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Review Article

Tight junction-related human diseases

Norimasa Sawada

Department of Pathology, Sapporo Medical University School of Medicine, Sapporo, Japan

Tight junctions are intercellular junctions adjacent to the apical ends of paracellular spaces. They have two classical functions, the barrier function and the fence function. The former regulates the passage of ions, water and various molecules through paracellular spaces, and is thus related to edema, jaundice, diarrhea and blood-borne metastasis. The latter function maintains cell polarity by forming a fence to prevent intermixing of molecules in the apical membrane with those in the lateral membrane. This function is deeply involved in cancer cell properties in terms of loss of cell polarity. Recently, two novel aspects of tight junctions have been reported. One is their involvement in signal transduction. The other is that fact that tight junctions are considered to be a crucial component of innate immunity. In addition, since some proteins comprising tight junctions work as receptors for viruses and extracellular stimuli, pathogenic bacteria and viruses target and affect the tight junction functions, leading to diseases. In this review, the relationship between tight junctions and human diseases will be described.

Key words: barrier function, cancer, claudin, fence function, human diseases, immunity, occludin, tight junctions

Establishment of a distinct internal environment is absolutely required for multicellular organisms to maintain life. For this purpose, all of their surfaces, the skin, gastrointestinal tract, respiratory tract, etc., are covered by various kinds of epithelia. In particular, for epithelial and endothelial sheets to work efficiently as a barrier,[1–5] paracellular spaces must be strictly sealed by tight junctions, which are characterized as a set of continuous and anastomosing strands at the apicalmost regions of the lateral cell membranes (Fig. 1).

Correspondence: Norimasa Sawada, MD, PhD, Department of Pathology, Sapporo Medical University School of Medicine, S1, W17, Sapporo 060-8556, Japan. Email: sawadan@sapmed.ac.jp

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The strict regulation of diffusion of solutes through paracellular spaces by tight junctions is referred to as the barrier function of the tight junction. When tight junctions of epithelial cells that cover the biliary tree and gastrointestinal tract become disordered, jaundice and diarrhea occur, respectively. Although vascular permeability depends on both the paracellular pathway and transcellular pathway of endothelial sheets, edema develops mainly as a result of the dysfunction of tight junctions between cells. Thus, dysfunction of tight junctions is considered to be intimately related to various pathological conditions.[6]

Epithelial cells have two distinct domains of the cell surface; the apical and basolateral cell membranes. Since the two domains play different roles, the compositions of proteins and lipids in the respective membrane domains are different. To prevent intermixing of molecules in the apical membrane with those in the lateral membrane, tight junctions continuously surrounding the apical pole work as a fence. This function of the tight junction is referred as the fence function. When the function is impaired, cells fail to perform their vectorial work, in terms of loss of cell polarity, and this is presumably deeply involved in cancer cell biology.

In addition to the above-mentioned functions of tight junctions, two properties of tight junctions are becoming clear. One is their involvement in signal transduction and the other is their participation in innate immunity. In this review, we will describe the properties of tight junctions, changes in tight junctions under pathological conditions, and the possible clinical application of tight junction research.

MOLECULAR COMPONENTS OF THE TIGHT JUNCTION (TABLE 1)

Integral membrane proteins

Of the proteins comprising tight junctions, the integral membrane proteins are the claudin family,[2,3,7] tight junction-associated MARVEL proteins (TAMPs)[5,8] composed of occludin, tricellulin[9] and MARVELD3, the immunoglobulin

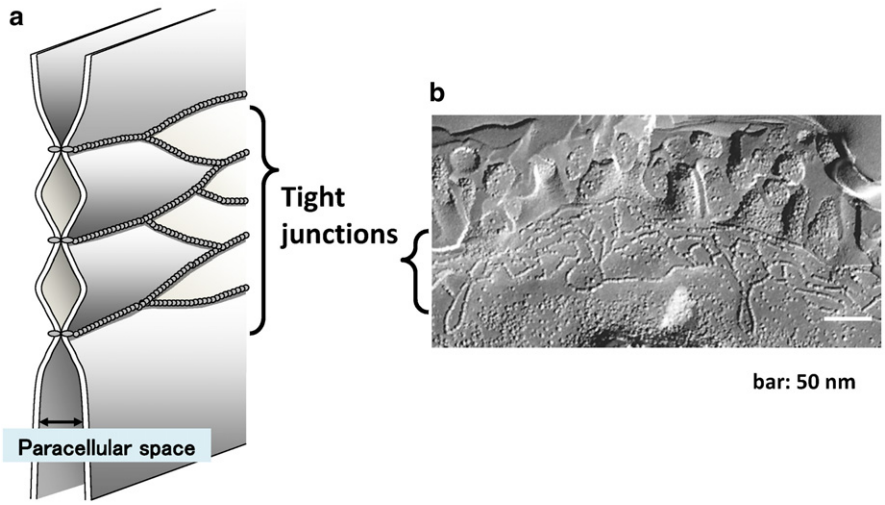

Figure 1 Morphology of tight junctions. (**a**) Schematic diagram of tight junction. (**b**) Tight junction strands on freeze-fracture replica.

