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The Organization of Tight Junctions in Epithelia: Implications for Mammary Gland Biology and Breast Tumorigenesis

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Abstract

Tight junctions (TJs), the most apical components of the cell–cell junctional complexes, play a crucial role in the establishment and maintenance of cell polarity within tissues. In secretory glandular tissues, such as the mammary gland, TJs are crucial for separating apical and basolateral domains. TJs also create the variable barrier regulating paracellular movement of molecules through epithelial sheets, thereby maintaining tissue homeostasis. Recent advances reveal that TJs exist as macromolecular complexes comprised of several types of membrane proteins, cytoskeletal proteins, and signaling molecules. Many of these components are regulated during mammary gland development and pregnancy cycles, and several have received much attention as possible “tumor suppressors” during progression to breast cancer.

Keywords

tight junctions; barrier; permeability; fence; polarity; PDZ domain; mammary gland

INTRODUCTION

Cell–cell interactions, as well as cell–ECM interactions, are indispensable for normal tissue architecture. In epithelial tissue, cell–cell interactions are mediated by junctional complexes that consist of tight junctions (TJs), adherens junctions (AJs), desmosomes, and gap junctions, each of which possesses unique morphological characteristics, composition, and functions (1). TJs directly contribute to the maintenance of cell surface polarity by forming a barrier “fence” that prevents diffusion of lipids and proteins between apical and basolateral domains in the plasma membrane (2). TJs also regulate transport of small molecules between cells and therefore allow epithelia to form a cellular barrier that separates compartments of different composition (3,4). This intercellular barrier formed by TJs has selectivity in molecular size and ion type as well as differences in permeability depending on the cell type and condition. TJs in the mammary gland have been mainly investigated in relation to lactogenesis. Many studies have shown that the structure and function of TJs are dynamically affected by lactogenic hormones, such as prolactin and glucocorticoids, as well as by progesterone. The integrity of TJs is crucial for milk secretion. During lactation, TJs in the alveoli epithelia are highly impermeable to avoid leakage of milk from the lumen, although they are leaky in the mammary epithelia of the pregnant animal.

In this review, we will first summarize structure, function, and molecular organization of TJs, then introduce the implications of this information for the mammary gland and breast cancer.

STRUCTURE OF TIGHT JUNCTIONS

TJs possess a characteristic structure, which is evident when ultrathin sectioned samples are analyzed by electron microscopy. Under these conditions, TJs are viewed as a series of fusion points between the outer leaflets of the membrane of adjacent cells (Fig. 1(A)). At these points (called “kissing” points), the intercellular space is eliminated completely. Using freeze-fracture replica electron microscopy (Fig. 1(B)), TJs appear as a network of continuous and branched intramembranous particles of fibrillary strands (TJ strands) on the protoplasmic face of the plasma membrane with complementary vacant grooves on the exoplasmic face (5). The number of TJ strands, as well as the frequency of their bifurcation, varies greatly with the cell type, producing marked variation in the morphology of TJ strand networks. Each TJ strand within the plasma membrane associates laterally with another TJ strand in the apposing membrane of adjacent cells and form paired strands of TJs, where the intercellular space is eliminated (2).

FUNCTIONS OF TIGHT JUNCTIONS

The Barrier Function and Tissue Homeostasis

The barrier function of TJs is necessary for maintaining homeostasis in various tissues. In intestinal tissues, barrier function regulates transport at the mucosal interface with the external environment. In vivo, paracellular fluxes through the epithelial sheets are strictly controlled by TJs, adapting permeability to physiological needs. Intestinal barrier function also aids in host defense mechanisms (6). Many pathogens, including bacteria and viruses, disrupt TJs during infections. Recently, it was shown that several pathogens bind and modify TJ components directly or exert their effect through the actin cytoskeleton, which associates with the cytoplasmic surface of TJs. For example, the fiber protein of reovirus binds to Junctional Adhesion Molecule (JAM, see below), an integral membrane component of TJs, allowing infection of the host cells and activation of NF- κ B leading to apoptosis (7). *Helicobacter pylori* CagA protein associates with the scaffolding protein ZO-1, causing an ectopic assembly of TJ components at sites of bacterial attachment and altering the composition and function of the apical junctional complex (8). Longterm CagA delivery to polarized epithelia causes a disruption of the epithelial barrier function and dysplastic alterations in epithelial cell morphology.

