REVIEW

Scaffolding proteins in the development and maintenance of the epidermal permeability barrier

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ABSTRACT

The skin of mammals and other terrestrial vertebrates protects the organism against the external environment, preventing heat, water and electrolyte loss, as well as entry of chemicals and pathogens. Impairments in the epidermal permeability barrier function are associated with the genesis and/or progression of a variety of pathological conditions, including genetic inflammatory diseases, microbial and viral infections, and photodamage induced by UV radiation.

In mammals, the outside-in epidermal permeability barrier is provided by the joint action of the outermost cornified layer, together with assembled tight junctions in granular keratinocytes found in the layers underneath. Tight junctions serve as both outside-in and inside-out barriers, and impede paracellular movements of ions, water, macromolecules and microorganisms. At the molecular level, tight junctions consist of integral membrane proteins that form an extracellular seal between adjacent cells, and associate with cytoplasmic scaffold proteins that serve as links with the actin cytoskeleton. In this review, we address the roles that scaffold proteins play specifically in the establishment and maintenance of the epidermal permeability barrier, and how various pathologies alter or impair their functions.

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Introduction

The complex architecture of the epidermis gives rise to the multifaceted nature of its barrier functions. The barrier characteristics of the epidermis can vary, depending on age, body site, species and health status. In mammals, the outside-in epidermal permeability barrier is provided at 2 levels: the first is the outermost cornified layer, whereas the second is formed through the assembly of tight junctions in granular keratinocytes found in the layers underneath the corneocytes. Tight junctions are also found in the epidermis of amphibians and reptiles, underlining the importance of this inner epidermal barrier across vertebrates. A large body of work has focused on deciphering the molecular basis for the formation of these diverse barriers and how their disruption can lead to cutaneous diseases. In this review, we summarize and discuss the physiologic roles of scaffold proteins in development and maintenance of the epidermal permeability barrier, and the pathological impact of their alterations on the epidermis.

As the outermost organ, the skin constitutes a watertight barrier against the external environment, allowing humans and other organisms to survive in terrestrial conditions. The skin shields all internal tissues against external insults, including chemicals, pathogens and physical agents. Structurally, the skin is composed of an inner dermis and an outer epidermis. The epidermis is a stratified squamous epithelium, which endows the skin with its extraordinary permeability barrier properties.^{1,2}

The epidermis is formed by one basal and several suprabasal layers of keratinocytes. The latter are the main cellular constituents of this tissue. Each epidermal layer contains keratinocytes at a particular stage of differentiation.³⁻⁵ The basal layer contacts extracellular matrix proteins on the basement membrane that separates the dermis from the epidermis, and is composed of undifferentiated keratinocytes, including stem cells and their transit-amplifying progeny. Basal keratinocytes have high proliferative capacity and are key for epidermal maintenance and regeneration after

injury.^{6,7} Expression of proliferation-inducing transcription factors, as well as keratins 5 and 14 are characteristic of these cells.^{4,8-11} Differentiation of basal keratinocytes gives rise to mature cells that form the suprabasal layers. Differentiated keratinocytes do not proliferate, but rather form strong intercellular adhesions (adherens and tight junctions, as well as desmosomes). A subset of differentiated keratinocytes is responsible for the barrier functions of the epidermis.¹²⁻¹⁴

Suprabasal keratinocytes exist in various differentiation states, and exhibit distinct molecular and functional characteristics, depending on their location within the epidermis. Several spinous cell layers are found immediately above the basal keratinocytes.¹⁵ Common to all spinous keratinocytes are the presence of abundant desmosomes and the expression of keratins 1 and 10, which are early differentiation markers that replace keratins 5 and 14. The robust cytokeratin network in spinous cells allows the strengthening of intercellular junctions, key for the mechanical resistance of the epidermis.¹⁶ Spinous keratinocytes however, also exhibit a degree of heterogeneity. Specifically, at later stages of differentiation, activation of a gene cluster termed the “epidermal differentiation complex” (EDC) in the outermost spinous keratinocytes results in the expression of the intermediate differentiation markers involucrin and loricrin.¹⁷

Overlaying the spinous cells are 3–5 granular cell layers, characterized by the presence of intracellular lamellar bodies and keratohyalin granules. Characteristic markers of granular keratinocytes are loricrin and filaggrin. Functionally, the stratum granulosum contains tight junctions, which form the basis for an important component of the permeability barrier properties in the epidermis. In addition, caspase-14, which is expressed in all suprabasal keratinocytes, is activated in granular cells, setting in motion cornification processes, the final steps of keratinocyte differentiation.^{18,19} During this stage, the nucleus and all cell organelles are degraded through poorly understood mechanisms, ultimately giving rise to the transition from the granular to the cornified layers.²⁰