Table 1 Tight junction-related proteins

1. Transmembrane proteins
Claudin family (cldn-1, ~27)
TAMPs (occludin, tricellulin, MarvelD3)
Immunoglobulin superfamily (JAM family, ESAM, CAR)
LSR(lypolysis-stimulated lipoprotein receptor)
2. Cytoplasmic proteins
a) PDZ domain-containing proteins
ZO-1, -2, -3, MAGI-1, -2, -3, MUPP-1, PAR-3, PAR-6, PALS-1, PATJ, mDlg, Scrib, afadin
b) Other proteins
Cingulin, Symplekin, heterotrimeric G protein, Rab3b, Rab13, ZONAB, huASH1, GEF-H1, aPKC, PP2A, PTEN, Pilt, CRB3, LYRIC, CASK/LIN-2, Merlin, Angiomotin/JEAP, TAZ/YAP, etc.

superfamily composed of the JAM family, CAR, and ESAM, Bves[10] and LSR (lypolysis-stimulated lipoprotein receptor).[11]

The claudin family, comprised of 27 members, is exclusively responsible for the formation of tight junction strands. Claudins are necessary and sufficient for the formation of tight junctions by homophilic and heterophilic binding to adjacent cells.[12,13] Regarding hereditary diseases, mutations of the claudin-1 gene cause neonatal sclerosing cholangitis with ichthyosis.[14] Those of claudin-14 cause deafness.[15] Some claudins are expected to form extracellular aqueous pores in paracellular spaces.[16,17] Distinct examples are claudin-16/paracellin-1[18] and claudin-19,[19] which are responsible for hereditary hypomagnesemia. Similarly, claudins are considered to form selective channels in tight junctions because of the existence of charged amino acids on the first extracellular loop.[17] Several claudins are expressed in one cell.[1,2] Their regulation is very complicated, as it is both claudin-specific and cell-specific. Claudin-1, -6 and -9 are reported to be required for the entry of hepatitis C virus (HCV) into hepatocytes after binding of HCV to CD81.[20] Claudins have been reported to recruit and promote the activation of pro-MMP-2.[21,22] Claudin-7, however, is reported to suppress expression of MMP-3.[23] In addition, claudin-7 interacts with Ep-CAM[24] and, in claudin-7-deficient mice, expression of integrin $\alpha 2$ is reduced and its localization altered in the intestinal epithelia.[23] These findings show that some claudins play as yet unknown roles in cellular processes other than tight junction functions.

Occludin was the first integral membrane protein of tight junctions discovered,[25] and can be clearly detected in tight junction strands by immunolabeling freeze-fracture replicas. However, occludin-deficient ES cells have the capability to develop tight junction strands indistinguishable from the normal strands.[26] Nevertheless, the immunohistochemical intensity of occludin in various tissues correlates well with the number of strands.[27] Of the tight junction proteins, occludin is the most ubiquitously expressed at the apicalmost basolateral membranes, and is the most reliable immunohistochemical marker for tight junctions.[28] Compared to claudins, occludin has a relatively long cytoplasmic c-terminus containing several phosphorylation sites and a coiled-coil domain that probably interacts with PKC-ζ, c-Yes, connexin26, and the regulatory subunit of phosphatidylinositol 3-kinase (PI3K) among others,[29] as well as occludin itself, ZO-1 and ZO-3.[1,2] Thus roles of occludin in signal transduction have been proposed (Fig. 2), and its involvement in apoptosis reported.[30,31] It, like claudin, is required for HCV infection.[20] However, the roles of occludin in regulation of tight junctions remain to be clarified.

Tricellulin is a member of the MARVELD3 subfamily. It is concentrated at tricellular contacts and is regulated by the c-JNK pathway in both normal and malignant pancreatic duct cells.[32] The C-terminus of tricellulin is highly similar to that of occludin, and mutations of tricelluilin cause deafness.[33]

JAM (junctional adhesion molecule)-A, JAM-B, JAM-D, CAR (coxsackievirus and adenovirus receptor) and ESAM (endothelial cell-selective adhesion molecule) belong to the immunoglobulin superfamily.[34] All of the members have extracellular V-type and C2-type immunoglobulin domains, a

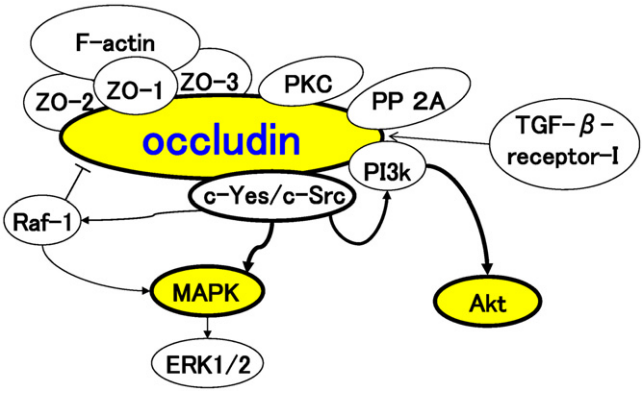

Figure 2 Possible participation of occludin in signal transduction.

