intestines, and skin) is composed of a seat of epithelial cells that separate the biological compartments in the body with different internal environments. The intercellular adhesion complex between epithelial cells consists of tight junctions, adherens junctions, and desmosomes and is fundamental to the construction of the epithelial cell seat and the maintenance of cellular polarity [1, 2].

Within the intercellular adhesion complex, tight junctions possess two distinct functions in the epithelium tissue (Figure 1(a)). They function as a barrier controlling molecular penetration of ions, solutes, water, and cells through intercellular space and act as a fence dividing apical and basolateral domains to compartmentalize the plasma membrane [3]. These characteristics of tight junctions allow epithelium to prevent pathogens and foreign substances from invading and to facilitate directional exchange of materials.

Tight junctions comprised 4 kinds of transmembrane proteins: occludin, claudins, junctional adhesion molecules (JAMs), and tricellulin as well as numerous cytosolic proteins (Figure 1(b)). The cytosolic proteins are roughly divided into two groups depending on the presence or absence of PDZ (PSD-95, Dlg, and ZO-1) domains: the PDZ proteins (ZO-1, -2, -3, Par-3, -6, and membrane-associated guanylate kinase protein [MAGI]-1, -2, -3, etc.) and the non-PDZ proteins (cingulin, heteromeric G proteins, atypical PKC [aPKC], rab-3b, -13, PTEN, etc.) [5, 6]. Thus, a growing body of studies has clarified the molecular components of tight junctions, but it is still obscure how they accumulate and form tight junctions.

The intercellular adhesion complex and cell polarity should be established during differentiation of stem cells into epithelial cells. In this paper, we focus on transmembrane proteins of tight junctions and highlight the participation of tight junctions, extracellular matrix, and nuclear receptors in epithelial differentiation.

FIGURE 1: Functions of tight junctions and molecular components. (a) Functions of tight junctions. Barrier function: tight junctions limit the penetration of intercellular material and impose selective permeability. Fence function: tight junctions restrict the diffusion of proteins and lipids in a cell membrane. (b) Schematic drawing of bicellular junction proteins. Transmembrane and scaffold proteins of tight junctions and polarity proteins are presented. These drawings are modified from a previously published review [4].

2. Transmembrane Proteins of Tight Junctions and Their Involvement in Epithelial Differentiation

2.1. Occludin. Occludin is a tetraspan membrane protein with two extracellular loops (EC1 and EC2), a short intracellular turn, and N- and C-terminal cytoplasmic domains [7]. Among these domains, the long C-terminal domain is phosphorylated at serine, threonine, and tyrosine residues by various protein kinases including src family kinase and CK2 [8, 9]. The C-terminal region of occludin also directly binds to ZO-1 [10], and the phosphorylation of some tyrosine residues prevents both the interaction with ZO-1 and the assembly at tight junctions [11]. In addition, the phosphorylation of some threonine and serine residues enhances occludin trafficking to tight junctions and the barrier function [9]. Although there are four splicing variants

in occludin [12], the biological significance of each variant in tight junctions is unclear.

Overexpression of full-length and mutated *occludin* gene in Madin-Darby canine kidney cells or *Xenopus* cells [13] suggests that occludin contributes to the barrier function of tight junctions. By contrast, *occludin*-deficient embryonic stem cells differentiate into polarized epithelial cells with well-developed tight junctions [14]. Moreover, *occludin*-null mice are born with normal structure and barrier function of tight junctions in the intestinal epithelial tissue [15], but they exhibit various phenotypes, such as growth retardation, hyperplasia of gastric mucosa, absence of cytoplasmic granules in striated duct cells of the salivary gland, thinning of the compact bone, brain calcification, and testicular atrophy. These phenotypes imply that occludin may be involved in neither epithelial differentiation nor the barrier function but in other unknown roles.

