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Review

Blood–Bile Barrier: Morphology, Regulation, and Pathophysiology

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The term blood–bile barrier (BBlB) refers to the physical structure within a hepatic lobule that compartmentalizes and hence segregates sinusoidal blood from canalicular bile. Thus, this barrier provides physiological protection in the liver, shielding the hepatocytes from bile toxicity and restricting the mixing of blood and bile. BBlB is primarily composed of tight junctions; however, adherens junction, desmosomes, gap junctions, and hepatocyte bile transporters also contribute to the barrier function of the BBlB. Recent findings also suggest that disruption of BBlB is associated with major hepatic diseases characterized by cholestasis and aberrations in BBlB thus may be a hallmark of many chronic liver diseases. Several molecular signaling pathways have now been shown to play a role in regulating the structure and function and eventually contribute to regulation of the BBlB function within the liver. In this review, we will discuss the structure and function of the BBlB, summarize the methods to assess the integrity and function of BBlB, discuss the role of BBlB in liver pathophysiology, and finally, discuss the mechanisms of BBlB regulation. Collectively, this review will demonstrate the significance of the BBlB in both liver homeostasis and hepatic dysfunction.

Key words: Blood–bile barrier (BBlB); Liver tight junction; Adherens junction; Paracellular space; Hepatocyte polarity

INTRODUCTION

Epithelial barriers are indispensable to the health and function of any normal tissue. These barriers function to exclude macromolecules from the epithelia, promote host defense against microorganisms, and protect from injury and inflammation. The inception of the term epithelial barrier dates back to the turn of the 19th century as an explanation of the property of a living brain to exclude certain dyes and pharmacologically active compounds when injected either into the bloodstream or into the cerebrospinal fluid of experimental animals[1]. Common examples of epithelial barriers in addition to the blood–brain barrier include the blood–placenta barrier, the blood–retina barrier, the blood–mucosal barrier, the blood–air barrier, and the blood–testis barrier. Another important and relatively less well-understood barrier is the blood–bile barrier (BBlB), which exists in the liver and segregates blood within hepatic sinusoids from the bile in the biliary canaliculi, thus restricting the mixing of blood and bile.

STRUCTURE OF BLOOD–BILE BARRIER

Liver, the largest internal organ and next to brain in functional complexity, performs a wide variety of metabolic and regulatory functions. Hepatocytes are the structural and functional units of liver and carry out many vital tasks including synthesis, detoxification, and metabolism of carbohydrates, protein, and lipids. In the adult liver, hepatocytes have three distinct membrane domains: sinusoidal/basal, lateral, and canalicular/apical[2] (Fig. 1). The sinusoidal membrane domains face the hepatic sinusoids,

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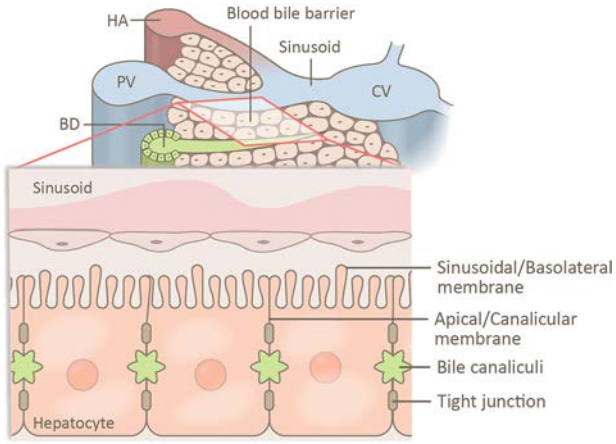

Figure 1. Barriers of the liver. Schematic depiction of the blood–bile barrier (BBlB) in the liver. The BBlB is composed of mainly tight junctions present at the apical membrane domain of the hepatocytes, which restricts the mixing of sinusoidal blood and bile. The hepatocytes polarize along the apical and basolateral membrane; the basolateral membrane faces the sinusoids, whereas the apical membrane faces the bile canaliculi. HA, hepatic artery; PV, portal vein; CV, central vein; BD, bile duct.

