

CHAPTER 13

GAP JUNCTIONS AND BLOOD-TISSUE BARRIERS

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Abstract: Gap junction is a cell-cell communication junction type found in virtually all mammalian epithelia and endothelia and provides the necessary “signals” to coordinate physiological events to maintain the homeostasis of an epithelium and/or endothelium under normal physiological condition and following changes in the cellular environment (e.g., stimuli from stress, growth, development, inflammation, infection). Recent studies have illustrated the significance of this junction type in the maintenance of different blood-tissue barriers, most notably the blood-brain barrier and blood-testis barrier, which are dynamic ultrastructures, undergoing restructuring in response to stimuli from the environment. In this chapter, we highlight and summarize the latest findings in the field regarding how changes at the gap junction, such as the result of a knock-out, knock-down, knock-in, or gap junction inhibition and/or its activation via the use of inhibitors and/or activators, would affect the integrity or permeability of the blood-tissue barriers. These findings illustrate that much research is needed to delineate the role of gap junction in the blood-tissue barriers, most notably its likely physiological role in mediating or regulating the transport of therapeutic drugs across the blood-tissue barriers.

INTRODUCTION

Intercellular communication is an important means to maintain tissue homeostasis in multi-cellular organisms. In animals, gap junction communication (GJIC) plays a crucial role to maintain the homeostasis of different types of epithelia as well as endothelia. Gap junctions (GJ) are sometimes compared to plasmodesmata in plants as they both allow direct transport of solutes across cells.¹ However, besides intercellular transport of

solutes via gap junction channels, GJ can also mediate solute transport between cells and extracellular space through GJ hemichannels.^{2,4} GJ proteins are the basic building blocks of GJ and include connexins in vertebrates, innexins in invertebrates and pannexins in both vertebrates and some invertebrates.⁵⁻⁷

In this chapter, we discuss the roles of gap junctions in regulating the junction dynamics in tissue barriers in mammals based on the latest findings in the field. It is noted that pannexins are more closely related to innexins than connexins^{8,9} and only form hemichannels.^{5,7} Pannexin-based hemichannels are found more recently in vertebrates and there are very few reports in the literature studying the relationship of pannexins and junction barriers. This chapter thus focuses on the functional relationships between connexins and blood-tissue barriers in mammals. Particular focus will be put on how GJ provides the crucial crosstalk between different junction types coexisting at the blood-testis barrier so that the immunological barrier can be maintained during blood-testis barrier restructuring at the time of preleptotene spermatocytes, many of which are connected by intercellular bridges in clones,^{10,11} in transit at the site.

CONNEXINS—THE BASICS: STRUCTURES, FUNCTIONS AND REGULATION

Connexin Family

Connexon is a functional unit of gap junctions and made up of a hexamer of gap junction proteins either of the same (homomeric) or different connexins (heteromeric). A connexon on the cell surface by itself is called a hemichannel while gap junction channel refers to connexons coupled between apposing cells^{2,3} (Fig. 1).

There are at least 20 connexins identified in humans and rodents (Table 1). Each mammalian cell type only expresses certain members of the connexin family. These tetraspan proteins are highly conserved in their intracellular N-terminal tail, four transmembrane domains and two extracellular loops, which are for recognition and coupling.^{2,3,12} The variability of connexins in terms of both length and sequence lies mostly in their intracellular loop and C-terminal tail. The C-terminal tail is the region where connexins interact with different modulators and interacting partners. Most phosphorylation sites of connexins (e.g., Cx43) are found on the C-terminal tail.¹³

Gap junction channel can be assembled between cells of the same cell type or of different cell types for heterocellular communication and the interaction of connexons can also be homotypic or heterotypic. Thus, a wide combination of gap junction channels can be formed between epithelial and endothelial cells.^{2,3}

Despite the similarities between different connexin family members and expression of different connexins in the same cell type, different connexins seem to process unique functions as well as shared functions,^{14,15} as illustrated in different knockout and/or knockin mouse models and genetic diseases in humans (Tables 1 and 2). For instance, while Cx43^{-/-} mice die at birth, homozygous Cx43 knockin Cx26 mice are viable at birth. Even though the percentage of homozygous Cx43 knockin Cx26 mice born is less than the expected Mendelian ratio and has lower survival rate, it illustrates that Cx26 can at least partially compensate for the loss of Cx43. But some vital functions of Cx43 cannot be compensated by Cx26 as gametogenesis in both homozygous males and females becomes impaired, resulting in infertility.¹⁵