In several tissues, such as brain and testis, barrier function is highly developed. The blood–brain barrier protects the brain from various harmful materials circulating in the blood by minimizing the entry of molecules into brain tissue (9). The blood–testis barrier in the testis creates a specialized microenvironment for germ cell development because developing germ cells do not have access to the systemic circulation (10). Although these well-developed barriers help tissues maintain homeostasis, they in turn prevent efficient delivery of therapeutic drugs, especially from entering into the central nervous system. Thus, research into the development of approaches to modulate barrier function for efficient drug delivery has received much attention (11).

Fence Function and Cell Polarity

TJs have been shown to function as an intramembranous diffusion fence that restricts the intermixing of lipid and protein components of apical and basolateral membrane domains (2, 12); in doing so, TJs are supposed to be critically involved in the regulation of cell polarity. Although the structural and molecular basis of the intramembranous diffusion fence still remains elusive, recent studies with *Drosophila* mutants, which have defects in epithelial polarity, support the idea that TJ components play major roles in the establishment of epithelial polarity (13,14). In *Drosophila*, three molecular complexes have been identified to regulate cell polarity. The first complex consists of Bazooka (Baz), Dm-Par6, and *Drosophila* atypical

PKC (DaPKC). The integral membrane protein Crumbs (Crb) and its associated proteins, Stardust (Sdt) and Discs Lost (Dlt), form the second complex. The third complex is comprised from Scribble (Scrib), Discs Large (Dlg), and Lethal Giant Larvae (Lgl). In *Drosophila*, embryonal epithelia lacking the expression of Baz, Dm-Par6, or DaPKC exhibit a disruption of apicobasal polarity. Mutants that have lost the expression of Crb, Sdt, or Dlt exhibit a similar phenotype. Overexpression of Crb leads to apical surface expansion and multilayering of epithelia; therefore, Crb appears to function as an apical determinant in *Drosophila* epithelia. The homologues of these three polarity genes are present in vertebrates, and two of them, Baz/Dm-Par6/DaPKC and Crb/Sdt/Dlt, are localized to the TJ region in epithelia. There are a number of each of the vertebrate homologues of *Drosophila* polarity determinants, and each is subdivided into protein families, indicating that the molecular mechanisms that regulate cell polarity are more complex in higher organisms. Further analyses, including mouse knockout studies, will improve our understanding of how TJs are involved in the establishment and maintenance of cell polarity.

Transcriptional Regulation and Cell Proliferation/Differentiation

Several TJ components have been shown to play a tentative role in the regulation of gene expression. Those proteins translocate from TJs to the nucleus and bind to specific transcriptional factors or function as transcriptional factors themselves. Human ASH1 (huASH1) was identified as a vertebrate homologue of *Drosophila* Ash1, a transcriptional factor that is involved in homeotic gene expression (15). huASH1 localizes at both TJs and the nucleus; thus it is speculated that huASH1 transduces adhesion-mediated signaling through TJs to the nucleus. The ZO-1-associated nucleic-acid binding (ZONAB) protein contains the consensus sequence of a Y-BOX-type transcriptional factor (16,17). In cells cultured at high confluence, ZONAB is localized to the TJ region. On the other hand, ZONAB is concentrated in the nucleus in cells cultured at low confluence. ZONAB associates with the promoter region of ErbB2 and CDK4, both of which regulate cell proliferation.

MOLECULAR ORGANIZATION OF TIGHT JUNCTIONS

In recent years, a growing number of proteins has been identified as TJ components. These can be generally categorized into following groups: 1) integral membrane proteins that constitute the TJ strands, 2) peripherally associated cytoplasmic proteins that organize the integral membrane proteins and connect them to actin filaments and/or to other cytoplasmic proteins, and 3) signaling proteins that may be involved in junction assembly, barrier regulation, and gene transcription.

Integral Membrane Proteins

Three types of integral membrane proteins are well-characterized as components of TJs (Fig. 2a).

Occludin—Occludin (~60 kDa) is the first molecule identified as an integral membrane protein localized at TJs (18). Occludin has four transmembrane domains, a long carboxy-terminal cytoplasmic domain and a short amino-terminal cytoplasmic domain. Two extracellular loops are similar in size and are enriched in tyrosine residues. Unusually, more than half of the residues in the first loop are comprised of tyrosines and glycines. Since antibodies against occludin exclusively labeled TJ strands in freeze fracture immunoreplica electron microscopy, occludin is thought to be directly incorporated into TJ strands. The expression level of occludin is correlated with the number of TJ strands in various tissues. These studies indicate that occludin is an essential component of TJ strands. A synthetic peptide corresponding to the second extracellular domain of occludin significantly reduces the expression level of occludin and alters TJ barrier function in cultured epithelial cells (19). This

effect is not a result of cell toxicity or of a general breakdown of cell–cell contacts because cell morphology is normal and localization of junctional components other than occludin is not altered. Surprisingly, however, epithelial cells in occludin knockout mice do not show any structural defect of TJ strands (20). These mice do show an abnormal phenotype in several tissues, but the physiological basis of the abnormality is still unclear.