Occludin interacts with a variety of cellular signaling molecules and may contribute to the signal transduction. For example, occludin binds to transforming growth factor [TGF]- β type I receptor and regulates TGF- β -dependent disassembly of tight junctions during epithelial-to-mesenchymal transition [16]. It also associates with nonreceptor tyrosine kinase c-Yes, aPKC, PI-3 kinase, and protein phosphatases 2A [6, 17]. Furthermore, occludin regulates the organization of actin and the directional migration in epithelial cells [18]. In addition, it is reported that occludin is concerned with apoptosis via activation of the small GTPase RhoA, mitogen-activated protein kinase (MAPK), and Akt signaling pathways [19, 20].

2.2. Claudins. The claudin family consists of 24 distinct members in human and mice with three others recently identified [4, 21, 22]. They are 18- to 27-kDa tetraspan proteins with N- and C-terminal cytoplasmic domains and two extracellular loop domains, and are expressed in tissue- and cell type-specific manners [3, 23]. In addition, isoforms of some claudins (e.g., claudin-10 and -18) are generated by alternative splicing and exhibit different expression patterns and functions [24, 25].

Claudin genes have few introns, and several pairs of them are located in close proximity in human and mouse genome. For example, in humans, *Claudin-3* and *Claudin-4* are located on chromosome 7, *Claudin-6* and *Claudin-9* on chromosome 16, *Claudin-8* and *Claudin-17* on chromosome 21, and *Claudin-22* and *Claudin-24* on chromosome 4 [26]. Gene duplication is assumed in these claudins, and the coordinated expression is reported at least for *Claudin-3* and *Claudin-4* genes [27].

Claudins are indeed the backbone of tight junctions, since they can reconstitute tight-junction strands even in fibroblasts [28]. All claudins except for claudin-12 possess a PDZ-binding motif and are capable of direct interaction with the proteins containing PDZ domain such as ZO-1, -2, -3, multi-PDZ domain protein (MUPP-1), and PALS-1 associated TJ protein via the cytoplasmic tail (PATJ) [29–32]. Hence, claudins are linked to the actin cytoskeleton through binding to the scaffolding proteins (ZO-1 and ZO-2), stabilizing tight junctions [33].

It should be noted that the posttranslational modification of claudins influences their localization and function. First, the phosphorylation of claudins is associated with tight-junctions assembly and paracellular permeability. For example, phosphorylation of claudin-3, -5, and -16 by protein kinase A facilitates tight-junctions assembly and functionality [34, 35]. Phosphorylation of claudin-1 and/or claudin-4 by MAPK and aPKC is also reported [36]. Second, palmitoylation of claudins at the dicysteine motif, which is conserved through the claudin family, is thought to increase claudin accumulation in tight junctions and enable their translocation to detergent-resistant plasma membranes (lipid rafts) [37]. Third, O-glycosylation in some claudins, claudin-1, -3, and -4 by O-linked N-acetylglucosamine transferase is predicted, but the function of this modification is unknown [38].

Physiological roles of claudins have been clarified from studies of transgenic and knockout mice or human diseases [39]. Mutations in *claudin-1* gene cause neonatal ichthyosis and sclerosing cholangitis in humans, and *claudin-1*-deficient mice result in neonatal death due to the disturbance of skin permeability [40, 41]. Claudin-2 knockout mice show no obvious phenotypic changes (e.g., of growth and behavior) [42]. The deletion of *claudin-2* gene causes the decrease in Na⁺ paracellular permeability [43]. *Claudin-7*-null mice die within 12 days of birth owing to severe salt wasting, chronic dehydration, and growth retardation, which are caused by the breakdown of NaCl homeostasis [44]. *Claudin-11* (also known as oligodendrocyte-specific protein) KO mice exhibit slowed CNS nerve conduction, markedly hindlimb weakness, and sterility in males due to the absence of tight-junction strands in CNS myelin and between Sertoli cells [45]. Mutations of *claudin-14* gene lead to autosomal recessive deafness in mice and humans, suggesting that claudin-14 is associated with the cation-restrictive barrier in outer hair cells of the cochlea in the ear [46, 47]. *Claudin-15*-deficient mice show uneventful birth and growth but manifest an enlarged upper small intestine due to increased proliferation of normal cryptic cells [48]. Moreover, in the adult small intestine of *Claudin-15*-deficient mice, luminal Na⁺ and K⁺ homeostasis is disturbed, and glucose absorption is significantly decreased [43]. Various mutations in *claudin-16* gene are seen in patients of familial hypomagnesemia with hypercalciuria and nephrocalcinosis, an autosomal recessive disease with severe Mg²⁺ and Ca²⁺ wasting [49]. In fact, claudin-16 is expressed in epithelial cells of the thick ascending loop of Henle [50]. Taken together with the finding that mutations in *claudin-19* gene also show similar phenotypes with abnormal Mg²⁺ reabsorption [51], it appears that claudin-16 and -19 modulate paracellular absorption of Mg²⁺ and Ca²⁺ in the kidney.