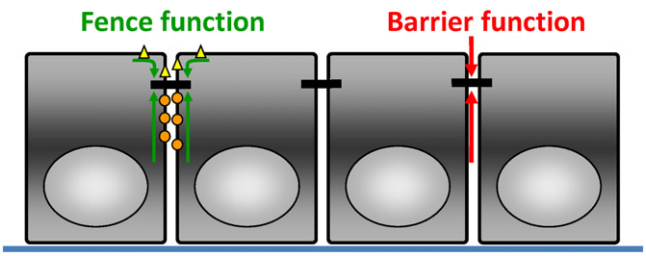

Figure 3 Schematic diagram of the fence and barrier functions of tight junctions. Fence function: tight junctions prevent intermixing of molecules in the apical membrane with those in the lateral membrane. Barrier function: tight junctions regulate diffusion of solutes through paracellular spaces.

single transmembrane region and a cytoplasmic tail. JAM-A lacks the capability to form tight junction strands. It is a ligand of lymphocyte function-associated antigen 1 (LFA-1)[35] and also a receptor for reovirus.[36] This group is considered to play roles in inflammatory reactions, particularly extravasation of inflammatory cells.[37]

Cytoplasmic proteins[1,2,6]

These tight junction proteins can be divided into two groups. One group consists of the PDZ domain-containing proteins ZO-1, ZO-2, ZO-3, ASIP/Par3, Par6, MAGI-1, MAGI-2, MAGI-3, AF-6/afadin, MUPP1 and PATJ. The other group is comprised of cingulin, 7H6 antigen, symplekin, heterotrimeric G proteins, aPKC, ZONAB, huASH1, Rab-3b, rab-13, PTEN, Pilt, angiomotin/JEAP[38] and protein phosphatase 2A.

Of these proteins, ZO-1, ZO-2 and cingulin can bind to actin filaments. ZO-1 and ZO-2 are essential for formation of tight junctions[39] because both interact with all of the integral membrane proteins and the latter interacts with claudins and JAM-A.[22] ZO-2 and ZO-3 also interact with claudins.[21] Mutation of ZO-2 causes hypercholanemia.[13] aPKC and PP 2A may regulate phosphorylation levels of the tight junction proteins to establish cell polarity and/or to regulate tight junction functions. PTEN lipid phosphatase antagonizes PI3K/Akt signaling and binds to MAGI-1 and MAGI-2. Recently, the Hippo pathway-related proteins YAP, TAZ, angiomotin/JEAP and Merlin were reported to localize at tight junctions.[40,41] Since tight junctions are functional multiprotein assemblies, each of the components and the related proteins may affect their functions. In this sense, understanding of life and the functions of each protein of tight junctions is required.

Regarding the biogenesis of tight junctions, in terms of polarization[42] it is speculated that formation of adherens junctions precedes formation of tight junctions. To establish the cell polarity of epithelial cells, in terms of development of the junctional complexes, E-cadherin and/or nectin first bind to the respective molecules on the surfaces of adjacent cells in a homophilic manner. Immediately thereafter, ZO-1 and JAM-A are recruited at spot-like primordial adherens junctions, and then Par3, Par6 and aPKC are recruited to JAM-A, and occludin and claudin are also recruited to ZO-1 complexes. Lastly, by unknown mechanisms probably involving an aPKC/Par complex and delivery of the integral membrane proteins, tight junctions are segregated from adherens junctions. Consistently, during polarization of mouse teratoma cell line F9, HNF-4α plays crucial roles in the formation of tight junctions[43] as well as adherens junctions.[44] Furthermore HNF-4α induces formation of microvilli on the apical surface.[45] These findings show that formation of tight junctions is very closely related to the establishment of cell polarity.

Roles of tight junctions in biology

Tight junctions, the apicalmost component of intercellular junctional complexes, separate the apical from the basolateral cell surface domains to maintain cell polarity (the fence function), and also regulate solute and water flow through the paracellular space (the barrier function). The formation and maintenance of tight junctions require ATP[46] and integrity of the actin cytoskeleton.[47] The barrier function is more deeply dependent on ATP[48] and actin[49] than the fence function. In addition, gap junctional intercellular communication strengthens tight junctions.[50] These properties of tight junctions suggest that the fence and barrier functions of the tight junction have a common feature of compartmentalization; the fence function is performed at the subcellular level and the barrier function is performed at the organ level.