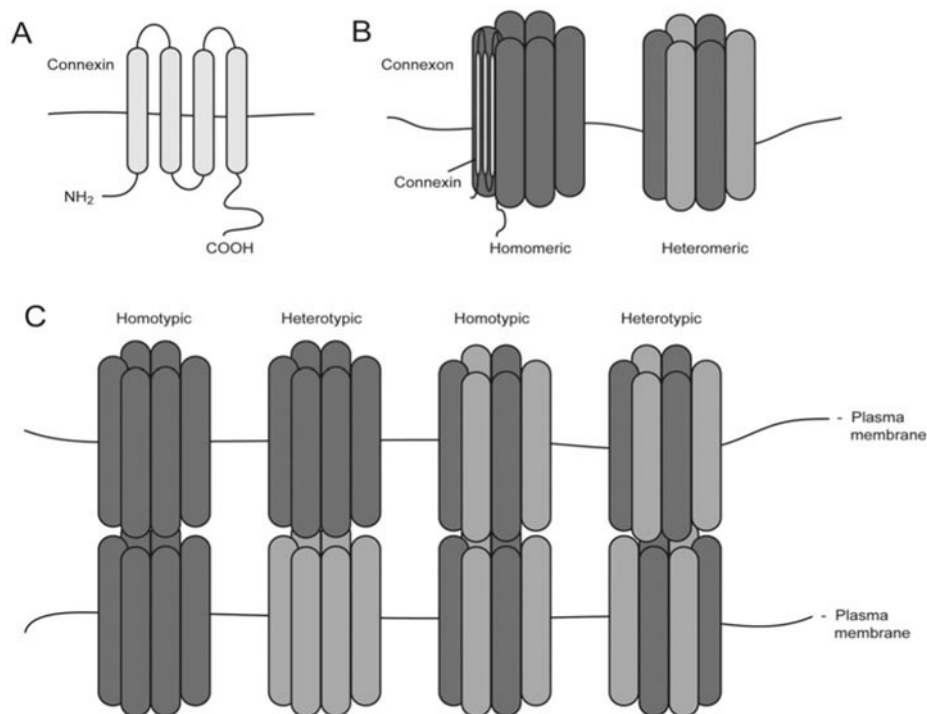


Figure 1. Schematic illustrations of the structure and organization of GJ. A) A connexin consists of four transmembrane domains, two extracellular loops and one intracellular loop. The variability of connexins lies mostly on the C-terminal tail that comes in different length and sequence and carries sites for phosphorylation and binding of interacting partners. B) Six connexins constitute a functional connexon. A connexon can be made up of the same type of connexins (homomeric) or of different types (heteromeric). An uncoupled connexon can also be called a hemichannel. C) GJ channel is formed between two compatible connexons on adjacent to that create a functional communication channel. The interaction of connexons could be homotypic or heterotypic, depending on the compatibility of individual connexins in a connexon.

Nomenclature of Connexins

Two systems of connexin nomenclature are currently used in parallel (Table 1). The conventional one names connexins according to their molecular sizes (in kDa).¹⁶ For example, Cx43 means a connexin protein of 43 kDa. This is a commonly used system which is also used in this chapter. This system nonetheless has its drawbacks due to the differences in molecular sizes in connexin orthologs even between humans and mice. The second system involves grouping connexins according to their sequence similarities and length of their cytoplasmic tails.¹⁶ Connexins are assigned into one of the several groups, namely α , β , γ , δ and ϵ , and a number according to the order of discovery. Cx43 is named GJA1 in the second system, which marks it as the first member discovered in the alpha group of gap junction proteins.

Table 1. Connexin family members and associated defects due to mutations

	Human	Mouse	Human Hereditary Disease(s)	Phenotype(s) of Knockout Mice
GJA1	Cx43	Cx43	Oculodentodigital dysplasia, ¹¹³ cardiac defects, ¹¹⁴ hearing loss ¹¹⁵	Neonatal lethality (lethal at birth) with abnormal cardiac development, ^{116,117} osteoblast dysfunction ¹¹⁸
GJA3	Cx46	Cx46	Cataract ¹¹⁹	Cataract ¹²⁰
GJA4	Cx37	Cx37	/	Female sterility ¹²¹
GJA5	Cx40	Cx40	Cardiac defects ¹²²	Cardiac defects ^{123,124}
GJA6	/	Cx33	/	/
GJA8	Cx50	Cx50	Cataract ¹²⁵	Microphthalmia, cataract ¹²⁶
GJA9	Cx59	/	/	/
GJA10	Cx62	Cx57	/	Reduction in visual field in retina ¹²⁷
GJB1	Cx32	Cx32	Charcot-Marie-Tooth disease (CMTX) ^{128,129}	Decreased glycogen mobilization, ¹³⁰ increased liver carcinogenesis, ¹³¹ mild myelination defects ¹³²
GJB2	Cx26	Cx26	Hearing loss, ¹³³⁻¹³⁵ epidermal disease ^{135,136}	Embryonic lethality ¹³⁷
GJB3	Cx31	Cx31	Non-syndromic hearing loss, ^{138,139} epidermal disease ⁸³	Placental dysfunction ¹⁴⁰
GJB4	Cx30.3	Cx30.3	Epidermal disease, ⁸⁵ hearing loss ¹¹⁵	/
GJB5	Cx31.1	Cx31.1	/	/
GJB6	Cx30	Cx30	Hearing loss, ¹⁴¹ ectodermal dysplasia ⁸⁴	Hearing loss, ¹⁴² behavioral changes to novel environment ¹⁴³
GJB7	Cx25	/	/	/
GJC1	Cx45	Cx45	/	Embryonic lethality due to vascular and cardiac defects ^{72,144}
GJC2	Cx46.6/ Cx47	Cx47	Pelizaeus-Merzbacher–like disease ¹⁴⁵	Mild myelination defects ¹⁴⁶
GJC3	Cx31.3/ Cx30.2	Cx29	Hearing loss ^{115,147}	/
GJD2	Cx36	Cx36	/	Visual transmission defects ¹⁴⁸
GJD3	Cx31.9	Cx30.2	/	Increase in cardiac impulse propagation ¹⁴⁹
GJD4	Cx40.1	Cx39	/	/
GJE1	Cx23	Cx23	/	/

Information about the recommended names of connexins and their corresponding molecular sizes in human and mouse is extracted from the UniProtKB database (<http://www.uniprot.org>, accession date: 11 May 2010). Molecular sizes of connexins in mouse are the same as those in rats although some members are yet to be identified in rats. “/”, not identified.