Claudins—The presence of TJ strands in occludin knockout cells suggested the existence of another integral membrane protein responsible for the formation of TJ strands. With the analysis of membrane fractions isolated from liver, two integral membrane proteins, claudin-1 and -2, were identified (21). A subsequent database search has revealed the existence of 24 members of the claudin family (3). All claudins encode 20–27 kDa proteins with four transmembrane domains, although these did not show any sequence similarity to occludin. Claudins have two extracellular loops, with one significantly longer than the second, and a short carboxyl intracellular tail. The last amino acids of this tail are highly conserved within the family and constitute PDZ binding motifs (see below).

There is accumulating evidence that claudins constitute the backbone of TJ strands. Immunoreplica electron microscopy revealed that claudins are exclusively localized on TJ strands. Exogenously expressed claudins conferred cell-aggregation activity to fibroblast cells and produced the formation of a large network of structures that looked like TJ strands concentrated at cell–cell contact sites (22). Occludin by itself cannot reconstitute such well-organized strands, but when it is co-introduced into fibroblast cells with claudin, it becomes incorporated into reconstituted claudin-based strands.

The expression pattern of claudins varies considerably among tissues and even within a single tissue. For example, in the kidney at least 12 distinct claudin genes are expressed (23). The kidney is comprised of nephrons, which have several structurally and functionally distinctive segments. It is known that the permeability of TJs varies significantly in each segment. The expression pattern of claudins exhibits a complicated segment-specific pattern, which might be responsible for the differential permeability of TJs in each segment (23). Claudins-2, -10, and -11 are expressed in the proximal segment, while claudins-3 and -8 are expressed in the distal tubule. In the thick ascending limb of Henle, four different claudins, claudins-3, -10, -11, and -16, are expressed. Interestingly, patients with hypomagnesemia syndrome, who manifest a selective defect in paracellular Mg^{2+} re-absorption in the thick ascending limb of Henle, have a mutation in claudin-16 gene (24).

Mutations in claudin are found in another human hereditary disease. Patients who suffer from autosomal recessive deafness, DFNB29, have mutations in the claudin-14 gene, which is expressed in the organ of Corti at the inner ear (25). TJs in the cochlea are thought to play crucial roles in the compartmentalization within the inner ear and provide structural support for the auditory neuroepithelium. Thus, mutations in claudin-14 likely impair the maintenance of the electrochemical gradient between the different compartments of the inner ear.

Several studies with claudin knockouts in mice have provided further evidence that they play an important role in physiological processes in vivo in a tissue specific manner. Claudin-5 is specifically expressed in endothelial cells, especially in high amounts in the brain endothelial cells which form the blood–brain barrier (26). Although claudin-5 knockout in endothelial cells of mice did not result in a general breakdown of TJs, paracellular permeability of small molecules (less than ~800 Da) was increased in a selective manner (27). TJs have been considered attractive targets for transient breakdown of the blood–brain barrier in therapies for various disorders of the central nervous system, and this result indicates that the blood–brain barrier can be manipulated to allow selective diffusion of small molecules by modulating claudin-5 expression in endothelial cells. Claudin-11 is localized at sertoli cell junctions and

myelin sheaths in the central nervous system. In claudin-11 knockout mice, TJ strands in myelin and between Sertoli cells are not observed with freeze fracture replica electron microscopy (28). Male claudin-11 knockout mice are sterile, their seminiferous tubules exhibit aberrant morphology and no spermatozoa are observed. This abnormality is presumably because of the disruption of the blood–testis barrier, which in turn inhibits the differentiation of early spermatocytes and eventually leads to cell death.

Genetic ablation of claudin-1 in mice induces neonatal death within 24 h after birth, although neither the morphology of keratinocyte TJs, the distribution of other TJ components, nor the organization of the epidermis in the layers is changed (29). Since the phenotype of null mice is associated with the rapid appearance of wrinkles in the skin, significant body dehydration might have occurred. The fact that claudin-1 could regulate permeability of water in the skin suggests TJs are critical regulators of permeability of stratified epithelia as well as of simple epithelia.