Under physiological conditions, epithelial cells maintain polarity and the functional multicellular sheet of epithelium maintains individual homeostasis via the fence function and the barrier function, respectively (Fig. 3). The fence function segregates growth factors that exist in the apical surface from their receptors on the basolateral cell surface, establishing

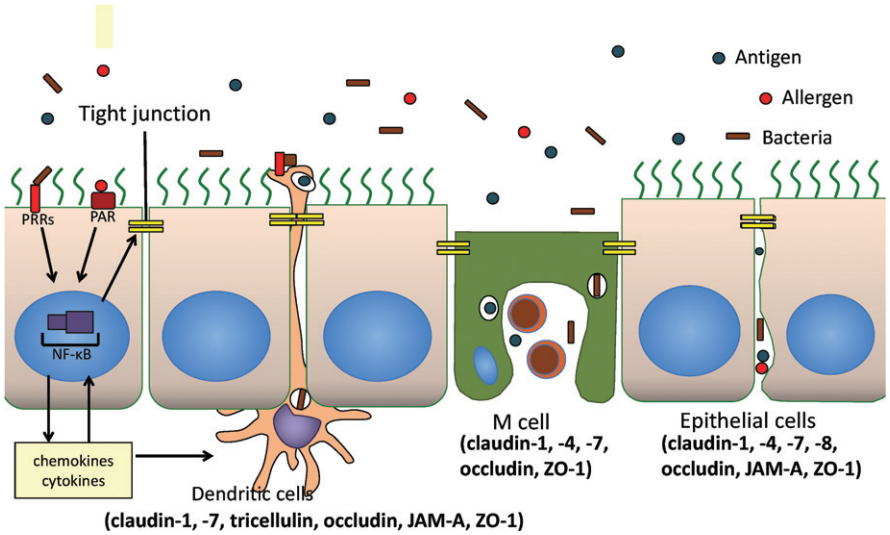

Figure 4 Schematic diagram of involvement of tight junctions in innate immunity. PRRs, pattern recognition receptors; PAR, protease-activated receptor.

cell polarity.[51] On the other hand, the barrier function prevents growth factors localized on the apical side from diffusing into paracellular spaces. Upon dysfunction of tight junctions, growth factors bind to their receptors on basolateral membranes though permeable tight junctions, resulting in quick responses to stimuli. This ligand-receptor segregation by tight junctions may be critical to maintain the physiological condition.[51]

The fence and the barrier functions of tight junctions are well recognized. Recently, two other roles of tight junctions have become clear. One is regulation of signal transduction.[52] Actually, occludin is capable of binding to many signal transduction-related molecules such as TGF-β,[53] and participates in apoptosis.[30] It has become clear that tight junctions tether some of the important molecules of the Hippo pathway, which is a growth suppressive pathway activated by unknown factors. In addition, Merlin (neurofibromatosis type II gene) is a tumor suppressor gene that shuttles between the nucleus and tight junctions.[54] Since the Hippo pathway is considered to be deeply related to contact inhibition, the question of how tight junctions are involved needs to be clarified, as do the stimulants of the pathway.

The other role is participation of tight junctions in the immune system (Fig. 4). This relationship has been investigated in mucosal immunity, though it is not fully clarified. It has been considered that tight junctions are very static components of innate immunity and a physical barrier against allergens, pollutants and bacteria. In other words, tight junctions have been thought to participate in innate immunity as a fort and immune cells are soldiers against various agents. In this context, dendritic cells residing between epithelial cells express occludin, claudin-1 and ZO-1, sending their dendrites outside through paracellular spaces in the colonic epithelium, probably to sample antigens in the intestine.[55] A similar relationship between dendritic cells and epithelial cells has been observed in the nasal mucosa,[56] and the epidermis.[57] These findings imply that dendritic cells may pass through intercellular spaces by using tight junction molecules to sense extrinsic noxious molecules.

In the human nasal mucosal epithelium, a key molecule of allergic rhinitis, thymic stromal lymphopoietin (TSLP), upregulates the functions of tight junctions with an increase of proteins, including claudin-7.[58] TSLP was also reported to upregulate claudin-7 expression in dendritic cells.[59] Regarding Toll-like receptor (TLR) signals, the TLR3 ligand poly (I:C) reduces JAM-A in human nasal epithelial cells.[60] In cutaneous tissue, several types of TLR, including TLR3, enhance tight junction functions in keratinocytes.[61] These observations show that tight junctions may play unknown but distinct roles in regulating innate immunity.

JAM-A and occludin seem to be involved in transendothelial diapedesis of inflammatory cells. JAM-A is a ligand of LFA-1 on the surface of the lymphocyte,[35] Leukocytes derived from JAM-A-null mice fail to pass between endothelial cells.[37,62] Occludin is expressed in activated T lymphocytes to make the diapedesis smooth with minimal effects on the tight junction function.[63] Occludin is also involved in transepithelial migration of neutrophils.[64] Recently, occludin was reported to be required for migration of delta/gamma lymphocytes between epithelial cells.[65] Furthermore, the relationship between claudin-4 and T cell maturation[66] shows involvement of tight junctions and the proteins in establishment of the immune system.