Table 2. Modulation of connexins and their effects on barrier integrity

	Modulation	Barrier Integrity	Other Observations	Tissue or Cell Line
Cx26	Overexpression	/	Prevent Na ⁺ /K ⁺ ATPase inhibitor ouabain-induced TJ barrier disruption, even in the presence of gap junction blockers 18β-glycyrrhetic acid or oleamide; Increase in Cldn14 expression	Human transformed bronchial epithelial cell line Calu-3 ¹⁵⁰
	Overexpression	Increase	Increase in Cldn4 expression; the increase in barrier integrity can be disrupted by oleic acid, taurocholic acid and 18α-glycyrrhetic acid	Human colonic cell line Caco-2 ¹⁵¹
	Ectopic expression with epidermis-specific promoter	Decrease	Disruption of epidermal barrier acquisition during development and recovery of epidermal barrier after wounding; increase in ATP release	Epidermis of genetically modified mice ⁸⁸
	Carriers of R134W Cx26 allele (loss of function mutant)	/	Increase in epidermal thickness	Population study of human epidermis ⁸⁷
	Overexpression of R134W mutant (loss of function mutant)	/	Increase in epidermal thickness	Coculture of human keratinocyte cell line nTERT and human cervical cancer cell line HeLa ⁸⁹
	Overexpression	/	Increase in invasion of enteric pathogen <i>Shigella flexneri</i> bacteria	Coculture of human keratinocyte cell line nTERT and human cervical cancer cell line HeLa ⁸⁹
	Carriers of 35delG Cx26 allele (loss of function mutant)	/	Increase in epidermal thickness	Population study of human epidermis ⁸⁶

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Table 2. Continued

	Modulation	Barrier Integrity	Other Observations	Tissue or Cell Line
	Overexpression	/	Increase the dissemination of enteropathogenic bacteria <i>Shigella flexneri</i> ; increase in ATP release	Human cervical cancer cell line HeLa ¹⁵²
Cx30	Knockout	Decrease	Independent of gap junction channel activity as GJ structures are lacking in normal mice	Intrastrial fluid–blood barrier in cochlear of <i>Cx30</i> ^{-/-} mice ¹⁵³
Cx32	Overexpression	No change	Induction of TJ strands and occludin level	Mouse hepatocyte cell line CHST8 ¹⁵⁴
	Ectopic expression	Slight increase	Increase in levels and/or localization at cell borders of occludin, claudin-1, ZO-1 and ZO-1, which can be reversed by 18 β -glycyrrhetic acid; these observations are absent in Cx26 or Cx43 transfectants	Immortalized Cx32-deficient mouse hepatocytes ¹⁵⁵
Cx43	Conditional knockout	/	Acceleration of wound closure	Epidermis of <i>Cx43</i> ^{Cre-ER(T)/fl} mice ⁹¹
	Knockdown with antisense oligo DNA	/	Acceleration of wound closure	Mice epidermis ⁹²
	Overexpression of Cx43K258Stop	Defective	Doubled half-life in Cx43K258Stop, which does not carry the C-terminal tail, as shown in HeLa cells	Epidermis of Cx43K258stop knockin mice ⁹⁰
	Knockdown with siRNA	No change	Disruption of barrier integrity only with a concurrent knockdown of Cx43 and desmosome protein plakophilin-2	Primary culture of Sertoli cells ⁶⁰

Life Cycle of Connexins

Connexins typically have short half-life of about 1.5-6 hours in mammalian cells.¹⁷ Majority of connexins, with the exception of Cx26, are translated in the rough endoplasmic reticulum (ER)¹⁸ and then oligomerize to form connexons in the

ER, ER–Golgi intermediate compartment or trans-Golgi network, depending on the individual connexins.^{18,19} Cx26, however, could be synthesized outside of the Golgi-based secretory pathway and inserted directly to the plasma membrane via microtubules.²⁰ All connexins are capable of forming monomeric channels. When cells express more than one connexin at one time, heteromeric connexons may be formed but the compatibility of connexins to form heteromeric connexons depends on individual protein structure. Thus, it is not surprising to find heteromeric connexons consisting of connexins in the same subgroup. For instance, Cx43 and Cx46 in the α subgroup²¹ and Cx26 and Cx30 in the β subgroup²² have been demonstrated to form heteromeric channels.

After oligomerization, connexons are inserted into the plasma membrane.^{18,23} Gap junction channels are assembled upon docking of connexons with compatible connexons on the apposing cell surface. Connexons until then remain “closed” to avoid unregulated and unwanted flow of materials from the intracellular compartment or between cells. The regulation of the opening of hemichannels and gap junction channels are to be discussed below. As mentioned above, connexon interactions can be homotypic or heterotypic. Connexons would sometimes aggregate to form gap junction plaques, which can contain up to thousands of connexons, between adjacent cells.^{18,19} Connexons can also be targeted to specific domains on the membrane,^{19,23} such as to cell adhesion site by microtubules.²⁴

In gap junction plaques, gap junction channels that need to be metabolically degraded are internalized at the central region as double membrane vesicles into one of the apposing cells while new connexons are recruited to the periphery of the plaque. The internalized structure is called annular gap junction or connexosome and is targeted to lysosomes for degradation.¹⁸

Gating of Connexons

Connexons or pannexons alike have gating mechanisms to avoid unwanted flow of solutes.⁷ Their openings are not “all or none” but are graded so that there are different levels of conductance, ranging between the fully “open” and “closed” state.^{7,25} Different connexins display different regulatory mechanisms. The factors regulating the opening of connexins include intracellular and extracellular calcium concentration, voltage, mechanical stress, intracellular pH, redox potential and phosphorylation status of connexins.^{7,12}

Phosphorylation of connexins serves as an important means for protein kinases in different signaling pathways to regulate the connexin gating.^{13,26} Studies have shown different phosphorylation patterns of connexins under various physiological conditions.^{27,28} For Cx43, many phosphorylation sites have been identified at its intracellular C-terminal tail.¹³ Phosphorylation of Cx43 by kinases such as c-Src, MAPK, protein kinase C would result in a decline in GJIC whilst protein kinase A and casein kinase 1 can phosphorylate Cx43 to induce an increase in GJIC. A shift in the phosphorylation level of Cx43 at Ser-368, an inhibitory form, has been detected at different phases of cell cycle²⁸ and during development from embryonic stage to adulthood in mice.²⁷ Hence, changes in the phosphorylation status of connexins plays a role to induce changes in GJIC under different physiological conditions.