Diamond *et al.* hypothesized that different types of paracellular channels exist in TJs, on the basis of their findings measuring electrophysiological epithelial permeability (30). Decades later, their hypothesis has been validated by *in vivo* and *in vitro* data described above indicating that claudins are paracellular channels which have selectivity for specific ions and play central roles in regulation in paracellular permeability. The diversity of the claudin expression pattern contributes to achieving and maintaining appropriate physiological homeostasis in response to each tissue's need.

Junctional Adhesion Molecule—Junctional adhesion molecule (JAM) is a ~40 kDa glycosylated protein localized at TJs in epithelial and endothelial cells (31). Five JAM-related proteins are reported so far. They have an extracellular region that contains two variable type Ig-like domains, a single transmembrane domain, and a short cytoplasmic domain. JAM-1, which is a ubiquitously expressed form in epithelia and endothelia, interacts with the PDZ domains of PAR-3 and ZO-1 through its carboxyl terminal PDZ binding motif (32,33). In freeze-fracture replica electron microscopy, JAM-1 shows an intimate spatial relationship with TJ strands. In fibroblasts, expression of exogenous JAM-1 leads to its concentration at cell–cell contact sites; however, TJ strand-like structures are not reconstituted. Instead, characteristic intramembrane-particle-free domains, which are occupied by laterally aggregated JAM-1 molecules, are generated. At the ultrastructural level, a freeze-fracture immuno-replica analysis with anti-JAM-1 antibody shows that JAM-1 is distributed on and around TJ strands. These data lead to a model in which JAM-1 in epithelial cells associates laterally as oligomers; these in turn aggregate with TJ strands composed of linear polymers of claudin and occludin.

Interestingly, intramembrane-particle-free domains are observed in the initial step of TJ assembly *in vivo*, and JAM-1 begins to concentrate at cell–cell contact sites earlier than occludin and claudin (34). Therefore, JAM-1 probably functions as the initial spatial cue for TJ formation, thereby restricting the free diffusion of proteins within the plasma membrane.

Several other integral membrane proteins, CAR, P0, OAP-1, have been reported to localize at TJs, although their functions have not been analyzed in detail (35–37).

Cytoplasmic Proteins

About 30 molecules have been identified as cytoplasmic components of TJs so far. These comprise mainly PDZ domain-containing proteins and non-PDZ proteins.

PDZ Domain-Containing Proteins—PDZ domain-containing proteins are considered to play major roles in the coordination of the assembly of large macromolecular complexes,

clustering TJ membrane proteins, and recruiting signaling proteins involved in cytoskeletal organization and gene expression.

Zonula Occludens (ZO-1, ZO-2, and ZO-3): Zonula occludens-1 (ZO-1), a peripheral membrane protein with a molecular mass of 220 kDa, was the first TJ component to be identified (38). When ZO-1 is immunoprecipitated from epithelial cell lysates, two proteins with molecular masses of 160 and 130 kDa are co-precipitated. Using specific antibodies, these proteins were shown to specifically localize at TJs and designated as ZO-2 and ZO-3, respectively (39). ZO-1, ZO-2, and ZO-3 have sequence similarities: each containing three PDZ domains (PDZ1, PDZ2, and PDZ3), one SH3 domain, and one guanylate kinase-like (GUK) domain (40,41). ZO-1 has homology with the tumor suppressor molecule *discs large* (Dlg) of *Drosophila* and with the postsynaptic density protein PSD95/SAP90 (42,43). The homologous domains of 80–100 amino acids among these three proteins are named “PDZ domains” (PSD95/DLG/ZO-1) (44). It is now well recognized that PDZ domains provide protein–protein interactions and exist in more than 100 proteins (45). Initially, PDZ domains were revealed to bind specifically to Ser/Thr–X–Val, a motif at the carboxyl-terminus of several channel proteins. Subsequent studies have shown that PDZ domains recognize more diverse 3–4 amino-acid sequences, most of which end in Val or Leu (46). Since PDZ domains are crucial in clustering and anchoring of transmembrane proteins, multiple PDZ domain-containing molecules function as scaffolds that bring together integral membrane, cytoskeletal, and signaling proteins at specific regions of the plasma membrane. In TJs, ZO-1, ZO-2, and ZO-3 recruit claudins and JAM by direct binding to their carboxyl terminal PDZ binding motif through their PDZ1 and PDZ3 domains, respectively (47; see Fig. 2b). Although occludin does not contain a PDZ binding motif, ZO-1, ZO-2, and ZO-3 directly bind to occludin at its carboxy-terminal tail via their GUK domains (48,49). The PDZ domain can mediate interactions with other PDZ domain-containing proteins, allowing the formation of a heterodimer comprised of two distinct PDZ proteins. In fact, ZO-2 and ZO-3 independently associate with ZO-1 through a PDZ-2/PDZ-2 interaction (50). ZO-1, ZO-2, and ZO-3 have the ability to assemble all integral membrane protein components of TJs as well as to bind directly to actin filaments; thus they probably play a crucial role in regulating the spatial organization of TJs and polarity in epithelia (51). Overexpression studies using mutant forms of ZO-1 or ZO-3 support this hypothesis. When deletion mutants of either ZO-1 or ZO-3 are overexpressed in cultured epithelial cells, barrier function and assembly of TJ components are impaired (52). Furthermore, transfection of ZO-1 mutants can influence morphology and gene expression in epithelial cells (17).