which are channels carrying blood from the hepatic artery and portal vein to the central vein and are lined loosely by hepatic sinusoidal endothelial cells. The canalicular membrane domain of hepatocytes forms the bile canalicular network that transports bile acids from hepatocytes to portal triad and into the intrahepatic bile ducts. The lateral plasma membrane domains of hepatocytes fuse alongside the bile canaliculi to form tight junctions (zonula occludens) that separate the apical/canalicular domain from the sinusoidal/basal surface by forming the BBlB (Fig. 1). Thus, the BBlB is mainly composed of tight junctions (TJs) between hepatocytes surrounding the bile canaliculi, where they function to seal the paracellular spaces between hepatocytes and at the same time impart and maintain hepatocyte polarity. Adherens junctions (AJs), desmosomes, and gap junctions (GJs) are also known to promote barrier function[3,4]. Hepatocytes also express highly polarized, specific transporters that actively regulate the BBlB by enabling the collection of bile inside the bile canaliculi[5,6]. Along with hepatocytes, barrier-promoting TJs are also present in cholangiocytes, which line the intrahepatic bile ducts and serve as conduits to transport bile from the liver to the extrahepatic biliary tree and eventually to the small intestine[7,8].

MOLECULAR COMPOSITION OF THE BBLB

Tight Junctions (TJs)

TJs are primarily responsible for the barrier properties of the BBlB. These structures are localized apically separating the apical membrane from the basolateral membrane. The three main families of hepatic TJ proteins are claudins, TJ-associated MARVEL proteins (TAMPs), and the cortical thymocyte marker in *Xenopus* (CTX)[9]. Occludin, MarvelD3, and tricellulin constitute the TAMP family of proteins[10]. The epithelial CTX family includes junctional adhesion molecule (JAM-A), coxsackievirus and adenovirus receptor (CAR), and CXADR-like membrane protein (CLMP)[11]. Except for JAM-A, all transmembrane proteins of TJs are tetraspanins with four transmembrane domains forming two extracellular loops and one intracellular loop. The C-terminal domain in the cytosol forms a complex with adaptor proteins such as zonula occludens-1 (ZO-1), ZO-2, and ZO-3[12]. These adaptor proteins interact with many other proteins and anchor themselves to the actin cytoskeleton. Interactions between different TJ proteins and the cytoskeleton are essential for the normal assembly and maintenance of TJ integrity. While the TAMP family of proteins has been implicated in influencing barrier function, proteins such as occludin also have critical signaling properties that regulate epithelial homeostasis[13–15]. The molecular composition of BBlB is depicted in Figure 2 and Table 1.

Claudins. The tetraspanin claudin family of proteins includes 31 family members in humans[14,16–19]. Ultrastructurally, claudins reside in TJs and serve to control the charge and size-selective properties of the paracellular space, thereby regulating barrier properties[10,20,21]. Claudins have been proposed to function either as “tight” plaque-forming claudins or as “leaky” pore-forming claudins. Tight claudins include claudin-1, -3, -4, -5, -6, -8, -12, -18, and -19[22], while leaky claudin-2 and -15 contribute to increased paracellular permeability to sodium

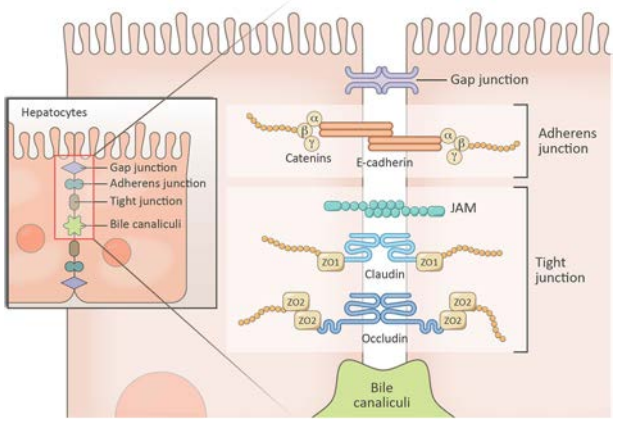

Figure 2. Structure of the BBlB in the liver. The hepatic junctions forming BBlB includes tight junctions, adherens junctions, desmosomes, and gap junctions. The tight junction is formed of claudins, occludin, ZOs, and JAMs. Catenins (-, -catenin, etc.) and cadherins associate with actin cytoskeleton to form the adherens junction. Gap junctions also provide barrier properties to the liver.