Selective Permeabilities of Connexins

Gap junction channels had been viewed as channels allowing passive nonspecific diffusion of solutes less than 1.0 to 1.5 kDa in molecular mass,^{29,30} ranging from

inorganic ions, ATP, cyclic nucleotides, siRNA, glucose to polypeptides.³¹ However, gap junction channels made of different connexins have been shown to process selective permeabilities (also called permselectivity) even towards similar solutes.^{2,31-35} These selectivities include ionic charge and molecular size, while other factors are still being identified. Early studies by Goldberg et al.^{33,36} provide a clear demonstration of this. Cx43 channels are more permeable to metabolites like ADP and ATP than Cx32 channels while Cx43 channels are less permeable to adenosine and calcein than Cx32 channels. Another example is the *in vitro* passage of siRNA through Cx43 channels, but not Cx26/Cx32 channels.³⁷ The rate of transfer is also inversely proportional to the length of siRNA.

For heteromeric channels, permeabilities are determined by their parental connexins. An early study has demonstrated the differences in permeabilities of homomeric Cx32 and heteromeric Cx26/Cx32 hemichannels.³⁸ While homomeric Cx32 hemichannels are similarly permeable to cAMP and cGMP, heteromeric Cx26/Cx32 hemichannels are more permeable to cGMP than cAMP. Heteromeric Cx43/Cx45 channels have also been shown to have unitary conductances that vary from their respective homomeric channels.³⁹ These functional diversities of connexins and connexons thus provide an explanation for the existence of a large number of connexins and human genetic diseases due to mutations in connexins (Table 1).

Modulators of Gap Junction Communication for Functional Studies

To perform functional studies of gap junction channels or hemichannels, modulation of their activities seems to be a necessity. Inhibition by RNAi or gene knockout model remains useful for the functional study of individual connexins but chemical modulators can also serve as convenient tools for functional studies (see Table 3). However, specificity of chemical modulators remains a concern.^{7,40} Some widely used modulators, such as 18 α -glycyrrhetic acid and oleamide, have indirect actions on connexins and likely affect other signaling pathways besides GJIC.⁴⁰ In addition, these inhibitors inhibit connexin and pannexins channels at similar concentrations.⁷ A comprehensive list of the effective concentration of these pharmacological inhibitors on connexin channels, hemichannels, pannexins hemichannels or other membrane channels, namely P2X₇, ATP channel and volume-regulated anion channel, has been provided by D'hondt et al.⁷

Additionally, mimetic peptides of connexins and pannexins were shown as specific inhibitors to study GJIC.⁴⁰ But they were later shown to exert steric inhibition rather than sequence-specific inhibition.⁴¹ Cross-reactivity is another concern using mimetic peptides for functional studies since pannexins mimetic peptide were shown to inhibit Cx46 channels as well as pannexins hemichannels.⁴¹ Therefore, much caution is needed to interpret results derived from studies using pharmacological inhibitors or mimetic peptides.

Assessment of Gap Junction Activity

GJIC or permeability of gap junctions is assessed by measuring the unitary conductance by patch-clamp technique or the flow of fluorescent or radioactive probes across gap junction channels or hemichannels.^{25,31} While unitary conductance is measured with the patch-clamp technique,⁴² the dye transfer assay has more varieties including the choice of dyes with different properties and different ways to introduce

Table 3. Gap junction blockers and their effects on barrier integrity

Chemical Modulator(s)	Concentration	Barrier Integrity	Other Observations	
18 β -glycyrrhetic acid	10 μ M	/	No observable changes in distribution of occludin, ZO-1 and NCAM of TJ and N-cadherin and β -catenin of AJ	Primary culture of embryonic chicken lens epithelial cells ¹⁵⁶
Oleic acid and taurocholic acid	3 mM and 4.5 mM respectively	Decrease	/	Human colonic cell line Caco-2 ¹⁵¹
18 β -glycyrrhetic acid or oleamide	5-20 μ M or 25-100 μ M respectively	Decrease	No significant change (in terms of protein level or distribution) in Cx40, Cx43, occludin, claudin-5, JAM-A, JAM-B, JAM-C and ZO-1	Primary porcine brain microvascular endothelial cells ⁷⁴
18 β -glycyrrhetic acid	20 μ M	Decrease	No significant change (in terms of protein level or distribution) of claudin-1 and ZO-1	Rat lung endothelial cell line RLE:rtTA:CL1 ⁷⁴
Octanol or 18 α -glycyrrhetic acid	500 μ M or 35 μ M respectively	/	Reduction of monocyte/macrophage transmigration across a blood brain barrier model induced by TNF α and IFN γ	Cocultures of human fetal astrocytes and human umbilical vein endothelial cell HUVEC and freshly isolated human monocytes ¹⁵⁷
Oleic acid (oleamide) or 18 α -glyceric acid	10 μ M or 10 μ M respectively	/	Decrease in enterocyte migration which is necessary for restitution of mucosal barrier	Primary culture of mouse intestinal epithelial cells ¹⁵⁸

the dye. Commonly used cell membrane impermeable dyes such as Lucifer yellow and neurobiotin, which are of different sizes and charges, can be introduced into selective cell or area of cells by microinjection, electroporation or scrape-loading.^{32,35,39,41,43,44} A cell membrane permeable dye named calcein AM can also be used to label epithelial cells in vitro. This dye is converted in living cells into cell membrane impermeable calcein.⁴⁵ The transfer of dye between cells can be assessed by incubating labeled cells with unlabeled ones⁴⁵ or using the fluorescence recovery after photobleaching technique.⁴⁶ To investigate the selective permeability towards specific metabolites, metabolites labeled with radioactive probes are also used.^{33,36}