The cornified envelope consists of 15–20 layers of dead, extensively cross-linked cornified keratinocytes (also termed corneocytes). The biophysical characteristics of the stratum corneum allow it to function as an efficient barrier against pathogens and chemicals, simultaneously providing the epidermis with its

mechanical strength.²¹ The formation of the cornified layer involves cross-linking of proteins at the cell periphery and loss of intracellular organelles and DNA. Concomitantly, strong and stable links form between corneocytes to generate a dense mesh-like structure.²⁰ Those corneocytes closest to the granular layer are arranged in a tight pattern, whereas those in the outermost layers are less tightly linked and undergo desquamation through processes that involve proteolysis by kallikreins and other enzymes.²⁰ In addition to forming a sealed envelope built with cross-linked proteins, corneocytes are also embedded in a lipid matrix composed of sebum secreted by the sebaceous glands, together with lipids extruded by the keratinocyte lamellar bodies. These lipid components endow the cornified layer with its water-repellent properties and are strongly connected via corneodesmosomes, ensuring the physical strength of the cornified envelope.^{20,21}

The lipids in the cornified layer play key roles in preventing excessive water loss and maintaining appropriate hydration of the epidermis.²² Together with the size of the corneocytes and their intercellular spaces, cornified envelope lipids are also key determinants of the types of substances that can be absorbed from the skin surface through diffusion.²³ These substances are typically non-polar compounds with molecular weight below 500 Da.²⁴

The tight junction barrier in the epidermis

Tight junctions constitute cell-cell seals that limit paracellular movements of molecules across epithelia. In renal, intestinal and other simple epithelia, tight junctions between adjacent cells are found in the uppermost regions of the lateral aspect of the cells, separating the basal and apical cell surfaces.²⁵ At the molecular level, the tight junction skeleton consists of integral membrane proteins that form a link between adjacent cells and control paracellular solute movements. In the epidermis, the tight junction transmembrane proteins claudins, tight junction-associated marvel protein (TAMP) and junctional adhesion molecule (JAM) are expressed.¹⁵ Associated with the transmembrane junctional proteins are cytoplasmic scaffold proteins that include Zonula occludens (ZO) and cingulin, which also function as hubs for a variety of factors involved in signaling and transcriptional regulation.^{13,26-29} The ZO family is composed of 3 members (ZO-1, ZO-2 and ZO-3) with ubiquitous tissue

distribution, and which provide the structural basis for the assembly of multiprotein complexes at the plasma membrane.³⁰ ZO proteins, which are exclusively present in metazoans, additionally associate with each other to form homo- or heterodimers that regulate tight junctions and various other signaling modules.

Core transmembrane components of the tight junctions

Claudins

Claudins are major structural components of tight junctions. They associate as homo- or heteroligomers, forming paracellular barriers and pores that determine their specific properties and their regulation of epidermal inside-out permeability toward small molecules.^{13,31,32} Claudins interact through their cytoplasmic PDZ domains with various scaffold proteins, and these interactions appear to be obligatory for the assembly of the junction complex.³³ Although a functional tight junction barrier is restricted to a subset of granular layers in the epidermis,¹³ different claudins are expressed throughout this tissue.¹⁵ In particular, claudins 1, 4, 6, 7, 10, 11, 12, 17 and 18 are found in the stratum granulosum.^{13,26,34-36} Alterations in claudin protein levels or deletion of the PDZ-containing cytoplasmic domain in claudin-6 induce pronounced epidermal permeability barrier defects.^{12,34,37}

Occludin

Occludin is a 65-kDa 4-transmembrane domain protein that associates with tight junctions and is present in the stratum granulosum of the epidermis.^{28,38} Occludin also binds to scaffold proteins present in tight junctions, including ZO-1.³⁹ Proteolytic cleavage of the occludin extracellular domain mediated by matrix metalloproteinases increases paracellular permeability.⁴⁰ Significantly, targeted inactivation of the *Ocln* gene in mice gives rise to morphologically intact tight junctions, but chronic inflammation potentially due to poor barrier function, in various internal epithelia,⁴¹ suggesting the possibility that occludin may additionally modulate epidermal tight junctions in a manner still poorly understood.