PAR: PAR (partitioning-defective) molecules were originally identified as essential genes for embryonic development in *Caenorhabditis elegans* (53). For example, mutations in PAR-3, which possesses three PDZ domains, alter the polarized distribution of other proteins involved in cell fate determination as well as the orientation of the mitotic spindles in successive cell cycles (54). *Drosophila* homologues of PAR proteins are crucial for the establishment of cell polarity and the formation of cell–cell junctions (see above) (55). The mammalian homologue of PAR-3 was isolated as a binding protein of aPKC (56). In mammalian epithelial cells, PAR-3 localizes at TJs through direct binding to the carboxyl terminus of JAM-1 via its first PDZ domain. PAR-3 forms a complex with another PAR family protein PAR-6, a single PDZ domain-containing molecule also shown to associate with aPKC and activated Cdc-42/Rac (57,58). Overexpression of PAR-3 or PAR-6 deletion mutants inhibits the initial formation of TJs, but does not disrupt established TJs. Expression of a dominant negative form of aPKC causes mislocalization of PAR-3 and cell surface polarity impairments, suggesting a role for the PAR protein complex in the apico-basal polarization of vertebrate epithelial cells.

Pals1, PATJ, MUPP1: The vertebrate homologues of the *Drosophila* polarity complex Crb/Std/Dlt were identified recently. Pals1, a homologue of Std, contains a single PDZ domain, SH3 domain, and GUK domain. Through its N-terminal region, Pals1 associates with PATJ

and MUPP1, both homologues of Dlt. PATJ and MUPP1 contain 10 and 13 PDZ domains, respectively (59,60). These proteins are thus thought to assist in the formation of macromolecular complexes at TJs. A recent study revealed that a ternary complex comprised of Crb, Pals1, and PATJ/MUPP1 is linked to TJs by interaction with ZO-3 in vertebrate epithelia.

MAGI: MAGI (MAGUK inverted) proteins contain six PDZ domains and a single GUK domain. Immunofluorescence studies have shown that these proteins localize at TJs; however, there is no information about their interaction with TJ components so far. MAGI-2 and MAGI-3 associate with PTEN through their second PDZ domains (61,62). PTEN is a tumor suppressor gene which is mutated in various types of cancer, but whether there is a connection between its tumor suppressor function and its binding ability to MAGI at TJs remains to be determined.

Non-PDZ Proteins—Other cytoplasmic components of TJs which do not possess PDZ domains appear to play a variety of roles, such as regulating vesicular traffic and contractility of actin cytoskeleton. However, the precise function of many of these components is still unknown (Table I).

Signaling Proteins

Several signaling molecules which regulate the assembly and/or disassembly of TJs have been discovered, mostly from pharmacological studies with specific activators and inhibitors.

Protein Kinase A—Elevation in intracellular cAMP, which activates protein kinase A (PKA), has been shown to promote epithelial barrier function. In addition, treatment of cells with the specific PKA inhibitor, H-89, attenuates the recovery of barrier function after a Ca^{2+} switch assay that is used to modify the assembly and disassembly of cell–cell contacts by changing extracellular Ca^{2+} concentration (72). Although several studies indicate that the reorganization of the cytoskeleton mediates PKA's action on TJs, detailed molecular mechanisms and physiological relevance remain to be elucidated.