CONNEXINS/GJ AND BARRIER FUNCTION

The physiological importance of connexins in various systems is demonstrated by the defects caused by mutations or ablation of connexins (Table 1). Our discussion in this section is limited to the roles of connexin-based gap junctions in maintaining blood-tissue barrier integrity. Readers are encouraged to consult other reviews for the roles of connexins in other systems, including the cardiac, neuronal and reproductive systems.^{4,47-49}

Interaction of Connexins and Junction Associated Proteins

Multiple junction proteins, including integral membrane proteins, scaffolding proteins and cytoskeletal proteins have been shown to interact with connexins.^{23,50} This information illustrates that connexins are part of the multiprotein junction complexes, suggesting that gap junctions may modulate different junction types in different epithelia.⁵¹

Interaction of tight junction members, such as occludin, claudin-1, claudin-5, ZO-1 and ZO-2, with connexins were mostly demonstrated by colocalization and co-immunoprecipitation.⁵⁰ Direct association of Cx43 with ZO-1 and ZO-2 was also demonstrated.^{52,53} The interaction of ZO-1 and Cx43 is important for the stabilization of Cx43 in the plasma membrane. The association of ZO-1 and F-actin binding protein drebrin with Cx43 was suggested for anchoring gap junction plaques to the actin cytoskeleton.²³ c-Src and ZO-1 also bind competitively to the C-terminal tail of Cx43.⁵⁴ The binding of c-Src to Cx43 and hence dissociation of ZO-1 from Cx43 was shown to drive the internalization of gap junction plaque from the cell membrane⁵⁵ and inhibit GJIC.^{56,57}

For adherens junction, an early study showed that the assembly of adherens junction and gap junction are interdependent. Although adherens junction are assembled at the cell-cell interface prior to gap junction formation, addition of antibodies against either N-cadherin or Cx43 could abolish the assembly of both junction types.⁵⁸ Cx43 can be transported to N-cadherin at existing adhesion site²⁴ and it was shown to colocalize and co-immunoprecipitate with AJ proteins N-cadherin and β -catenin.^{59,60} These reports thus supported a close physical and functional association between AJ and GJ.

GJ is also working closely with desmosomes in heart and reproductive organs. For instance, arrhythmogenic right ventricular cardiomyopathy is a hereditary disease of heart muscle caused by mutations in desmosomal proteins including plakoglobin and plakophilin-2.⁶¹ Patients with this disease had a lower level of Cx43 at the cell surface.^{62,63} The knockdown of plakophilin-2 by RNAi was shown to cause a reduction in Cx43 level and GJIC^{63,64} while plakophilin-2 can physically associate with Cx43.^{60,64} In the ovary and testis, junction complexes bearing ultrastructural properties of both desmosomes and GJ have been identified and are named desmosome-like or desmosome-gap junction.⁶⁵⁻⁶⁸

In short, connexins interact with various junction proteins and their associated scaffolding and signaling proteins in a tissue-dependent manner. The implications of these associations in regulating the homeostasis of barrier integrity are discussed below.

Endothelial Blood-Tissue Barrier

Various blood-tissue barriers are formed by TJ barrier between endothelial vascular cells and these include the blood-brain barrier, inner blood-retinal (also known as blood-ocular) barrier.^{69,70} Additional reinforcement by epithelial cells, such as pericytes, also contribute to the blood-brain barrier.⁷⁰ Some barriers, such as blood-aqueous barrier

and blood-retinal barrier, consist of more than one layer of TJ barrier formed by both vascular and epithelial cells.⁷¹

As shown in knockout animals, some connexins are required for proper vascular development. For instance, Cx45 is present in the endothelial cells and smooth muscle cells of all blood vessels. Embryonic lethality in *Cx45*^{-/-} mice was accompanied by defects in vascular development.⁷² Perinatal death was noted in *Cx37*^{-/-}/*Cx40*^{-/-} double knockout mice, but not in single *Cx37*^{-/-} or *Cx40*^{-/-} knockout mice. Vascular defects in *Cx37*^{-/-}/*Cx40*^{-/-} double knockout mice were exhibited by hemorrhages in certain tissues, in particular testis and intestine.⁷³ These studies illustrate the importance of connexins in vascular development. In addition, studies have suggested the importance of gap junction activity in the maintenance of tight junction barrier integrity of vascular cells. In primary cultures of vascular cells from porcine brain, addition of GJ blocker, 18 β -glycyrrhetic acid (5-20 μ M) or oleamide (25-100 μ M), can significantly inhibit the barrier integrity.⁷⁴ In another study of rat blood-brain barrier, the reversible barrier disruption induced by ultrasound was accompanied by a redistribution of gap junction plaques. An increase in size of Cx43 and Cx36 based-GJ plaques was observed during blood-brain barrier disruption.⁷⁵ These reports illustrate the importance of connexins and GJ in vascular development and maintenance of endothelial vascular barrier.

Blood-Testis Barrier

Blood-testis barrier is notably different from the endothelial blood-tissue barrier mentioned above (see Fig. 2). This barrier is constituted by adjacent Sertoli cells residing in the seminiferous epithelium, near the basement membrane in adult mammalian testes, instead of endothelial vascular cells found in the interstitium. Secondly, it is formed at the basal, instead of the apical, side of Sertoli cells. Thirdly, its structural components include not only tight junctions, but also atypical adherens junctions (basal ectoplasmic specialization), desmosome-gap junctions and GJ.^{66,76,77} Major functions of this barrier include providing immunological protection to developing germ cells and creating a microenvironment for the development of postmeiotic male germ cells, known as the apical compartment, during spermatogenesis^{77,78} (Fig. 2).