Thus, tight junctions seem to be integrated into the immune system. These findings might result from that fact that tight junction proteins are able to interact between heterotypic cells, i.e. epithelial cells and dendritic cells,[55,56] like a glue and/or a lubricant. In the future, the roles of tight junctions in immunity will be clarified and how the tight junctional barrier

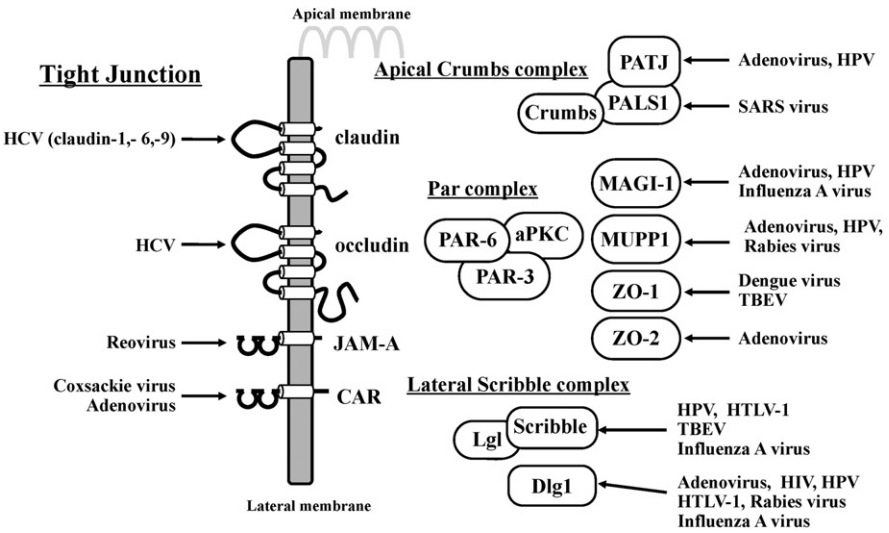

Figure 5 Human viruses and tight junction-related proteins. HCV, hepatitis C virus; HIV, human immunodeficiency virus; HPV, human papillomavirus; HTLV, human lymphotropic virus; SARS, severe acute respiratory syndrome; TBEV, tick-borne encephalitis virus.

is integrated into the immune system, coping with all kinds of agents, should be elucidated.

HUMAN DISEASES RELATED TO TIGHT JUNCTIONS

Infectious diseases

Claudin-3 and -4 are receptors for the enterotoxin of *Clostridium perfringens* (CPE), which is a common cause of food poisoning.[67,68] When CPE binds claudin-4 expressed in MDCK cells, the complexes internalize like other ligand-receptor complexes, and then the function of tight junctions becomes disordered.[69] Very interestingly, this phenomenon is observed only when the basolateral surface of the cell is exposed to CPE.[70] The *Helicobacter pylori* toxin CagA causes an increase in paracellular permeability of intestinal cells by inactivating Par1/MARK kinase.[71] As a result, the barrier function of gastric foveolar epithelium deteriorates and microvilli disappear.[72]

Hemagglutinin/protease (HA/P) produced by *Vibrio cholerae* digests occluding.[73] *Vibrio cholerae* causes severe diarrhea via the combination of cholerea toxin, zonula occludens toxin (ZOT) and HA/P. The second mechanism is a change in actin organization. This organization takes place in a wide variety of cellular conditions, including regulation of tight junctions. In particular, some kinds of bacteria directly affect Rho and myosin light chain kinase activities in tight junction functions.[6] Toxins from *Clostridium diphtheriae*, *Clostridium difficile* and enteropathogenic *Escherichia coli* change Rho activity by modification of amino acids to cause severe colitis, the former two causing psuedomembranous colitis. Enteropathogenic *Escherichia coli* also induces myosin light chain phosphorylation, resulting in diarrhea. ZOT of *V. cholerae* activates PKC, resulting in loss of tight junctions.

In viral infection, occludin and claudins are coreceptors of hepatitis C virus, CAR is a receptor of adenoviruses and Coxsackie viruses and JAM-A is a reovirus receptor.[34] In addition, pathogenic viruses such as those causing influenza target the PDZ domain of cytoplasmic tight junction-related proteins (Fig. 5).[74]

Malabsorption of Ca ions due to vitamin D deficiency

Calcium plays a fundamental role in various physiological functions such as bone mineralization, blood coagulation, neuromuscular transmission and muscle contraction, as well as cell–cell adhesion and intracellular signaling. Ca^{2+} is absorbed in the intestinal mucosa via two distinct routes, the transcellular and paracellular pathways.[75,76] The molecular basis for paracellular Ca^{2+} absorption, which occurs throughout the intestine, is largely unknown. Recent studies have disclosed that claudins are the major determinant of the barrier function of tight junctions. Importantly, the first extracellular loop, in which there is a wide variation in the position and number of charged amino acids depending on each claudin, is known to create paracellular pores (channels) for cations or anions between neighboring cells.[13] The expression of putative cation-permissive claudin-2, -7, -12 and -15 in the intestine of vitamin D receptor-deficient mice was compared to that of wild mice,[77] clearly showing a decrease of claudin-2 and -12. This was confirmed by approaches using RNAi and overexpression in the vitamin D-responsive intestinal cell line Caco-2.[78] These two claudins are essential for Ca^{2+} absorption between intestinal epithelial cells, providing a novel mechanism underlying vitamin D-dependent intestinal Ca^{2+} transport. Thus, vitamin D deficiency rickets affects Ca^{2+} absorption in the intestine via the paracellular pathway as well as the transcellular pathway.