2.3. Junctional Adhesion Molecules (JAMs). JAMs belong to the immunoglobulin (Ig) superfamily and are N-glycosylated transmembrane proteins of tight junctions [5, 52]. They consist of two extracellular Ig-like domains, a single transmembrane region, and a C-terminal cytoplasmic domain that ends in a canonical PDZ domain-binding site. JAMs are classified into two subgroups by their sequence and structure. JAM-A (also referred as JAM/JAM-1/106 antigen/F11R), JAM-B (also known as VE-JAM/mJAM-3/hJAM-2), and JAM-C (also known as mJAM-2/hJAM-3) have a class II PDZ-binding domain at C-terminal ends, which directly interact with proteins containing PDZ domains: ZO-1 [53], AF-6/afadin [54], MUPP1 [30], and PAR-3 [55]. On the other hand, coxsackie and adenovirus receptor (CAR), endothelial cell-selective adhesion molecule (ESAM), and JAM4 contain a class I PDZ domain-binding motif at their C-terminal ends, associate with Ligand-of-Numb protein X1 and MAGI-1 [56–59]. In addition, it has been reported that JAM-A, JAM-B, and JAM-C interact with integrins α L β 2 (LFA-1), α 4 β 1 (VAL-4), and α M β 2 (Mac-1), respectively, via the extracellular domains [60, 61].

JAM-A contributes to the formation of tight junctions and cell polarity through homophilic binding and inter-

TABLE 1: Nuclear receptors induce the expression of claudins.

Nuclear receptors	Induced claudin expression	Cells	References
Retinoic acid receptor	Cldn1, Cldn4	Human nasal epithelium	[83]
	Cldn3	Human urothelium	[84]
	Cldn6, Cldn7	Mouse F9 stem cell	[81]
	Cldn23	Mouse epidermis	[82]
Hepatocyte nuclear factor 4 α (HNF4 α)	Cldn6, Cldn7	Mouse F9 stem cell	[85, 86]
	Cldn1	Mouse hepatocyte	[87]
Androgen receptor	Cldn3	Mouse Sertoli cell	[88]
Progesterone receptor	Cldn3, Cldn4	Mouse amniotic epithelium	[89]
Corticoid receptor	Cldn3, Cldn4	Gill epithelium	[90]
Vitamin D receptor	Cldn2, Cldn12	Mouse intestinal Caco-2 cell	[91]

action with PAR-3/aPKC/PAR-6 complex in epithelial cells [62, 63]. JAM-B and JAM-C also associate with PAR-3 via PDZ-binding domain [52]. JAM-B and JAM-C are expressed in Sertoli cells and spermatids, and JAM-C is essential for the polarization of round spermatids [64]. By contrast, ESAM is observed in endothelial cells and platelets, and the deletion of *ESAM* gene increases VEGF-induced permeability in mice endothelial cells [65–67]. Interestingly, JAM-C is expressed in undifferentiated embryonic stem cells more abundantly than in differentiated cells [68]. However, JAM-C mutant mice do not show developmental defects. Further studies are required to elucidate the role of JAMs in epithelialization.