Scaffolding proteins of the tight junctions

Zonula occludens (ZO) proteins

Some of the most prominent and best characterized scaffold proteins on the cytoplasmic aspect of the epithelial tight junction are the ZO proteins, 3 forms of

which are known in mammals (ZO-1, ZO-2, ZO-3).^{25,42} These members of the membrane-associated guanylate kinase (MAGUK) family are characterized by the presence of 3 N-terminal PDZ domains, a central Src homology 3 (SH3) region, and a catalytically inactive C-terminal domain with homology to guanylate kinase.⁴³⁻⁴⁵ ZO proteins are targeted to the plasma membrane and localize immediately underneath the tight junctions, through binding to occludin and claudins, as well as to phosphoinositides in the plasma membrane.^{25,46} ZO-1, the 225-kDa first discovered member, has both nuclear localization and nuclear export signals, and is found in the nucleus during remodelling of intercellular contacts.⁴⁷ ZO-1 is expressed in the granular keratinocyte layers, and its abundance in differentiating keratinocytes is positively regulated by p38 δ mitogen-activated protein kinase.⁴⁸ Both ZO-1 and ZO-2 have also been detected in the upper spinous layers, where they may fulfill additional functions independent of tight junctions.¹⁵

Cingulin

Cingulin is a 140-kDa peripheral membrane protein composed of coiled-coil domains, which binds to ZO-1 at tight junction plaques.⁴⁹ Although targeted gene inactivation or silencing of cingulin expression does not disrupt tight junction formation in simple epithelia, this protein plays both structural and signaling roles.⁵⁰ Cingulin exhibits actin-bundling activity *in vitro*, and has been implicated in anchoring of both actin filaments and a planar apical network of microtubules to the tight junctions. The anchoring and organization of microtubules mediated by cingulin requires phosphorylation of this protein at tight junction sites by adenosine monophosphate protein kinase (AMPK), further illustrating the importance that cingulin has as a signaling hub.^{51,52}

Assembly of the tight junctions in the epidermis

Epithelial cells both in simple and stratified epithelia establish apical-basal polarity through the assembly of tight junctions. In culture, undifferentiated epidermal keratinocytes do not form cell-cell adhesions, as the culture conditions do not provide sufficiently high extracellular Ca²⁺ concentrations to allow establishment of intercellular junctions.⁵³ Upon increasing the extracellular Ca²⁺ concentration, keratinocytes begin a process of differentiation, mimicking suprabasal

cells. They form a sealed epithelial sheet characterized by the formation of desmosomes, adherens and tight junctions.⁵⁴ Immediately following Ca^{2+} stimulation, cultured keratinocytes extend numerous F-actin-rich filopodia, which slide along those formed by adjacent cells. E-cadherin complexes also containing catenins cluster at the tips of these filopodia, which become sites where prominent actin fibers also polymerize and extend toward the cortical F-actin cytoskeleton.⁵⁴ ZO-1 is present in these structures, indicating that during the early stages of cell-cell junction formation, components of both adherens (E-cadherin, catenins) and tight junctions (ZO-1) are delivered from intracellular stores to sites of cell-cell contact.⁵⁵ Significantly, in cultured keratinocytes and other vertebrate cells, the formation of early cadherin-containing adhesions is an essential prerequisite for the subsequent assembly and maintenance of tight junctions.⁵⁶

In epidermal tissues, claudins 1, 4, 7, 11, 12 and 18, occludin, ZO-1, ZO-2, cingulin and Multi-PDZ Domain Protein 1 (MUPP-1) localize to cell-cell borders in the stratum granulosum, and are associated with the functional tight junction barrier. Notably, some tight junction proteins also localize to all epidermal layers (e.g. claudins 1, 7, and 12, Junctional Adhesion Molecule (JAM)-A, and MUPP1), or to the spinous keratinocytes (e.g., ZO-1, ZO-2, claudins 4, 6, 18).^{26-28,35,57-62} Similar to observations in cultured keratinocytes, disruption of cadherin-containing adherens junctions *in vivo* results in impairment in tight junction formation. Targeted inactivation of *Cdh1* in mice, which encodes E-cadherin, results in extensive transepidermal water loss and loss of functional tight junctions in the stratum granulosum.⁶³ In these mice, paracellular diffusion of ions was also increased due to enhanced tight junction permeability. These defects were associated with altered distribution of ZO-1 and claudin-1 at the membrane of granular keratinocytes. Thus, although the absence of E-cadherin in the epidermis does not completely obliterate tight junctions, it prevents assembly of key constituents and proper barrier function. A more pronounced phenotype is observed in mouse epidermis lacking both E- and P-cadherin, in which localization to cell-cell borders of occludin, claudin-1 and ZO-1 is perturbed, impairing tight junction assembly in the granular layer.⁶⁴ Thus, cadherins play critical roles in the assembly and/or stability of epidermal tight junctions, both in culture and *in vivo*.