Diabetic retinopathy

Tight junctions of endothelial sheets in vivo are leaky in general, because a wide variety of substances must be exchanged between the blood and organs through paracellular pathways as well as transcellular pathways of the sheets. In certain organs such as the brain and retina, however, endothelial cells possessing well-developed tight junctions form a blood-brain barrier (BBB),[79] Interestingly, the endothelial cells forming the blood-tissue barrier in the brain,[80,81] retina[82] and testis[83] express receptors for glial cell line-derived neurotrophic factor (GDNF), which is secreted from astrocytes.[84] In diabetic retinopathy, the blood-retinal barrier (BRB) is leaky due to VEGF.[85] Glyceraldehyde-conjugated advanced glycation end-products (AGEs) induce VEGF, making tight junctions of the BBB-forming endothelial cells highly permeable and also decease GDNF, making the BBB less permeable in astrocytes.[86] Inversely, retinoic acid and RAR-α show opposite effects on astrocytes. In the early stage of diabetic retinopathy, VEGF makes the BRB highly permeable. RAR-α reduces VEGF expression in astrocytes, resulting in a marked decrease in the leakage of dye from the BRB.[87] It is very clear that dysfunction of tight junctions of the BRB initially causes diabetic retinopathy. However, these results suggest that it is possible to indirectly treat diabetic retinopathy using a paracrine mechanism. In this case, astrocytes may be the most suitable target for treatment in the early phase of diabetic retinopathy in terms of maintaining the impermeability of the BRB.

The above-mentioned conditions are examples of human diseases related to the barrier function of tight junctions. Since tight junctions are located at the apicalmost areas of basolateral spaces between epithelial, endothelial or mesothelial cells, they are involved in a wide variety of pathological conditions where the physiological regulation of the passage of ions, molecules, and inflammatory cells may be affected. In Table 2, human diseases involving dysfunction of tight junctions are listed. Inflammation is always accompanied by an increase in vascular permeability, in part caused by VEGF, which primarily affects the barrier function of tight junctions via both phosphorylation and down-regulation of occluding.[95,96] Cancer cells also secrete VEGF to induce angiogenesis, presumably to intravasate and extravasate, in other words, to metastasize. Thus, various diseases in which VEGF production is involved may cause tight junction dysfunction. To a greater or lesser degree, many cytokines affect the functions of tight junctions under pathological conditions.

Table 2 Human diseases related to tight junctions

I. Disturbance of the barrier fynction
1. Hereditary diseases
Hypomagnesemia
Deafness
Neonatal sclerosing cholangitis with ichthyosis
Familial hypercholanemia
2. Vascular system[37]
Edema
Endotoxinemia
Cytokinemia
Diabetic retinopathy
Multiple sclerosis
Blood-borne metastasis
3. Gastrointestinal tract[88,89]
Bacterial gastritis
Pseudomembranous colitis
Crohn's disease
Ulcerative colitis
Celiac disease
Collagenous Colitis
Malabsorption of Ca ions in vitamin D deficiency
4. Liver[90]
Jaundice
Primary biliary cirrhosis
Primary sclerosing cholangitis
5. Respiratory tract[91,92]
Asthma
Adult (or acute) respiratory distress syndrome (ARDS)
Nasal allergy
6. Cutaneous tissue[93]
Atopic dermatitis
7. Bacterial infection[94]
Vibrio cholerae, Helicobacter pylori,
Clostridium perfringens, Clostridium diphtheria,
Clostridium difficile, enteropathogenic Escherichia coli
8. Viral infections:[74]
Reovirus, adenovirus, coxsackievirus, rotavirus. HIV,
Hepatitis C virus, RS virus etc.
II. Disturbance of the fence function
Cancer cells
Oncogenic papillomavirus infection

Neoplasms

First of all, discussion of the relationship between tight junctions and human cancer must be divided into two aspects. One is changes in tight junctions as a cellular apparatus and the other is changes in a certain components of tight junctions, such as claudin.

Changes in tight junctions

In general, cancer cells lose their specific functions and polarity with a decrease in the development of tight junctions. In well-differentiated adenocarcinomas derived from human colon and endometrium, comparable amounts of occludin are detected, and with further dedifferentiation cancer cells lose tight junctions.[97,98] These findings show that cancer cells irreversibly and progressively lose tight junctions with dedifferentiation by means of genetic and epigenetic changes (Fig. 6).

On the other hand, once malignant transformation occurs, the degree of differentiation in cancer cells can reversibly