Protein Kinase C—Phorbol esters and diacylglycerol, potent activators of classical protein kinase C (cPKC) and novel PKC (nPKC), modulate TJ assembly in cultured epithelia. Phorbol esters have been shown to induce the disassembly of TJs in many cell types (73). Several PKC inhibitors affect both assembly and disassembly of TJs; therefore PKCs may regulate the dynamics of TJ formation. Although it is not clear how cPKC and nPKC are involved in regulation of TJs, recent work has shown that aPKC, which is not stimulated by phorbol esters nor diacylglycerol, plays a crucial role in TJ regulation. aPKC directly associates with the polarity proteins PAR-3 and PAR-6 (see above). Overexpression of a dominant-negative mutant of aPKC impairs the TJ barrier and polarity as well as preventing the accumulation of TJ components at cell–cell contact sites. Since this effect is observed only before TJ formation is completed, kinase activity of aPKC is probably required during the dynamic establishment of the junctional structures and epithelial cell polarity (70).

Heterotrimeric G-Proteins—Treatment of cells with AlF_4 , a known activator of heterotrimeric G-protein α subunits ($G\alpha$), is shown to increase the barrier function of TJs (71). Several $G\alpha$ family members are expressed in epithelial cells, and among them, $G\alpha_{i2}$ and $G\alpha_{12}$ localize at cell–cell contact sites close to TJs. Overexpression of $G\alpha_{i2}$ decreases the paracellular permeability. On the other hand, $G\alpha_{12}$, which associates with ZO-1, increases permeability when overexpressed in epithelial cells. How different types of $G\alpha$ proteins regulate TJ function in opposite ways is not clear. Nevertheless these studies show that $G\alpha$ subunits are involved in regulation of TJs.

STRUCTURE AND FUNCTIONS OF TIGHT JUNCTIONS IN THE MAMMARY GLAND

In mammary epithelia, permeability of TJs is closely related to milk secretion, with TJ strands varying significantly in composition between pregnant and lactating animals. Several studies with freeze-fracture electron microscopy have revealed morphological changes of TJs in the mammary gland during lactation (74). In pregnancy, the number of TJ strands in mammary epithelia is smaller than that observed in the lactating animals. Furthermore, TJ strands are less organized and show fewer branched networks. These quantitative and qualitative changes of TJ strands allow them to function as appropriate barriers for diffusion to and from the pericellular environment in the mammary gland.

Hormones such as progesterone, prolactin, and glucocorticoid appear to be involved in the modification of TJ strands (75,76). Progesterone is known to play a role in the establishment and maintenance of pregnancy. Reduction of progesterone levels at parturition induces extensive changes in the mammary gland that are important for onset of lactation. In vivo studies have revealed that withdrawal of progesterone induces the closure of TJs in mammary epithelia in the pregnant mice. Cell culture studies have confirmed these findings (77).

Glucocorticoids are essential for maintenance of lactation and play a role in regulating lactogenesis. Addition of dexamethasone, a synthetic glucocorticoid, to cultured mammary cells significantly reduces permeability, and enhances the barrier function of TJs. Administration of glucocorticoids in vivo also enhances TJ barrier function in the lactating mammary epithelia and prevents the associated reduction in milk secretion. Molecular events which connect glucocorticoid signaling to TJ regulation encompass a complicated multistep cascade. There is evidence suggesting the involvement of PI3K, ras, and rho signaling in this cascade (78,79). Although precise mechanisms are not clear, the modification of ZO-1 phosphorylation also seems to be related to the enhancement of the TJ barrier with the addition of glucocorticoids (80).

Prolactin induces alveolar growth of the mammary gland and stimulates milk production associated with lactogenesis. Several early studies in vivo had shown that prolactin modulates the TJ barrier. However, it was not clear if prolactin acted directly on this process. Because prolactin treatment inhibits apoptosis during involution of the mammary gland, it is possible that prolactin decreases permeability of the mammary epithelia by preventing alveolar cell death. Recent studies using cultured mammary epithelial cells have shown that prolactin as well as glucocorticoids enhance the expression levels of occludin and ZO-1 (81). Therefore, signals from prolactin may affect the organization of TJs directly.

Milk secretion and the TJ barrier are regulated coordinately. A decrease in permeability in response to prolactin or glucocorticoid is accompanied by an increase in the rate of milk secretion. Mammary epithelial cells treated with glucocorticoids enhance TJ barrier function as well as the synthesis of milk components (75). Since it is necessary for mammary epithelial cells to tighten the TJ barrier before maximal milk secretion can occur, these two events may be regulated by the linked signaling pathways.