Nature of paracellular transport through tight junctions: Inside-out and outside-in barrier properties

Tight junctions are major determinants of paracellular barrier function in all epithelial cells. Two types of permeability barriers regulated by tight junctions have been defined in terms of the types of substances whose movement they regulate.⁶⁵ The first one has been termed the "pore pathway," and is characterized by high capacity, and allows permeability of small uncharged solutes and specific ions. Pore pathway characteristics are mainly determined by the particular claudin forms found at the tight junction, and may also be regulated by protein-protein interactions. The second type of permeability barrier is termed the "leak pathway." It has low capacity, is permeable to larger macromolecules, and does not exhibit selectivity toward ions.⁶⁵ ZO-1, occludin and the actin cytoskeleton play key roles in the modulation of the leak pathway.⁶⁵

The organization of tight junctions in the multilayered epidermis is not identical to that described in simple epithelia. For example, the functions associated with tight junction assembly in the epidermis have been described in terms of inside-out and outside-in permeability barriers, depending, respectively, on whether a substance leaves the skin toward the external environment, or enters the skin from the outside.¹⁵ In addition, epidermal tight junctions do not necessarily modulate permeability to cations, anions, small molecules, tracers, antigens and water in identical manners.

The inside-out permeability barrier in the epidermis is important to prevent fluid and ion loss from the organism toward the exterior. Loss of tight junctions causes increased transepidermal water loss *in vivo*, which, depending on its severity and the presence of other epidermal abnormalities, may be lethal.¹³ Lanthanum has been used as a tracer to demonstrate an inside-out barrier toward ions in the stratum granulosum *in situ*,⁶⁶ and the existence of a tight junction-associated barrier toward the small 557-Da solute sulfo-NHS-LC-biotin in the stratum granulosum *in vivo* has also been demonstrated.¹³ In contrast, the demonstration of outside-in permeability barrier characteristics of the epidermal tight junctions has been more challenging. This is due to the existence of the stratum corneum barrier, which impedes passage of

many solutes, and experimental manipulations that remove the stratum corneum barrier can also damage the underlying tight junctions.⁶⁷

The pore and leak pathways associated with tight junctions have been investigated at the cellular level in keratinocyte monolayers induced to differentiate and to assemble strong cell-cell junctions by culture in growth medium supplemented with 1.0–1.8 mM Ca^{2+} .^{68,69} Transepithelial resistance (TER) has been used as a measure of the barrier function toward ions provided by tight junctions in submerged human and mouse keratinocyte monolayers.^{68,69} Upon induction of differentiation by Ca^{2+} , pronounced increases in TER are observed in confluent keratinocyte monolayers, which provide a measure of tight junction assembly and functionality.^{68,69} Further, the use of 2-path impedance spectroscopy has demonstrated that the increases in TER associated with decreased ion permeability in Ca^{2+} -treated human keratinocyte monolayers are primarily due to decreased paracellular permeability, with only very minor contributions from changes in tight junction-independent transcellular permeability.⁷⁰

The paracellular ion permeability barrier in human keratinocyte monolayers treated with Ca^{2+} is selective: anions (e.g., Cl^-) are less permeable than divalent cations (e.g., Ca^{2+}), which, in turn, are less permeable than monovalent cations (e.g., Na^+).⁷⁰ In culture, the permeability barrier to ions reaches maximum levels only after 48 h of culture in medium with high Ca^{2+} ,⁷⁰ indicating that this interval is necessary for the full maturation of tight junctions in cultured keratinocytes. Similarly, paracellular transepithelial water fluxes also decrease with the formation of tight junctions in human keratinocyte monolayers.⁷⁰

Examination of the leak pathway in cultured keratinocyte monolayers has also demonstrated the existence of a permeability barrier toward larger molecules, such as fluorescein (332 Da), and fluorescein-labeled dextrans of small and medium molecular weights (3–40 kDa).^{71–74} Under these conditions, the barrier toward these larger solutes is observed at earlier times after the induction of tight junction assembly, compared with the formation of barriers toward ions.⁷⁰ Significantly, silencing of either claudin 1, claudin 4, occludin or ZO-1 in Ca^{2+} -treated human keratinocyte monolayers reduced paracellular TER, and abrogated the permeability barrier to ions, small- and intermediate-size macromolecules, further confirming

the involvement of tight junctions in these properties of epidermal cells.⁷⁰

Scaffold proteins involved regulation of tight junctions in the epidermis

Polarity proteins

In addition to ZO scaffold proteins, which are found at tight junction plaques, other adaptor proteins modulate tight junction assembly and/or function. Tight junctions are key determinants of cell polarity in simple epithelia.²⁵ Because of its stratified architecture, the epidermis also exhibits cell polarity, although the manner in which it is organized is not identical to that in simple epithelia. The apical-basal axis of epithelial cells is also characterized by the expression of polarity proteins such as the PAR (Partition-defective), Crumbs and Scribble protein complexes.⁷⁵