Table 1. Molecular Atlas of Blood–Bile Barrier (BBlB): Junctional Proteins and Their Association With Hepatopathophysiology

Cellular Junction/ Component	Abbreviation	Gene	Location	Function	Association With Hepatic Pathophysiology	References
Tight junction						
Claudins	Claudin 1	CLDN1	Hepatocytes and cholangiocytes	Seals the barrier	Cirrhosis, hepatocellular carcinoma, HCV entry, cholestasis, primary sclerosing cholangitis, aging	23, 33, 89, 107, 111, 149
	Claudin 2	CLDN2	Predominantly in hepatocytes	Bile canaliculi formation, downstream target of Wnt pathway	Hepatocyte polarity, cholestasis, hepatocellular carcinoma	22, 24, 27, 34, 55, 83, 89, 182
	Claudin 3	CLDN3	Hepatocytes and cholangiocytes	Seals the barrier	Chronic liver injury, aging	27, 33, 89, 153, 154
	Claudin 4	CLDN4	Not known	Normally absent; expression is associated with liver diseases	Cirrhosis, hepatocellular carcinoma	22, 26, 119, 123, 128
	Claudin 5	CLDN5	Hepatocytes	Seals the barrier	Chronic liver injury	22, 27, 119
	Claudin 6	CLDN6	Weak expression in hepatocytes	Present in liver	HCV entry	22, 112
	Claudin 7	CLDN7	Predominantly cholangiocytes	Associated with barrier function	Cirrhosis, HCC, chronic liver injury	22, 119, 122
	Claudin 8	CLDN8	Weak expression in hepatocytes	Seals the barrier	Liver injury	22, 123
	Claudin 9	CLDN9	Weak expression in hepatocytes	Associated with barrier function	HCV entry	22, 112
	Claudin 10	CLDN10	10a: not present; 10B: present in hepatocytes	Present in liver	HCC	26, 120
	Claudin 11	CLDN11	Weak expression in hepatocytes	Present in liver	HCC	22
	Claudin 12	CLDN12	Predominantly in hepatocytes	Present in liver	Not known	22
	Claudin 13	CLDN13	Not known	Absent in liver	Not known	
	Claudin 14	CLDN14	Present in hepatocytes	Present in liver	Not known	
	Claudin 15	CLDN15	Weak expression in hepatocytes	Intrahepatic biliary duct biogenesis	Hereditary cholestasis	22
	Claudin 16	CLDN16	Weak expression in hepatocytes	Weakly expressed in liver	Not known	
	Claudin 17	CLDN17	Very weak expression in liver	Not known	Not known	
	Claudin 18	CLDN18	Not known	Not known	Not known	22, 127
	Claudin 19	CLDN19	Weak expression in hepatocytes	Present in liver	Not known	22
	Claudin 20	CLDN20	Weak expression in hepatocytes	Not known	Not known	

(*continued*)

Table 1. (Continued)