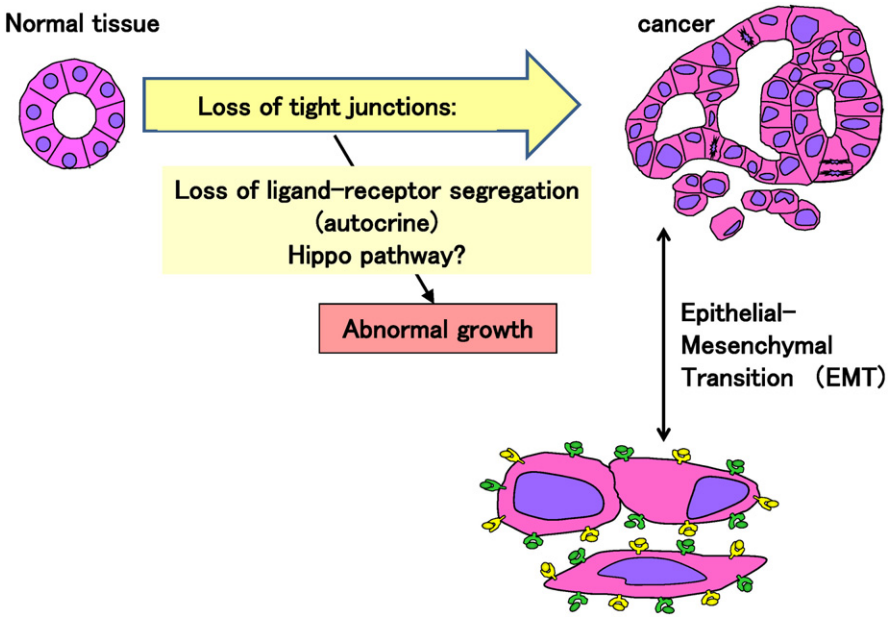

Figure 6 Schematic diagram of changes of tight junctions during carcinogenesis. EMT, epithelial–mesenchymal transition.

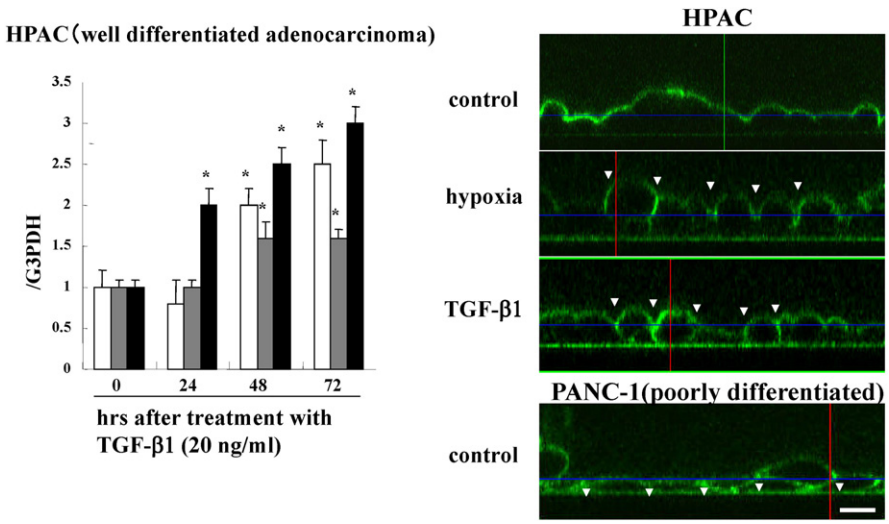

Figure 7 Changes of tight junction fence function during epithelial–mesenchymal transition of well-differentiated adenocarcinoma of the pancreas induced by TGF-β. (**a**) TGF-β-induced expression of Snail, Slug and SIP1. (**b**) Visualization of the cell membranes. TGF-β and hypoxia reversibly cause the fence function of well-differentiated adenocarcinoma (HPAC) to deteriorate, resembling that of poorly differentiated adenocarcinoma (PANC-1). In well-differentiated adenocarcinoma, tight junctions prevent basolateral membranes from being labeled by BODIPY-sphingomyelin without the stimulation. Arrow heads show cell-cell contacts. □, Snail; ▒, Slug; ■, SIP1.

change in terms of structural atypia. This reversible change is called the epithelial-mesenchymal transition (EMT).[99,100] The EMT is essential for the development of the body; i.e. normal cells often migrate during embryogenesis. Regarding cancer cells, however, EMT is deeply associated with the malignant properties of invasion and metastasis. In the process of invasion and metastasis, cancer cells detach from cell nests and change from epithelial to mesenchymal shape. Epithelial-mesenchymal transition is accompanied by loss of occludin and claudins as well as E-cadherin. The genes of these proteins contain an E-box in their promoter.[101]

The loss of tight junctions is visualized by the fence function. As shown in Fig. 7,[102] well-differentiated adenocarcoma cells of the pancreas still possessing the fence function phenotypically change into poorly differentiated adenocarcinoma and lose the function with EMT-inducing treatments such as TGF-β-treatment and hypoxia. Both treatments significantly induce Slug, SIP1 and Snail in the well-differentiated adenocarcoma cells,[102] These transcriptional factors bind to E-box in the promoter of E-cadherin and tight junction protein genes, probably resulting in undergoing EMT. This change is reversible, indicating that cancer cells undergo EMT and MET, presumably induced by cytokines such as TGF-β, not genetic or epigenetic changes.