2.4. Tricellulin. Tricellulin, a tetraspan protein that concentrates at tricellular contacts of epithelial cells, was identified by using gene chip analysis to compare parental epithelial cells and cells undergoing EMT [69]. It is phosphorylated by CK1, and its expression is repressed by SNAI1, a zinc-finger type transcription factor that plays an important role in EMT [70]. In addition, tricellulin concentrates at tricellular tight junctions in cochlear vestibular epithelial cells and the recessive mutations of *tricellulin* gene cause nonsyndromic deafness [71]. Moreover, tricellulin is related to the epithelial barrier and organization of both bicellular and tricellular tight junctions. Interestingly, tricellulin is incorporated into claudin-based tight junctions independently of ZO-1 binding and is translocated from bicellular to tricellular tight junctions in the presence of occludin [72].

3. Possible Involvement of Claudins in Epithelial Differentiation

During embryonic development in mice, claudin-6 is first detected in the epithelial zone of embryonic bodies at least in part via the bone morphogenic protein-signaling pathway [73], and afterwards observed in several types of epithelial tissues such as the epiblast and hypoblast (to E7.5), the definitive endoderm (to E8.5), the entire gut, optic vesicles, and a small region of the forebrain (at E9.5) [74]. Although these results imply that claudin-6 is involved in epithelialization, the examined claudin-6-null mice show no

abnormality [74]. Other claudins might compensate for the function of claudin-6.

The epithelial-mesenchymal transition (EMT) is a process during which epithelial cells convert to mesenchymal cell morphology. This occurs in normal developmental processes including mesoderm and neural-crest formation and in the invasive process in tumors of epithelial origin. EMT has two steps: loss of intercellular adhesion (adherens and tight junctions) and acquiring cell motility [75]. Snail directly binds to the promoter of *E-cadherin*, *claudin-3*, *claudin-4*, and *claudin-7* and represses the expression of those genes [76], thereby inducing EMT. Therefore, not only E-cadherin, but also claudins are possibly related to EMT.

In the development of gut tube, endodermal cells differentiate into gut epithelial cells and form a lumen as the cells polarize. Claudin-15 associates with single lumen formation and forms ion-permeable pores from the study of knockdown of it using morpholino in zebrafish [77]. These findings also suggest that claudins directly relate to epithelial differentiation.

4. Involvement of Nuclear Receptors in Epithelial Differentiation

Retinoids have numerous biological effects on vertebrate development, differentiation, proliferation, and homeostasis through two types of nuclear receptors, retinoic acid receptors (RARs) and retinoid X receptors (RXRs) [78, 79]. Using a mouse F9 stem-cell line, we previously showed that various RXR/RAR heterodimers exerted both specific and redundant functions in endodermal (epithelium-like cells) differentiation [80]. In addition, we found that the RXR α /RAR γ pair mediated the induced expression of tight-junctions molecules (occludin, claudin-6, and claudin-7) and the establishment of both polarized epithelial morphology and functional tight junctions (Table 1) [81]. It is also reported that retinoid receptors and the kinase IKK1 cooperatively regulate claudin-23 expression in keratinocytes and participate in the formation of the epidermal barrier (Table 1) [82].

Hepatocyte nuclear factor 4 α (HNF4 α), another member of the nuclear receptor superfamily, transcriptionally con-

FIGURE 2: Models of HNF4 α -triggered formation of tight junctions and microvilli in F9 cells. (a) In an undifferentiated state, several junctional proteins are accumulated to premature junctions. (b) HNF4 α provokes the formation of junctional complexes and microvilli via induction of expression of occludin [OCLN], claudin-6 [CLDN6], claudin-7 [CLDN7], and ezrin/radixin/moesin-binding phosphoprotein 50 [EBP50].