Cellular Junction/ Component	Abbreviation	Gene	Location	Function	Association With Hepatic Pathophysiology	References
	Claudin 21	CLDN21	Not known	Present in fetal liver	Paracellular barrier	
	Claudin 22	CLDN22	Not known	Not known	Not known	
	Claudin 23	CLDN23	Not known	Not known	Not known	
	Claudin 24	CLDN24	Not known	Not known	Not known	
	Claudin 25	CLDN25	Not known	Not known	Not known	
	Claudin 26	CLDN26	Not known	Not known	Not known	
	Claudin 27	CLDN27	Not known	Not known	Not known	
	Claudin 28	CLDN28	Not known	Not known	Not known	
	Claudin 29	CLDN29	Not known	Not known	Not known	
	Claudin 30	CLDN30	Not known	Not known	Not known	
	Claudin 31	CLDN31	Not known	Not known	Not known	
Occludin	Occludin	OCLN	Predominantly hepatocytes	Acts as a signaling molecule; no role in barrier identified	Chronic liver injury, cholestasis, cirrhosis, HCV entry, HCC, leukocyte trafficking	26, 33, 34, 53, 54, 55, 89, 111, 113, 119, 135, 142, 146, 149, 153, 154, 177, 184, 185, 186, 87
	Occludin1B	OCLN1B	Not known	Present in liver	Not known	25
JAMs	JAM-A	F11R	Hepatocytes and cholangiocytes	Vascular remodeling, hepatocyte polarity	Liver fibrosis, cholestasis	36, 37, 38, 43, 44, 45
	JAM-B	JAM-2	Hepatocytes and cholangiocytes	Vascular remodeling, barrier properties	Autoimmune liver disease, PSC, liver fibrosis	36, 37, 38, 43, 44, 45
	JAM-C	JAM-3	Hepatocytes and cholangiocytes	Maintenance of bile duct structure, barrier properties, leukocyte recruitment	Autoimmune liver disease	36, 37, 38, 43, 44, 45
Membrane-associated guanylate kinase	ZO-1	TJP1	Hepatocytes and cholangiocytes	Acts as an adaptor protein between the TJ and actin cytoskeleton	Liver cholestasis, HCC	6, 12, 45, 51, 52, 56, 119, 133, 134, 149, 184
	ZO-2	TJP2	Hepatocytes and cholangiocytes	Establishes link with the actin cytoskeleton; seals the barrier	PFIC, chronic liver injury, HCC	12, 33, 53, 54, 55, 83, 134, 168
	ZO-3	TJP3	Hepatocytes and cholangiocytes	Present in the liver, acts as an adaptor protein	Not much known	12, 56

Coxsackievirus–adenovirus receptor	CAR1	CAR1	Hepatocytes and cholangiocytes	Expressed mainly in cholangiocyte TJ, barrier function in liver	Viral entry, tissue permeability	46, 47, 48, 119
	CAR2	CAR2	Hepatocytes and cholangiocytes	Barrier function in liver, homeostasis, expressed in cholangiocyte TJ	Viral entry, tissue permeability	46, 47, 48, 119
	CAR3	CAR3	Hepatocytes and cholangiocytes	Barrier function in liver, homeostasis,	Viral entry, tissue permeability	46, 47, 48, 119
Adherens junction						
Cadherins	E-cadherin	Cdh1	Hepatocytes and cholangiocytes	Cell–cell adhesion, cell migration,	Liver fibrosis, HCC, cholestasis, PSC, Liver development	34, 60, 61, 99
Catenins	-catenin	CTNNA1	Hepatocytes, cholangiocytes, endothelial cells	Cell–cell adhesion, actin cytoskeleton organization	Cholestasis	60, 61, 62,
	-catenin	CTNNB1	Hepatocytes, cholangiocytes, endothelial cells	Cell–cell adhesion, actin cytoskeleton organization, hepatocyte polarity, barrier function	Liver fibrosis, HCC, cholestasis, PSC, liver development, regeneration, PFIC, chronic liver injury	29, 34, 52, 59, 61, 77, 88, 90, 156, 182
	P-120	CTNND1	Hepatocytes, cholangiocytes, endothelial cells	Cell–cell adhesion, actin cytoskeleton organization, intrahepatic bile duct development	Cholangiocarcinoma, loss of bile duct structure	
Desmosomes						
Desmosomal proteins	-catenin	JUP	Hepatocytes and cholangiocytes	Cell–cell contact, hepatocyte polarity	Liver fibrosis, HCC, cholestasis, PFIC	34, 68, 69, 70, 88, 90
	Desmoglein	DSG	Hepatocytes and cholangiocytes	Cell–cell adhesion, E-cadherin trafficking	Irritable bowel syndrome	5, 68
	Desmoplakin	DSP		Not known	Not known	68
Gap junctions						
Connexin hemichannels	Connexin-26	Cx-26	Predominantly expressed by hepatocytes; uniformly distributed	Cell–cell communication; required for BBlB	Liver regeneration, liver growth	3, 66, 67
	Connexin-32	Cx-32	Predominantly expressed by hepatocytes; localized in periportal acinar area	Cell–cell communication; protects against acetaminophen-induced injury	Reduced bile flow, aberrant bile duct structure, cholestasis, liver injury	3, 66, 67