Release from growth suppression includes two mechanisms. One is loss of the functions of tight junctions, which lose ligand-receptor segregation.[51] When the barrier function is lost, growth factors that normally exist in the apical mucus can bind to its receptors normally located on the basolateral surface of the cells. As a result, cell proliferation occurs in an

Table 3 Tissue expression of claudins in various cancers

	Claudin									
	1	2	3	4	5	7	10	16	18	23
Breast	↑↓		↑↓	↑↓		↓				
Lung										
Adenocarcinoma	↓				↑					
SCC	↑				↓					
Esophagus (SCC)	↑↓		↑	↑		↑↓				
Stomach	↑		↑	↑↓		↑			↑↓	↓
Colon, rectum	↑↓	↑	↑	↑↓	↑	↑↓				
Hepatocellular carcinoma	↑↓	↑		↓		↑↓	↑			
Biliary duct				↑					↑	
Pancreas duct			↑	↑	↑				↑	
Bladder				↑↓						
Kidney	↑		↑	↑		↑				
Prostate	↑		↑	↑	↓	↑				
Ovary (epithelial tumor)	↓		↓	↑	↑	↑		↑		
Uterus, cervix	↓	↓		↓		↓				
Uterus, body			↑	↑		↓				

SCC, squamous cell carcinoma; ↑, increase; ↓, decrease; ↑↓, variable.

autocrine manner.[51] The other feasible mechanism is related to signal transduction. Tight junctions work as docks of cell cycle-regulating molecules[52] and as a possible target of the Hippo pathway suppressive of cell proliferation and crucial for organ size regulation.[40] Tight junctions tether Hippo signal-related molecules such as TAZ/YAP, and tumor suppressor gene Merlin (neurofibromatosis type 2) binds to the tight junction protein PATJ.[41]

Changes of expression of claudin family

Changes of expression of claudins are summarized in Table 3.[4,103] Expression of some claudin family members is significantly altered by epigenetic regulation in human cancer,[104–106] though tight junctions deteriorate in cancer. At present, we cannot draw any conclusion about the relationship between claudin expression and human cancer because the expression occurs in a cell type-specific fashion. In addition, like claudin-7,[23] claudin-3 and -8 are representatively detected on lateral membranes as well as in tight junction regions. These claudins localized on the basolateral surface might have unknown roles in cell functions in addition to forming tight junctions. Further characterization of each member of the claudin family and immunostaining panels for various human cancers are required to elucidate why claudin expression is changed regardless of tight junction formation,[5]

Are tight junctions really suppressive of tumorigenesis? Possibly, yes. However, the above-mentioned findings show that loss of tight junctions in cancer cells is a secondary and/or late event of carcinogenesis, though tight junctions are considered to be deeply involved in tumorigenesis and invasion. On the other hand, tight junctions of endothelial and mesothelial cells of the host possibly act as a barrier against disordered migration leading to metastasis and dissemination of cancer cells.[107–108]

PROMISING APPROACHES TO BEDSIDE MEDICINE

Grossly, there are three prospective areas of application: drug delivery systems (DDSs), markers for cancer and target molecules for therapy. Regarding DDSs, development of drugs to open tight junctions will help to treat brain tumors by drugs,[109] and to administer biologically active peptides.[110,111] To invent new drug delivery techniques, we should follow the strategies of pathogenic agents such as allergens and viruses. In contrast to opening of tight junctions, it is theoretically possible that closing tight junctions could result in selective uptake of materials via a transcellular pathway like the BBB. Thus, it is also feasible that dendritic cells or M cells could be solely stimulated by closing tight junctions of surrounding epithelial cells,[55,112] possibly resulting in a new method of vaccination.

As a marker for early stages of malignant lesions in the bile duct[113] and pancreas,[114] claudin-18 is promising. It is also expected to be a target molecule for cancer therapy.[106] Exosomes derived from the blood of patients with ovarian cancer contain claudi-4.[115] However, further study to complete the profile of claudin expression in human cancer is required.

Regarding cancer therapy, CPE seems to be effective for treatment of cancer expressing claudin-4.[116] Thus, this claudin is highlighted as a target molecule for cancer therapy. Since normal prostate epithelial cells and pancreas duct epithelial cells are highly insensitive to CPE,[70,117] CPE-modified materials are expected to be new drugs for advanced or hormone-resistant carcinoma as well as a tight junction opener.[118,119]

Development of agents making tight junctions of endothelial cells close might also be highly useful as new anti-inflammatory drugs and anti-metastatic drugs. Such agents, in particular, are very promising for prevention of diabetic

retinopathy, the most common cause of losing sight. In the future, much better understanding of the molecular mechanisms of regulation of the functions will be required to apply the cell biology of tight junctions to medicine and to make tight junctions open or close with complete control.

CONCLUDING REMARKS

Fifteen years ago, no one could have imagined the recent advances in tight junction research. Now we are struggling to apply the outcome of the research to clinical medicine. The surprising advances are considerably due to the outstanding contributions of Dr Shoichiro Tsukita and his colleagues who discovered occludin and tricellulin, as well as the claudin family. He proposed the field of Barriology, based on the barrier function of tight junctions. Until recently, research on tight junctions was exclusively on the fence and barrier functions at the cellular level. Now it is becoming clear that tight junctions are deeply involved in immunity. However, the study of this issue is still in the early stage. With advances in this area, tight junction research may contribute to the development of drugs, vaccination and drug delivery systems. Further extensive study is required to clarify the issues concerning tight junction functions.[5] On the other hand, each of the tight junction proteins, in particular each of the claudins, may play distinct roles in cell biology in addition to the formation of tight junctions. This issue should be also clarified.

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