STRUCTURE AND FUNCTIONS OF TIGHT JUNCTIONS IN BREAST CANCER: POSSIBLE ROLES AS TUMOR SUPPRESSORS

Most cancers, including breast cancer, originate from epithelial tissues and are characterized by aberrant growth control, and loss of differentiation and tissue architecture. It is a fundamental property of cancer cells that their mutual adhesiveness is significantly weaker than that of the corresponding normal cells. Reduced cell–cell interactions allow cancer cells

to disobey the social order, resulting in destruction of overall tissue architecture, the morphological hallmark of malignancy. Loss of contact inhibition, which reflects disorder in the signal transduction pathways that connect cell–cell interactions are typical of both early (loss of cell polarity and growth control) and late (invasion and metastasis) stages of tumor progression (82). It has been shown that loss or reduced expression of E-cadherin, the prominent cell–cell adhesion molecule localized at AJs, is linked to tumor progression in epithelial cancers (83).

Recent studies have shown that several TJ components are directly or indirectly involved in breast cancer progression. Since ZO-1 has sequence similarity to Dlg, a known tumor suppressor in *Drosophila*, ZO-1 could also possess a potential to function as a tumor suppressor gene in mammalian epithelia. In fact, decreased ZO-1 staining is observed in several invasive breast cancer cell lines. Furthermore, reduction of ZO-1 signal is correlated with decreased tumor differentiation in human breast cancer samples, specifically with a decrease in the glandular differentiation of the tumor (84). Although it is still not clear if the reduction of ZO-1 expression is a consequence of destruction of cell–cell interactions or a primary event, loss of heterozygosity of ZO-1 in a number of breast tumors indicate that ZO-1 could be directly involved in progression of malignancy in breast cancer. A study using the tumorigenic breast cell line MCF-7 showed that activation of insulin like growth factor I receptor (IGF-IR) led to upregulation of ZO-1 expression. The same study also showed that IGF-IR activation enhanced E-cadherin-mediated cell–cell adhesion and reduced invasive ability of tumorigenic cells, although the expression of E-cadherin was not changed (85). This result suggests that ZO-1 could reduce metastatic ability of certain types of breast cancer cells in response to growth factor signaling.

Analysis of the expression of ZO-2 in several types of human cancer specimens have revealed that ZO-2 is downregulated in most of breast adenocarcinomas, although ZO-2 expression is rarely abnormal in colon or prostate adenocarcinomas (86). Significant reduction of ZO-2 expression is also detected in many breast cancer cell lines. ZO-2 is a cellular target of adenovirus type 9 oncogenic determinant E4 protein. Adenovirus type 9 E4 is unique in its ability to promote tumors specifically in the mammary gland in animals. Association of E4 proteins with ZO-2 leads to aberrant sequestration of ZO-2 within the cytoplasm. Overexpression of mutant ZO-2 lacking the E4 binding domain inhibits transformation of cells by E4 protein; thus association and sequestration of ZO-2 is probably crucial for the tumorigenic properties of E4 protein (87). These studies provide suggestive evidence that scaffolding proteins localized at TJs have tumor suppressor potential in breast epithelial cells.

Recent advances in comparative gene expression profiling studies, such as cDNA microarray or SAGE analyses, have identified differentially expressed genes in various types of cancer. Claudins have been reported to show a high incidence of dysregulated expression in various types of cancer, including breast cancer. For example, claudin-7 is significantly reduced in expression in many breast cancer cell lines and in primary breast carcinomas compared to normal mammary cells at both mRNA and protein levels. Claudin-7 is lost in most invasive ductal carcinomas, although the expression of claudin-3 and -4 are not affected (88,89). Another study has shown that the expression of claudin-1 is lost or significantly decreased in several breast cancer cell lines (90). These studies suggest that down-modulation of some of the claudins is correlated with progression of breast tumors, although the molecular mechanisms behind the loss is not fully determined yet. In contrast, claudin-1 is overexpressed in colon cancer cell lines and claudin-3 and -4 are overexpressed in ovarian cancer cell lines (91-93); therefore it appears that deregulation of claudin expression is controlled differently in different kinds of cancer. The results nevertheless indicate that significant changes of claudin expression in cancer may provide a valuable prognostic marker and help predict the grade of the tumors.

CONCLUDING REMARKS

While the structural features of TJs in the mammary gland were determined a number of years ago, the molecular composition and the mechanisms of assembly remain largely unknown. In addition, the availability of current imaging techniques warrant revisiting the older structural analysis. Recent advances in the understanding of the molecular organization of TJs in other organisms and in cultured cells should also help us to analyze how TJs are involved regulating mammary gland function. It is expected that studies aimed at elucidating the precise roles of TJ components in normal mammary gland function and breast tumor progression will help in development of useful markers and treatments in breast cancer.