Of the various connexins expressed by Sertoli cells,⁴⁸ Cx43 is a promising candidate that regulates the integrity of blood-testis barrier. While spermatogenesis defects were not reported in mice with Cx31, Cx32, Cx37, Cx40, or Cx46 knockout,⁴⁸ Sertoli cell specific Cx43 knockout causes impaired spermatogenesis, leading to infertility in homozygous male mice.⁷⁹ Further analysis reveals that these Sertoli cells without Cx43 stay in the proliferative phase without differentiation.⁸⁰ Since the establishment of functional blood-testis barrier has been associated with differentiation of Sertoli cells,⁸¹ it is tempting to speculate that Cx43 may be a prerequisite for the establishment of blood-testis barrier.

A recent report from our research group indicated that Cx43 co-operates with desmosomal protein plakophilin-2 in the maintenance of the blood-testis barrier integrity. Simultaneous knockdown of both Cx43 and plakophilin-2, but not either one alone, would disrupt the barrier integrity in primary culture of Sertoli cells.⁶⁰ In addition, a dual-knockdown of desmoglein-2 and desmocollin-2, which are integral membrane proteins of desmosomes, would perturb the TJ-permeability integrity in primary Sertoli cell cultures, partly via enhancing the rate of endocytosis of TJ protein CAR.⁸² We

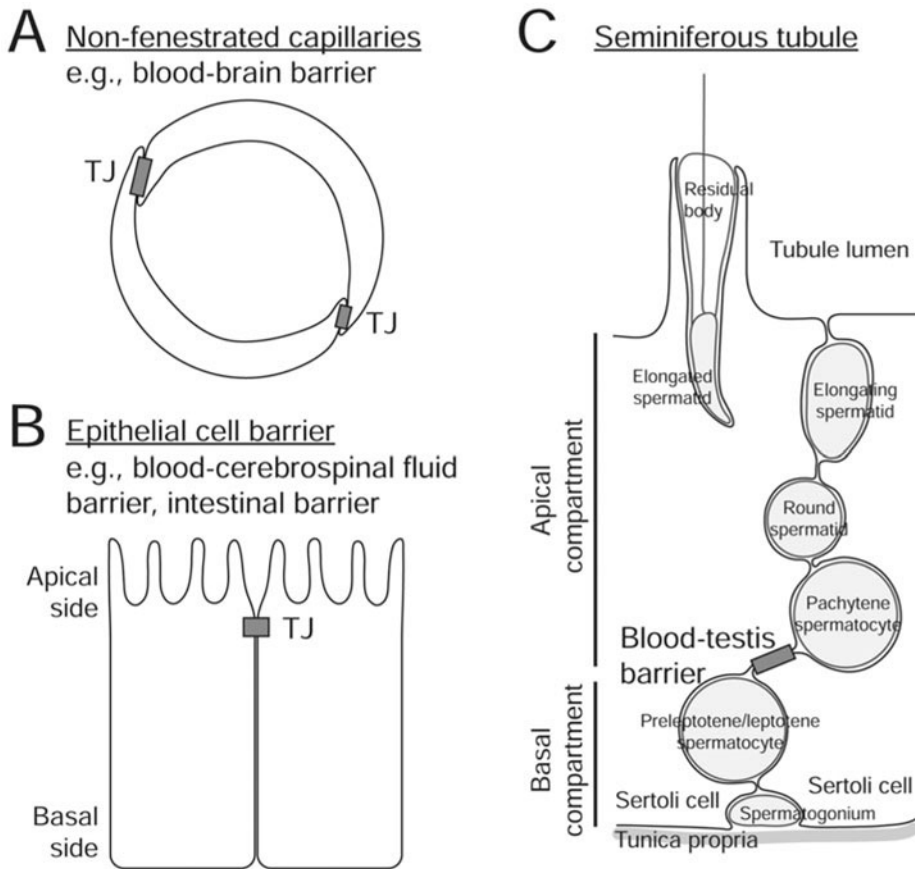


Figure 2. This figure illustrates the major morphological features of various types of blood-tissue barriers. A) Blood-tissue barriers, including blood-brain barrier, could be constituted by TJ formed between endothelial vascular cells in nonfenestrated capillaries. B) Blood-tissue barriers could also be formed by epithelial cells. At the blood-cerebrospinal fluid barrier, the blood vessel is fenestrated (without TJ) and TJs formed at the apical region of adjacent choroid plexus epithelial cells constitute the barrier. C) The blood-testis barrier is located in the seminiferous epithelium of the seminiferous tubule, which is formed near the basal region of adjacent Sertoli cells. Different junction complexes have been identified at this site, including TJ, basal ES (an atypical AJ), desmosome-like junction and GJ. The blood-testis barrier also segregates the seminiferous epithelium into the basal and apical (or adluminal) compartment, so that meiosis and the entire events of postmeiotic germ cell development (i.e., spermiogenesis) take place behind this immunological barrier in a specialized microenvironment.

postulate that when primary spermatocytes are in transit at the blood-testis barrier, such as at Stage VIII of the seminiferous epithelial cycle, the AJ, GJ and desmosome formed between Sertoli cells would be replaced by those between Sertoli cell and spermatocytes (Fig. 3). From these reports, a reduction of GJ and desmosome between adjacent Sertoli cells would induce blood-testis barrier disruption. This includes a decline in the steady-state levels of TJ and AJ proteins at the Sertoli cell surface, which is partly mediated by an increase in endocytosis of junction proteins. Thus, it resulted in an increase in the permeability at the apical region of the translocating spermatocytes to