The best-studied polarity proteins are the PAR proteins, which are necessary for the establishment of cell polarity and for the proper formation of tight junctions.^{76,77} The *par* genes were first identified in *Caenorhabditis elegans* as regulators of zygote anterior-posterior polarity.⁷⁸ Since their discovery, polarity proteins have been shown to play important roles in other organisms, such as in *Xenopus*, where they regulate gastrulation,⁷⁹ and in mammals, where they are involved in epithelial polarization,^{80,81} as well as in neuronal dendritic spine morphogenesis.⁸² The mammalian homologs Par3 and Par6, together with atypical protein kinase C (aPKC), form the “polarity complex.” Par3 and Par6 contain PDZ domains, which enable them to bind to each other and act as scaffolds for other proteins. Par6 functions as an adaptor protein connecting Par3 with aPKC and the small GTPase Cdc42, when activated and bound to GTP (Cdc42-GTP).⁸³ These interactions are important for tight junction formation.⁸³ Par6 inhibits aPKC kinase activity. This inhibition is relieved through interaction of Par6 with Cdc42-GTP, which induces a conformational change in Par6, no longer allowing it to inhibit aPKC. In this manner, the latter can then phosphorylate its substrates.⁶⁸ Par3/Par6/aPKC proteins are not always found together in a complex. One of the substrates of aPKC is Par3, and phosphorylation of Par3 results in its partial dissociation from the complex. Another polarity protein known as Crumbs completely displaces Par3, resulting in localization of

Par6 and aPKC at the apical membrane and of Par3 at tight junctions.⁸⁴

Par proteins, along with aPKC, are regulators of epithelial polarization and tight junction assembly, maturation and function. In Madin-Darby Canine Kidney (MDCK) epithelial cells, Par3, in complex with ASIP (atypical PKC isotype specific interacting protein), is involved in the regulation of tight junction formation. The role of Par3 in this context is not limited to functioning as a structural protein.⁸⁵ RNAi-mediated silencing of Par3 in mammalian epithelial cells significantly delays the formation of tight junctions by disrupting the localization of other polarity markers such as aPKC.⁸⁶ Significantly, Par3 silencing also resulted in a delay in the formation of adherens junctions.⁸⁶

In the epidermis, polarity is mainly established along the basal-to-apical axis, but the mechanisms that regulate polarized protein distribution throughout the different epidermal layers are not well understood. Par3 is present in cultured primary keratinocytes and in all living epidermal layers.⁸⁷ In keratinocyte monolayers, Par3 associates with Tiam1, and this association is necessary for assembly and barrier function of tight junctions.⁷² In this system, Tiam1 functions upstream from the polarity complex, mediating activation of the small GTPase Rac1, which in turn activates the polarity complex.⁷² Whether this mechanism is operative in the assembly of tight junctions in granular keratinocytes *in vivo* has yet to be determined. Par3 co-localizes with ZO-1 in stratum granulosum keratinocytes, and is required for proper aPKC localization to tight junctions and signaling.⁸⁷ Genetic inactivation of Par3 in newborn mouse epidermis results in pronounced perturbations in the tight junction permeability barrier to small solutes and increased transepidermal water loss, as well as reduced ZO-1, claudin-1 and occludin levels and their abnormal localization in granular keratinocytes.⁸⁰ In spite of these abnormalities, these mice survived to adulthood, at which time tight junction function and protein expression had normalized, indicating the possibility of activation of compensatory mechanisms.

In newborn mouse epidermis, aPKC ι/λ has been found co-localized at cell-cell borders with Par3 and Par6 specifically in granular keratinocytes.⁶⁸ Further, in response to Ca²⁺-induced differentiation in primary keratinocytes, recruitment of ZO-1 and occludin at cell-cell contacts, tight junction assembly and

barrier function are accelerated in the presence of exogenously expressed aPKC ι/λ . Of note, epidermis-restricted inactivation of the gene that encodes aPKC ι/λ was not reported to result in any changes in tight junction function that affected viability, although it caused pronounced defects in hair follicle stem cell maintenance.⁸⁸ Thus, the precise role that aPKC ι/λ plays in the assembly, maintenance and function of epidermal tight junctions *in vivo* remains to be elucidated.

Afadin

Afadin is a scaffold protein containing a PDZ domain, which localizes to cell-cell junctions and also binds F-actin.⁸⁹ In epidermal keratinocytes and other epithelial cells, afadin interacts with the 4 members of the nectin transmembrane adhesion protein family, connecting them with the actin cytoskeleton.⁸⁹ Afadin is also required for nectin signaling at tight junctions, through its interactions with ZO-1.⁸⁹ Afadin is involved in functionally linking adherens and tight junctions, especially during junction assembly and remodeling, although it appears to be dispensable for epidermal stratification and viability in mice.⁹⁰ Recently, an additional, unexpected role for afadin in tight junction assembly that involves its interactions with the Ephrin receptor EphA2 has been described.⁹¹ EphA2 is a transmembrane receptor tyrosine kinase expressed in a polarized fashion in the upper suprabasal layers of the human epidermis.⁹¹ This receptor fulfills several functions in the epidermis, including protection against chemically-induced carcinogenesis,⁹² modulation of keratinocyte adhesion and tight junction formation.⁹³ Afadin binds to EphA2, and in the absence of the latter the subcellular distribution of afadin and occludin changes from mainly junctional to cytoplasmic.⁹¹ These changes are also associated with defects in the tight junction permeability barrier to ions and a dextran tracer. Thus, the interactions of afadin with EphA2 are essential for tight junction assembly.