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Abbreviations used

TJs	tight junctions
AJs	adherens junctions
ECM	extracellular matrix
JAM	junctional adhesion molecule
PKA	protein kinase A
PKC	protein kinase C

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Fig. 1.

(A) Ultrastructural image of a tight junction captured with a transmission electron microscope using thin-sectioned intestinal epithelial cells. Arrows indicate “kissing points,” an indistinguishable distance between two adjacent cell membranes; Mv, microvilli; TJ, tight junction. (B) Tight junction strands visualized by freeze-fracture replica electron microscopy of intestinal epithelial cells. Tight junction strands are observed as a network of continuous and anastomosing intramembranous particles of fibrillary strands on the protoplasmic face (arrows), with complementary vacant grooves on the exoplasmic face (arrowheads); Mv, microvilli (Courtesy of S. Tsukita *et al.*, from *Nat. Rev. Mol. Cell Biol.* 2001;2:285–293).

Fig. 2.

(A) Direct interactions between TJ membrane proteins and PDZ proteins. ZO-1, ZO-2, and ZO-3 bind to occludin and claudins through the GUK and PDZ1 domain, respectively. PDZ9 and PDZ10 of MUPP1 bind to claudins and JAM, respectively. JAM associates with ZO-1 through its PDZ3 domain and with PAR-3 through its PDZ1 domain. The specific binding domains to each membrane proteins in PDZ proteins are highlighted. (B) Schematic representative of the molecular organization of TJs. TJ strands mainly consist of claudins. However, evidence suggests that occludin and JAM are also incorporated into TJ strands via interaction with PDZ proteins. PDZ proteins associate with each other, with other cytoplasmic

components such as non-PDZ proteins and signaling molecules, and with actin filaments. Thus, PDZ proteins function as a scaffold to organize multiple protein networks at TJs.

Table I

Molecular Componentets of Tight Junctions

Proteins	Size (kDa)	Associating proteins at TJs	Possible functions	References
TJ Integral membrane proteins				
Occludins	~60	ZO-1, ZO-2, ZO-3	Regular of TJ barrier	18
Claudins	20-27	ZO-1, ZO-2, ZO-3 MUPP1	Formation of TJ strands, regulation of selective permeability	21
JAM	~45	ZO-1, PAR-3, MUPP1	Regulation of Polarity	31
CAR	~46	ZO-1	?	37
P0	~25	N.D.	?	35
OAP-1	~30	Claudin-11	?	36
PDZ-domain containing TJ cytoplasmic proteins				
ZO-1	~220	Occludin, Claudin, JAM, CAR, ZO-2, ZO-3, Cingulin, ZONAB	Formation of molecular complex	38
ZO-2	~160	Occludin, Claudin, ZO-1, Cingulin	Formation of molecular complex	40
ZO-3	~130	Occludin, Claudin, ZO-1, PATZ	Formation of molecular complex	41
PAR-3	~180	JAM, PAR-6, aPKC	Formation of molecular complex	56
PAR-6	~37	PAR-3, aPKC	Formation of molecular complex	57
MUPP1	~250	Claudin, JAM	Formation of molecular complex	59
Pals1	~70	PATJ	Formation of molecular complex	60
PATJ	~200	Pals1, ZO-3	Formation of molecular complex	60
MAGI-1	~130	N.D.	?	63
MAGI-2	~160	PTEN	?	61
MAGI-3	~125	PTEN	?	62
Other TJ cytoplasmic proteins				
Cingulin	140-160	JAM, ZO-1, ZO-2	Linking of TJs to actomyosin filaments	64
7H6 antigen	~155	N.D.	?	65
Symplekin	110-150	N.D.	?	66
Rab3B, Rab13	~25	N.D.	Vesicle transport	67

Proteins	Size (kDa)	Associating proteins at TJs	Possible functions	References
JEAP	~100	N.D.	?	68
PILT	~85	N.D.	?	69
ZONAB	47-55	ZO-1	Transcriptional regulation	16
huASH1	~330	N.D.	Transcriptional regulation	15
aPKC (PKC ζ/λ)	~70	PAR-3, PAR-3,	Regulation of Polarity and assembly of TJs	70
G <i>ai</i> 2	~40	N.D.	Regulation of assembly of TJs	71
G <i>α</i> 12	~40	ZO-1	Regulation of assembly of TJs	71
PTEN	~54	MAGI-2, MAGI-2	Phosphatase, tumor suppressor	61

N.D., not determined.