and water[22]. Claudin-1, -2, -3, -4, -5, -7, and -10 have been studied in the liver[23–28] . Recent studies indicate the emerging role of these relatively small-sized TJ proteins as signaling molecules[29]. Claudin-1 has also been identified as a receptor for hepatitis C virus (HCV).

Occludin. Occludin was the first TJ protein to be discovered[30]. It has similar structural and functional features to that of claudins, being a molecule with four transmembrane domains, which utilizes a similar binding mechanism to other TJ proteins (Fig. 2)[31]. However, it differs from claudins (25 kDa) in its molecular weight (65 kDa)[13,32]. A variant of occludin, occludin 1B[25], has been identified and differs from occludin in having an extended amino-terminus. Both are found in mouse livers[5,15,33]. The role of occludin in barrier function is controversial as occludin-null mice do not manifest a significant difference in barrier function[30]. Occludin is also emerging as a signaling molecule, and there is increasing evidence of it being regulated by posttranslational modifications[34,35]. Occludin also serves as a receptor for the HCV.

JAMs. JAM[36–38] is a single-pass TJ protein with its amino-terminus in the extracellular region and carboxyl-terminus in the cytoplasm (Fig. 3)[38]. JAM protein from one cell couples with JAM protein of an adjacent cell to form a barrier. At least four members of JAM have been identified, and JAM-1/A, JAM-2/B, and JAM-3/C have been studied in mouse livers[39–42]. Beside barrier function, JAMs are also associated with liver fibrosis, stellate cell activation, leukocyte recruitment, and integrin binding to enhance transendothelial migration in the liver[41,43–45].

CARs. CAR was initially discovered as a new class of viral receptor belonging to the immunoglobulin-like family[46]. Further studies demonstrated that CAR also had TJ functions[46,47]. Among the three isoforms of CAR (i.e., CAR-1, CAR-2, and CAR-3), CAR-2 is present in hepatocytes, and both CAR-1 and CAR-3 are associated with cholangiocytes in mouse livers[48]. CARs act as an integral membrane protein to provide TJ integrity and stability[49].

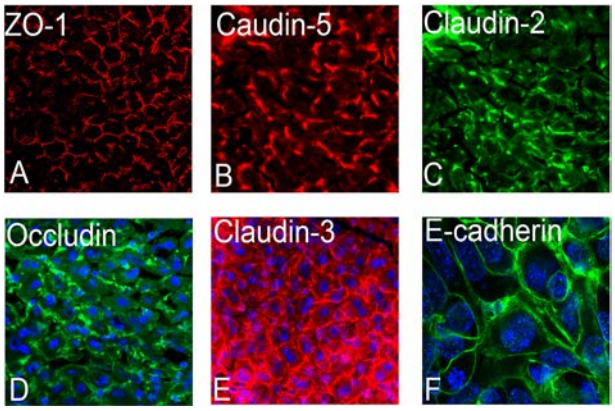

Figure 3. Immunofluorescence analysis of chief junctional components of the BBlB in the liver. (A) ZO-1, (B) claudin-5, (C) claudin-2, (D) occludin, (E) claudin-3, (F) E-cadherin staining in mouse liver tissue section exhibit junctional localization.