Integrin-linked kinase

Integrin-linked kinase (ILK) is a 51-kDa scaffold protein with no catalytic activity, that associates with integrins and localizes to cell-cell junctions in cultured differentiated keratinocytes, in a Ca²⁺-dependent manner.^{94,95} In the epidermis, ILK is expressed in all

living keratinocyte layers, as well as in hair follicles,⁹⁶ and is important for maintenance of follicular and interfollicular keratinocyte cell polarity. Epidermis-restricted *Ilk* gene inactivation during embryogenesis abrogates the apical distribution of E- and P-cadherin in developing hair follicles, and alters the cortical actin cytoskeleton in follicles and in the interfollicular epidermis.^{97,98} In cultured keratinocytes, Ca^{2+} induction of differentiation and cell-cell junction formation involves activation of the G-protein coupled Ca^{2+} -sensing receptor (CaSR), which in turn triggers activation of RhoA and delivery of E-cadherin to form adherens junctions.^{99,100} *In vivo*, CaSR is also essential for the establishment of the tight junction barrier in mice.¹⁰¹ In the absence of ILK, signaling through CaSR is altered and RhoA activation is impaired in cultured keratinocytes.¹⁰² Under these conditions, E-cadherin, and ZO-1 fail to translocate to the cell membrane, through mechanisms that involve impaired RhoA activation and endosomal delivery of junction proteins to the plasma membrane.¹⁰³ As a result, adherens and tight junctions fail to form. These alterations are reflected in barrier defects *in vivo*, as ILK-deficient epidermis exhibits altered ZO-1 and claudin-1 distribution in granular layers, reduced ZO-1 expression and tight junction formation. These abnormalities are also associated with increased permeability to medium molecular-weight tracers, indicating impairment of paracellular tight-junction barrier properties toward macromolecules.¹⁰²

Disruption of tight junctions and barrier properties in epidermal diseases

Several human disorders and alterations are associated with disruption of tight junctions, through mechanisms that target various components of these structures directly or indirectly. These diseases include hereditary disorders involving genetic mutations, inflammatory processes and responses to pathogens and environmental insults.

Photoaging and UV radiation

The chronic exposure of the skin to UV radiation and other environmental insults, together with chronological aging, alters several properties of the skin.¹⁰⁴ UV-A light (λ 320–400 nm) is able to penetrate deeper into the dermis and is associated with changes in dermal collagen and dermal elasticity.¹⁰⁵ In contrast, the

range of penetration of UV-B radiation (λ 290–315 nm) is largely limited to the epidermis, causing damage due to the production of reactive oxygen species, macromolecule modification and degradation, and DNA damage.^{106–108} Few studies have investigated the consequences on epidermal tight junctions upon UV irradiation.

In addition to damaging macromolecules, UV radiation also severely disrupts the cutaneous permeability barrier. UV-B irradiation of human skin xenografts in immunodeficient mice results in increased transepidermal water loss, which remains at a maximum 24–72 h after irradiation, and returns to normal levels by 144 h.⁶⁹ These alterations were reportedly accompanied by loss of inside-out barrier capacity in the upper stratum granulosum, which was also re-established 144 h post-irradiation. Similar disruptions in the tight junction barrier were observed in UV-irradiated human skin organotypic cultures⁶⁹ and in a hairless mouse model.¹⁰⁹ At the cellular level, these alterations were accompanied by disassembly of tight junctions, through mechanisms that involved abnormal Rac1 activation of the polarity complex, specifically atypical PKC.⁶⁹ Although the status of the scaffold protein Par3 was not evaluated in these experiments, given that it is a direct target for Rac1 during tight junction assembly, it is possible that UV radiation also alters the localization and/or adaptor activity of Par3. These are important issues for future research.

Ichthyosis

Neonatal ichthyosis schlerosing cholangitis (NISCH) is a very rare genetic disease in which affected individuals possess nonsense mutations in the *CLDN1* gene, resulting in complete absence of Claudin-1 protein.¹¹⁰ This syndrome results in cholangitis, skin abnormalities including sparse eyelashes and eyebrows, hypotrichosis and alopecia, as well as formation of a compact and hyperkeratotic stratum corneum.^{110,111} Although electron microscopic analyses of affected skin in some patients have revealed apparently normal tight junctions, the status of the functional barrier has never been examined. However, significant phenotypic differences in several individuals carrying the same mutation have been observed, suggesting the possibility that genetic modifiers may also contribute to the severity of this disorder.¹¹² Importantly, the role of claudin-1 and/or the mechanisms activated to

compensate for its loss in humans appear to differ from those in mice, as inactivation of the *Cldn1* gene in the latter results in perinatal lethality with full penetrance.¹³ Also requiring further investigation are any alterations that may occur in tight junction-associated scaffold proteins and other components of these structures in the absence of claudin-1 in humans.