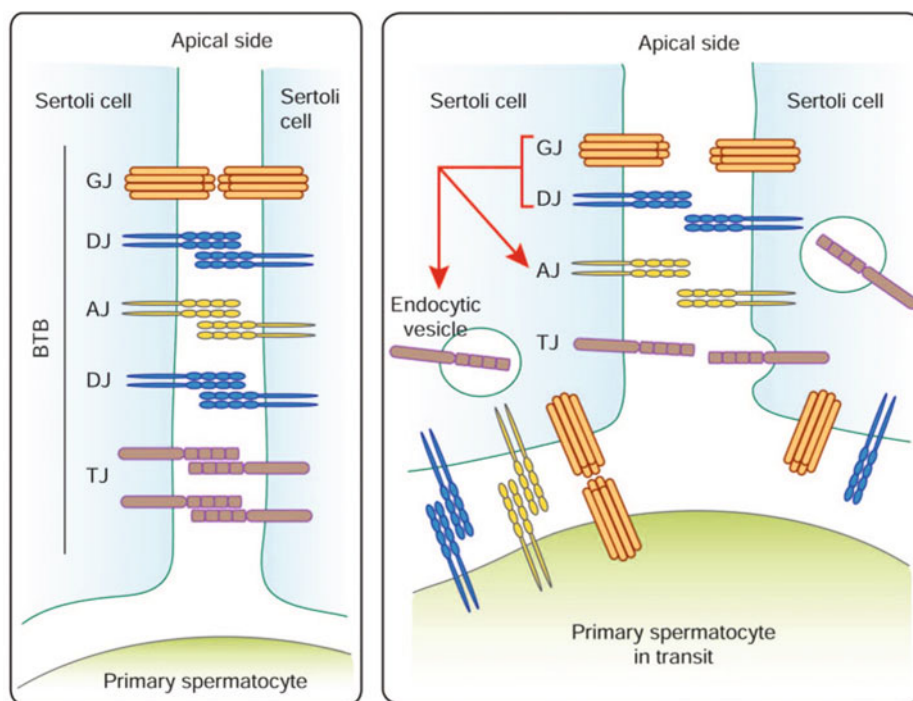


Figure 3. Schematic illustration of the roles of GJ and desmosome at the blood-testis barrier. The blood-testis barrier (BTB) remains intact at most stages of the seminiferous epithelial cycle and consists of coexisting TJ, AJ, GJ and desmosome-like junction (DJ) (left panel). Primary spermatocytes migrate across the BTB at Stage VIII of the seminiferous epithelial cycle in the rat testis (right panel). The AJ, DJ and GJ formed between Sertoli cells are likely replaced by those between Sertoli cell and spermatocyte since it is now known that many of AJ, DJ and GJ proteins are also found in germ cells, such as spermatocytes.⁷⁶⁻⁷⁷ A reduction of GJ and DJ between adjacent Sertoli cells would destabilize the BTB, inducing its disruption. This involves a decline in the steady-state levels of TJ and AJ proteins at the Sertoli cell surface, which is partly mediated by an increase in endocytosis of junction proteins. The net result is an increase in the permeability at the apical region of the translocating spermatocytes to facilitate their transit at the BTB. However, “new” TJ, AJ, DJ and GJ are formed behind the spermatocytes in transit before the “old” junctions are being disrupted, so that the immunological barrier can be maintained.

facilitate its translocation. Cx43 hence likely serves as a regulator of the blood-testis barrier homeostasis by maintaining the crucial crosstalk among different coexisting junction types at the blood-testis barrier (Fig. 3).

Epidermal Barrier

Epidermal barrier of mammalian skin serves as the first line of defense against pathogens and other harmful substances.¹ At least nine connexins are expressed at different layers of epidermis except the uppermost layer called stratum corneum.² Influences of connexins on epidermal barrier integrity are exemplified by their effects on epidermal thickness and wound repair process. Multiple human hereditary diseases in skin with mutations in Cx26, Cx30, Cx30.3 and Cx31, have been discovered.⁸³⁻⁸⁵ This illustrates

the necessity of connexins in maintaining the epidermis homeostasis and epidermal barrier. For instance, Cx30 mutants could result in hidrotic ectodermal dysplasia,⁸⁴ with symptoms including eczematous dermatitis.

Cx26 in particular has been the focus of much research since the loss of function mutants of Cx26 that lead to nonsyndromic hearing loss would give heterozygous individuals an advantageous edge of an increase in epidermal thickness.^{86,87} An ectopic Cx26 overexpression in mice epidermis would however disrupt the epidermal barrier development and wound healing process.⁸⁸ Another study using cocultures of keratinocytes and HeLa cells demonstrated that the invasion of enteric pathogen *S. flexneri* could be enhanced by overexpression of Cx26, but not its loss of function mutant.⁸⁹ These reports collectively illustrate the inhibitory effect of Cx26 on the establishment, recovery and hence integrity of epidermal barrier.

Cx43, which displays a broad expression profile in epidermis, has been shown to regulate the epidermal barrier in animal studies even though Cx43 mutants are yet to be associated with skin abnormalities in humans. Knockin mice with Cx43 carrying no C-terminal tail (Cx43K258Stop) have perinatal death due to epidermal barrier defects. The truncated Cx43 mutant without C-terminal tail also form GJ channels and has a doubled half-life than Cx43.⁹⁰ Mice having an epidermis-specific Cx43 knockout or knockdown display an acceleration of wound closure.^{91,92} These illustrate that a decline in Cx43 level is probably required for epidermal barrier establishment and wound repair.