ZOs. ZO proteins of the MAGUK (membrane-associated guanylate kinase) family are an integral component of TJs. They function as scaffolding proteins linking other TJ proteins with the actin cytoskeleton[50]. ZO-1, also known as the tight junction protein-1 (TJP1), is a 220-kDa protein that is expressed in hepatocytes and cholangiocytes[45,51–53]. Loss of ZO-1 leads to increased transepithelial permeability and liver cholestasis[54] in rats. Reduced levels of ZO-1 are associated with liver tumors and hepatocellular carcinoma[55].

ZO-2 has a similar structure and function to that of ZO-1, and it localizes to hepatocytes and bile duct epithelium. ZO-2 promotes the activity of other TJ proteins including claudins and occludin. Loss of function mutations in ZO-2 have been reported in a subset of patients with progressive familial intrahepatic cholestasis (PFIC)[56] and familial hypercholanemia (FHC)[57,58].

Very little is known about the expression and function of ZO-3 due to lack of antibodies that can distinguish ZO-3 from ZO-1 and -2[59]. However, studies have shown that ZO-3 localization is excluded from TJs with a high level of ZO-1 and 2[12].

Actin Cytoskeleton. Filamentous actin is distributed along the plasma membrane of hepatocytes and cholangiocytes, concentrating at the apical membrane domain. The actin cytoskeleton of hepatocytes and cholangiocytes promotes BBlB function by providing anchorage of the TJ proteins to the adaptor molecules. Loss of actin can disrupt the junctional stability leading to BBlB disruption[60]. F-actin misexpression has been linked to increased viral entry and disruption of cell adhesion due to barrier deficiency[61]. A complete description of the role and regulation of actin cytoskeleton is outside the scope of this review, and readers are referred to these references[60–62].

Adherens Junction (AJs)

AJs are situated below TJs in the basal region of the lateral plasma membrane and promote homotypic cell–cell adhesion in epithelial tissue. Cadherins, a group of transmembrane AJ proteins, interact on one end with cadherins from another cell, while their cytoplasmic end interacts with catenins to form links with the actin cytoskeleton to thus promote homotypic cell–cell adhesion. AJ protein -catenin links E-cadherin to the actin cytoskeleton through binding to -catenin[63]. Catenins including - and -catenin are also involved in the trafficking and post-translational modification of E-cadherin[64]. Recent studies indicate a potential role of AJs in providing additional barrier function in the liver. Loss of -catenin in the liver

leads to drastically altered bile canaliculi, inflammation, loss of microvilli, and jaundice resulting in a cholestasis-like phenotype[65]. Loss of -catenin led to a notably milder phenotype associated with mild cholestasis and increase in basal hepatic bile acids[29,66]. Further studies revealed that loss of -catenin in hepatocyte-specific -catenin knockouts was compensated by an increase in -catenin[67,67]. More recently, we showed the true functionality of this redundancy when conditional elimination of both - and -catenin from hepatic epithelia led to progressive intrahepatic cholestasis[34]. Further analysis revealed a complex interplay of these AJ proteins with TJ proteins, especially claudins and occludin, leading to a notable defect in BBlB.

Gap Junctions (GJs)

GJs are specialized intercellular connections formed between the cytoplasm of two cells that promote regulated movements of ions, molecules, and electric impulses between cells[68,69]. GJs are increasingly associated with regulation of BBlB. Misexpression of GJ structure caused by environmental toxins and drug-induced liver injury (DILI) is shown to promote chronic and acute liver diseases[6,70,71]. Connexins are key GJ proteins, and associate with TJ protein occludin and ZO-1 to promote BBlB integrity[6]. Studies have also shown the potential use of GJ proteins as therapeutic targets in DILI. Inhibition of GJ protein connexin-32 protects mice against acetaminophen-induced liver injury, suggesting a potential use of these proteins to limit DILI and promote drug safety[72]. For a detailed review on the role of gap junction in BBlB maintenance readers are referred to this review[6].