trols the expression of a large number of target genes involved in nutrient and drug metabolism, hematopoiesis, and blood coagulation [92–94]. It is initially detected in primitive endoderm and subsequently in visceral endoderm (VE) during early mouse development [95]. In adults, HNF4 α is expressed in a variety of epithelial cells of the liver, kidney, intestine, pancreas, and stomach. HNF4 α also participates in the differentiation of VE cells and hepatocytes [96, 97]. Interestingly, we demonstrated that HNF4 α triggered the expression of tight-junction molecules (occludin, claudin-6, and claudin-7) and translocated tight junction proteins (ZO-1, -2, -3, JAM-B, and JAM-C) and cell polarity proteins (PAR-3, PAR6, aPKC, CRB3, Pals1, and PATJ) to tight junc-

tions as well as the formation of functional tight-junctions and epithelial polarity [85, 86] (Figure 2 and Table 1). HNF4 α also induced the expression of ezrin/radixin/moesin-(ERM-) binding phosphoprotein 50 and phosphorylation and apical concentration of ERM proteins [98] (Figure 2). Thus, HNF4 α initiates the formation of tight-junctions and microvilli and induces differentiation in epithelial cells.

Androgen and estrogen receptors induce the differentiation of prostate and mammary gland epithelium, respectively [99]. Several other nuclear receptors, including those of progesterone, corticoid, vitamin D, and PPARgamma, induce the expression of claudins in various cells (Table 1) [83,

84, 89–91]. Taken collectively, it is strongly suggested that nuclear receptors induce the expression of tight junctions and cell-polarity proteins and provoke epithelial differentiation with the formation of cell junctions and cell polarity.

5. Involvement of Extracellular Matrix in Epithelial Differentiation

Extracellular matrix (ECM, also known as basement membrane) is composed of various proteins, such as collagen, laminin, fibronectin, heparan sulfate proteoglycan, and nidogen, and these proteins are expressed in an organ- and development-dependent manner [100, 101]. In epithelial tissue, ECM functions to support epithelial cells and to stimulate their proliferation. ECM also seems to be essential for the differentiation of epithelial cells [102]. For instance, laminin and collagen type IV promote the differentiation of intestinal epithelial cells [103], and ECM functions to increase the ability of proliferation and differentiation of human renal cells and to maintain the differentiated epithelial cells for a long term [104].

Concerning the relationship between ECM and tight junctions, ECM proteins, especially fibronectin, increase the expression of claudin-18 and occludin along the plasma membrane in alveolar epithelial cells, and enhance the barrier function [105]. In turn, claudins recruit certain types of matrix metalloproteinases (MMPs, which serve as proteinases for ECM) onto cell surfaces and enhance the activation of MMP [106]. Thus, ECM and tight junctions influence the expression and function of each other's components in ways that suggest exciting avenues for further research.

6. Conclusion

Our knowledge of the molecular nature of tight junctions is still expanding. For instance, MarvelD3 has similar structure to occludin and tricellulin and is able to partially compensate for the deletion of those genes [107]. Bves/Pop1a, which belongs to the Popeye family Popdc, modulates RhoA and ZONAB/DbpA, a γ -box transcription factor, and associates with tight-junctions proteins via ZO-1 in epithelial cells [4, 108–110]. In addition, a novel RhoGEF (p114RhoGEF) is identified to be involved in maturation of tight junctions via restricted activation of RhoA [111]. Thus, a variety of extracellular and intracellular proteins appear to participate in the formation and function of tight junctions in epithelial cells.

However, there remain several open questions in tight junctions. (1) Where are tight-junctions proteins assembled (e.g., cytoplasmic versus junctional assembly)? (2) How are they recruited into the junctions (e.g., do cargo proteins exit)? (3) What accounts for the selectivity of heteromeric or homomeric claudin-claudin interactions? (4) What is the functional relevance of posttranslational modification of tight-junctions proteins? In addition, it should also be determined how tight-junctions proteins regulate epithelial differentiation.

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