CONNEXIN-MEDIATED BYSTANDER EFFECTS

Connexins Mediating Harmful Signals

The above discussion illustrates the regulatory roles of connexins on the homeostasis of different blood-tissue barriers. Most barriers serve primarily as selective permeability barrier to isolate and protect cells behind the barriers from harmful substances such as pathogens.¹ While connexins could regulate barrier integrity, they could also be responsible for mediating harmful signals under pathological conditions. For instance, in intestinal barrier, Cx43 hemichannel was recently shown to mediate infection of enteric pathogen *Citrobacter rodentium*.⁹³ Water loss following *C. rodentium* incubation, as assessed by the water content in distal colon, was significantly reduced in heterozygous *Cx43*^{+/-} mice. In addition, connexins have been implicated in tumorigenesis.⁹⁴ Tumor cell migration and attachment during metastasis was shown to be induced by Cx43.⁹⁵ This is probably due to the close structural association of connexins and adhesion molecules as discussed above so that an alteration of Cx43 would lead to changes in cell adhesion and cell migration.

Bystander Killing by Connexins

Due to their versatility, gap junction channels or hemichannels are capable of transferring harmful signals between neighboring cells. Bystander effect is a term used to describe the spread and amplification of harmful signals from cells directly exposed to insults to neighboring cells. These insults include radiation, inflammation and viral transfection.⁹⁶⁻⁹⁹ Exposure to very low influences of α -particles could induce DNA damage in non-irradiated cells in skin and lung fibroblasts cultures.⁹⁹ After spinal cord

injury, Cx43 was upregulated. Rats with a knockdown of Cx43 by Cx43 antisense oligodeoxynucleotides showed reduced inflammation and a faster functional recovery.⁹⁸ GJIC was also shown to be responsible for mediating the transfer of apoptotic signals from HIV-infected astrocytes to non-infected ones.⁹⁷

Bystander effect can be observed even across an intact barrier. A recent study has shown the damage caused by indirect exposure to cobalt-chromium nanoparticles in human fibroblast cells across an intact layer of BeWo cells.¹⁰⁰ The DNA damage resulted in fibroblast cells was reduced by gap junction mimetic peptide GAP26 while it was potentiated by antiarrhythmic peptide AAP10,¹⁰⁰ an upregulator of GJIC.¹⁰¹ Furthermore, regional X-ray irradiation of the lower body part of mice induced DNA damage and apoptosis in mouse cerebella, which are behind the blood-brain barrier. The use of GJIC inhibitor 12-*O*-tetradecanoylphorbol-13-acetate could reduce these bystander effects.¹⁰²

Potential Uses of Connexin-Bystander Effect

Apart from the bystander deaths mediated by gap junctions, bystander effect is beneficial under certain circumstances. Preconditioning in heart and brain involves exposing bystander cells to stress but not yet damaging stimuli, which results in better resistance towards higher and damaging levels of stimuli during subsequent exposures.^{103,104} Studies utilizing Cx43-deficient mice reported the absence of preconditioning in heart and brain of Cx43-deficient mice, illustrating Cx43 as a prerequisite for preconditioning.^{105,106} The role of connexins in preconditioning in heart has been recently reviewed.¹⁰⁷ In addition, the possibility of utilizing the bystander effect in cancer therapy has been explored. A recent review discussed the possibility of taking advantage of the bystander effect in radiation-related cancer therapy to amplify the harmful effects of radioactive isotopes or external radiation to tumor cells.¹⁰⁸ A potential gene therapy for cancer treatment involves the targeted introduction of thymidine kinase gene by virus into tumor cells, which is necessary for the processing of an antiviral drug named ganciclovir into its toxic phosphorylated form.^{94,109} It has been shown that GJ would again increase the range of the toxicity due to its bystander effect.^{96,110} These studies draw attentions not only to the safety of medical use of nanoparticles and radiation, but also to the potential uses of gap junctions to amplify signals, such as during cancer therapy.

CONCLUSION AND FUTURE PERSPECTIVES

Herein we summarize some of the latest findings in the field regarding the role of GJ and GJIC in the normal functioning of blood-tissue barriers. Earlier morphological studies have shown that in most blood-tissue barriers with the exception of the blood-testis barrier, GJs are present in discrete cellular localization at the paracellular site, being segregated from the tight and anchoring junctions.¹ Recent studies have shown that some GJ are present in the junctional complexes besides the GJ plaques to provide the necessary communications between cells to maintain the homeostasis of an epithelium including the TJ barrier function.¹ We also provide an updated molecular model regarding the crucial role of GJ in the blood-testis barrier dynamics by coordinating different *coexisting* junction types at the blood-testis barrier to facilitate the transit of primary spermatocytes, namely preleptotene spermatocytes, while maintaining the immunological barrier integrity (see Fig. 3).

Based on the latest findings that support this model, it is very likely that GJ is working beyond its “traditional” role of serving as a channel for the transport of chemical signals between cells. Perhaps other important biomolecules, such as electrolytes, ions, small molecular drugs, and paracrine factors are being actively transported across adjacent cells in a cell epithelium to synchronize cellular events. As a result, an entire epithelium can respond to the challenge of an external cue and/or stimulus during a complex molecular event, such as growth, differentiation, development and spermatogenesis.

In light of the recent advances in the role of GJ in blood-tissue barriers, such as the blood-testis barrier, which determines and/or dictates which drug(s) and how much of a drug can traverse the barrier to enter the apical compartment, it is also possible that GJ is working in concert with drug transporters, such as influx pumps (e.g., p-glycoprotein) or efflux pumps (e.g., Oatp3), at the blood-testis barrier. This possibility is important and it should be carefully evaluated in future studies to better understand the role of GJ in drug transport at the blood-testis barrier since such studies would have significant impacts to therapeutically manage illnesses. For instance, anti-viral drugs that effectively to reduce the AIDS/HIV-1 viral loads in the blood of AIDS patients fail to reduce the viral content in the semen,^{111,112} making the male reproductive tract a safe haven for HIV-1 mutation. If these drugs could traverse the blood-testis barrier as effectively as in other organs, this would minimize the transmission of AIDS from infected individuals to their partners.

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