Ichthyosis vulgaris is a hereditary, autosomal semi-dominant skin disease mainly caused by loss-of-function mutations in the *FLG* gene, which encodes filaggrin.¹¹³ Although filaggrin is not directly involved in tight junction assembly, it is an important contributor to the stratum corneum inside-out barrier, and its absence also affects the tight junction barrier. Both filaggrin-deficient humans and mice exhibit impaired inside-out and outside-in barrier function, as evidenced, respectively, by increased transepidermal water loss and small molecule penetration.¹¹⁴⁻¹¹⁷ Significantly, loss of filaggrin in humans, but not in mice, is accompanied by gene dosage-dependent decreases in occludin and ZO-1 immunoreactivity in granular keratinocytes,¹¹⁴ although additional studies are needed to elucidate the molecular mechanisms involved in these alterations.

Atopic dermatitis

Atopic dermatitis is a chronic inflammatory disease, characterized by T-helper type 2 (Th2) inflammation and epidermal barrier defects.¹¹⁸ Atopic dermatitis is a relatively common disease, with a prevalence of about 20% in children, and up to 10% in adults.¹¹⁸ Atopic dermatitis can arise as a consequence of multiple abnormalities, including *FLG* gene mutations, which result in the absence of filaggrin or of biologically active filaggrin peptides, leading to disruptions in the stratum corneum.¹¹⁹⁻¹²² Various single nucleotide polymorphisms in the *CLDN1* gene, which encodes claudin-1 have been associated with some cohorts of individuals affected by atopic dermatitis, as has reduced expression of claudins -1 and -23.⁷¹ Experimentally, silencing and downregulation of claudin-1 in cultured human keratinocytes^{70,74} or genetic inactivation of *Cldn1* in mice¹²³ impairs both tight junctions and the stratum corneum barrier. These observations, together with the alterations in tight junctions consequent to filaggrin loss, are consistent with the possibility of reciprocal regulation of the tight junction and stratum granulosum barriers.

Scaffold proteins associated with tight junctions have received scarce attention in human atopic dermatitis or animal models of this disease. Decreased ZO-1 expression has been reported in filaggrin-deficient patients, and has been proposed as a contributor to the epidermal barrier abnormalities in these individuals.¹¹⁴ Similarly, a recent study described decreased and discontinuous ZO-1 immunoreactivity in the affected areas of the epidermis in a canine model of atopic dermatitis,¹²⁴ suggesting that ZO-1 abnormalities may also be key contributors to the paracellular barrier defects present in individuals affected by this disease. The complexity of the abnormalities present in atopic dermatitis is increased by the role of inflammation in affected skin, which also contributes to the reduced stratum corneum barrier properties and the increased paracellular permeability through tight junctions.¹²⁵

Infection

The skin is a target for infection by a variety of pathogens, including viruses, bacteria and fungi. The defensive ability of the skin against invading pathogens results from the combined action of its physical barrier function, together with its immune antimicrobial barrier capabilities.¹²⁶ During infection, tight junctions and epidermal integrity can be affected through a variety of mechanisms, including direct effects of the invading pathogens and production of inflammatory mediators in response to immune response activation. Major viral agents that target the skin are the herpes simplex and the human papilloma viruses. Herpes simplex virus 1 (HSV-1) invades human hosts through skin abrasions or mucosal surfaces, establishes life-long infections, which can lead to atopic dermatitis and disseminated skin infections.¹²⁷ Cell-cell junctions provide an important barrier to viruses, and their components can be used by these pathogens as attachment receptors that mediate virus fusion with the keratinocyte plasma membrane.¹²⁸ HSV-1 does not infect intact mouse epidermis or intact human skin equivalents, but it can infect wounded human skin equivalents.¹²⁹ The formation of mature tight junctions prevents HSV-1 invasion of keratinocyte monolayers. Significantly, disruption of tight junction assembly due to Par3 deficiency in mouse keratinocyte cultures results in increased HSV-1 infection upon exposure to this virus,¹²⁹ demonstrating that the

ability of HSV-1 to invade stratified keratinocytes is only maintained in conditions in which the functional tight barrier is disrupted, thus facilitating virus access to its attachment receptors.