Desmosomes

Desmosomes are structures present in the lateral side of the plasma membrane through which two cells are attached. Desmosomes are composed of cadherin family of proteins including desmoglein, desmocollin, and -catenin[73]. Relatively less studied than TJs or AJs, desmosomes are also important in BBlB maintenance. Previously, we have shown that loss of desmosomal protein -catenin alone lack adhesive defect due to compensation by -catenin[67,74]. However, our recent data shows that -catenin and -catenin acts together to promote the stability of TJ proteins occludin and claudin-2[34]. Loss of - and -catenin in the liver leads to loss of E-cadherin, occludin, and claudin-2, causing a breach in BBlB and cholestasis[34]. Figure 3 shows the expression pattern of TJ and AJ proteins in the liver.

Biliary Transporters

In addition to junctional proteins providing selective permeability and tightness of the BBlB, bile transporters also promote BBlB function (Fig. 3). Bile transporters are categorized based on their localization within hepatocytes. Bile acids are synthesized in hepatocytes from cholesterol via classic pathway through the action of cyp7a1 or an alternate pathway that utilizes cyp27a1. Bile acids are also taken up from the portal blood into the hepatocytes by the basolateral transporters (Fig. 4). These include the sodium taurocholate cotransporting polypeptide (NTCP), organic anion transporters (OATP1-2), and organic cation transporters (OCTs). Once inside the hepatocytes, bile acids undergo intracellular transport to reach the bile canaliculi[75,76]. Upon reaching the canalicular membrane, transport of bile acids across the canalicular membrane occurs with the help of apical/canalicular transporters in an ATP-dependent manner mediated by bile salt export pump (BSEP), ATP-binding cassette subfamily G member 5 (ABCG5), ATP-binding cassette subfamily G member 8 (ABCG8), multidrug resistant-associated protein 2 (MRP2), and multidrug resistant protein (MDRs) 2, 3 (Fig. 4). Bile acids can also travel back into the liver sinusoids with the help of efflux transporters consisting of the MRP group of proteins (MRP 1, 3, 4, 5, 6)[75]. More detailed information on bile acid transporters can be found elsewhere[29,77,78]. Misexpression of bile transporters can cause hepatocyte accumulation of bile acids, and progressive toxicity and injury leading to leakage of BBlB[79–81]. A direct influence of bile transporters on the function of TJ proteins cannot be ruled out due to their close proximity in the hepatocyte membrane.

FUNCTIONS OF BLOOD–BILE BARRIER

The BBlB promotes many essential functions in liver including generation and maintenance of hepatocyte

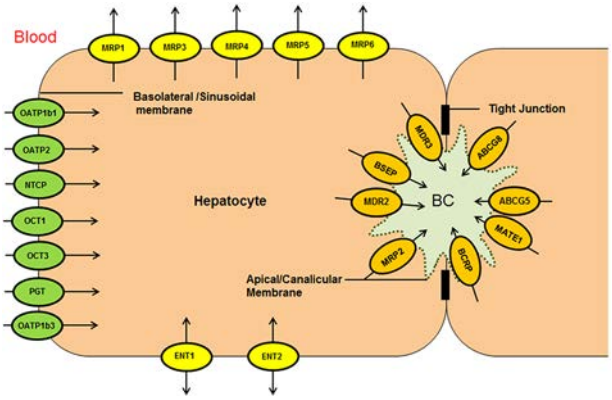

Figure 4. Localization of bile transporters in the liver. Schematic diagram showing the localization of important bile transporters in the hepatocyte membranes. Basolateral influx transporters including NTCP, OATP-1, and OCT promotes the movement of bile acid precursors from sinusoids to hepatocytes, whereas basolateral efflux transporters MRP-1, 3, 4, 5, 6 efflux them back to sinusoids. Apical/canalicular transporters send bile acids to bile canaliculi.

polarity and regulation of bile acid secretion and bile flow (Fig. 5).