Human papilloma virus (HPV) infections are key risk factors for the development of cervical, as well as head and neck, carcinomas. Various HPV proteins are able to interact and interfere with the functions of multiple cellular proteins. The high-risk HPV16 E6 protein targets PDZ-containing proteins involved in cell polarity and intercellular junction formation. In particular, E6 binds to Par3, altering its subcellular localization, and interfering with tight junction assembly.¹³⁰ Tight junction proteins also modulate cell proliferation and transformation, and it is possible that the interference of the HPV E6 proteins with Par3 function and tight junction assembly contributes to viral infection and replication, as well as to subsequent cell transformation and tumorigenesis.

Aside from pathogenic viruses, major contributors to bacterial skin infections are *Staphylococcus aureus* and *Streptococcus pyogenes*.¹³¹ *S. aureus* infections produce abscess formation and worsening of atopic dermatitis and other inflammatory conditions, and cause considerable morbidity and mortality.^{132,133} Multiple mechanisms are involved in *S. aureus* invasion, including expression of an array of proteins that mediate bacterial attachment to the keratinocyte plasma membrane and extracellular matrix proteins, as well as production of toxins that disrupt the epithelial barrier. Some toxins also mediate proteolytic cleavage of desmoglein-1 and desmosome disassembly.¹³³ *S. aureus* also elicits inflammatory responses, which collectively contribute to changes in the skin barrier. The changes in tight junctions associated with staphylococcal infections are complex and are dependent on the staphylococcal strain analyzed. For example, early after *S. aureus* infection of spontaneously immortalized HaCaT keratinocytes, there is a disassembly of tight junctions, as evidenced by marked decreases in ZO-1, claudin-1 and occludin immunoreactivity at cell-cell contacts, without substantial alterations in the abundance of these proteins.¹³⁴ In these cells, localization of aPKC to tight junctions was also severely affected, indicating the possibility that *S. aureus* also targets, directly or indirectly, Par3 and/or Par6. These changes were also accompanied by loss of tight junction barrier functions associated with impeding paracellular ion transport.¹³⁴ Similar decreases were observed upon infection of porcine skin explants and

in specimens of human skin diagnosed with generalized *S. aureus* infection (impetigo contagiosa).¹³⁴ In these studies, no changes in adherens junctions or desmosomes were observed with the *S. aureus* strains used, suggesting the possibility that exfoliative toxins from some strains may preferentially target tight junction components.

Host defense peptides (also termed antimicrobial peptides) are produced by keratinocytes and other cell types in response to bacteria.¹³⁵ Over 2,000 different human host defense peptides are known. They function by killing pathogens and by modulating various biologic processes, including stimulation of chemotaxis, production of cytokines and chemokines, regulation of epithelial cell apoptosis, and suppression of pro-inflammatory responses. Three host defense peptides with key roles in cutaneous protection are cathelicidin, LL-37, β -defensins, and S100A7 (psoriasin).¹³⁵ In intact skin, LL-37 is barely detectable in keratinocytes, but it is strongly upregulated during infections or after injury.¹³⁶ Significantly, LL-37 upregulates the expression of tight junction proteins, and increases tight junction barrier function by reducing paracellular fluxes in cultured keratinocytes.¹³⁷ Mechanistically, LL-37 induces these effects through activation of the Par3/Par6/aPKC pathway, as well as through activation of Rac1 and glycogen synthase kinase (GSK)-3.¹³⁷ LL-37 functions synergistically with other antibacterial peptides produced by nonpathogenic commensal bacteria that colonize healthy skin, and contributes to decreased *S. aureus* colonization in skin from individuals with atopic dermatitis.¹³⁸ An important area for future research will be to determine if antibacterial peptides from nonpathogenic commensals also synergize with LL-37 in modulating the paracellular barrier function in the epidermis.

Integrin-linked kinase also plays key roles in the susceptibility of the skin to *S. aureus* invasion.¹³⁹ Specifically, staphylococcal penetration of skin explants increased over 30-fold in specimens from mice with epidermis-restricted inactivation of the *Ilk* gene. Given that ILK-deficient keratinocytes exhibit severely impaired internalization of these bacteria, a major component of the increased invasion observed is likely associated with the loss of the paracellular permeability barrier in these animals.¹⁰² Significantly, ILK-deficient epidermis also exhibits upregulation of antimicrobial peptides, such as psoriasin,¹⁴⁰ in agreement with the known cutaneous responses to its increased susceptibility to infection.

Conclusions

Although the contribution of scaffold proteins to the existence of functional tight junctions in the epidermis has been firmly established for well over a decade, many aspects of their biologic roles and their regulation remain unexplored. In particular, much remains unknown about which and how scaffold proteins contribute to orchestrate such diverse events as differentiation, intercellular junction assembly, cytoskeletal dynamics and control of paracellular solute movements. Future research will undoubtedly unearth novel mechanisms and identify additional adaptor proteins involved in the regulation of the epidermal permeability barrier in health and in disease.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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