Hepatocyte Polarity

During prenatal development at around embryonic day 14 (E14) to E17, rat hepatocytes are distorted and irregular in shape with no polarization. They also show only limited contact with one another due to an almost complete absence of tight and gap junctions[82]. Between approximately E17 and E21, junctions appear, and the cells assume an acinar arrangement and surround dilated bile canaliculi[83,84]. Hepatocytes exhibits polarity at this stage that is characterized by differentiation of the plasma membrane into morphological and functional domains (Fig. 4)[85,86]. The apical or bile canalicular domain, which is in contact with bile, is specialized for bile secretion, absorption and is characterized by numerous microvilli. The basolateral domain includes both the lateral membrane, which is involved in cell–cell adhesion and thus is marked by junctional complexes, and the basal or sinusoidal membrane, which is specialized for the exchange of metabolites with the blood and is characterized by irregular microvilli and many coated pits. Loss of TJ leads to aberrant bile canalicular structure and impaired polarity[85]. Loss of TJ protein claudin-1 and ZO-2 is associated with depolarization-associated liver injury and cholestasis. Mutation causing disruption of ZO-2 and claudin-2 can also lead to loss of hepatocyte polarity[58,87]. Whereas TJ proteins are indispensable for polarity, studies show a rather dispensable role of AJ protein in promoting and maintaining hepatocyte polarity[88].

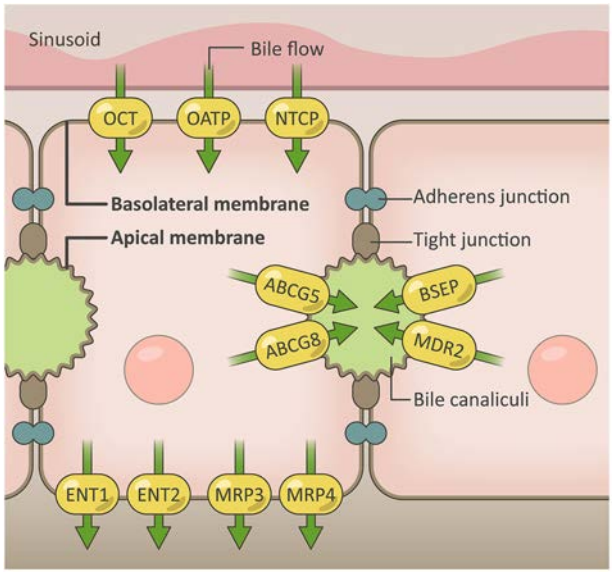

Figure 5. Major functions of the BBlB. Schematic depiction of major functions of the BBlB. The polarized localization of junctional proteins at the apical membrane separates the hepatocyte membrane into apical and basolateral domains by generation of hepatocyte polarity. Bile acid precursors enter into the hepatocyte through the transporters present in the basolateral membrane and after further processing go to bile canaliculi through the canalicular transporters. The flow of bile is shown in green lines. Bile transporters are shown by oval boxes.

Bile Secretion

One of the key functions of the liver is bile formation and secretion[89]. Bile acids, the chief constituent of bile, are 1,000 times more concentrated in bile compared to portal blood[90]. This is achieved by the active transport of bile acids synthesized in the hepatocytes and captured from the sinusoidal blood across hepatocytes into bile canaliculi. TJs promote the selective transport of choleophilic bile components and prevent the regurgitation of bile acids. Loss of TJ protein claudin-2 prevents the formation of bile canaliculi leading to aberrant bile secretion, although the exact mechanism by which claudin-2 regulates bile canaliculi formation needs to be elucidated[87]. Accumulation of bile acids in hepatocytes causes further loss of BBlB function by altering the localization of TJ proteins[91].

Along with TJs, AJs and GJs also regulate bile secretion. We have shown that loss of AJ protein -catenin causes reduced bile secretion and morphological defects in bile canaliculi characterized by dilatation, tortuosity, and loss of canalicular microvilli[66]. Similar, albeit stronger, morphological abnormalities are associated with loss of both - and -catenin, suggesting a strong influence of AJs in bile secretion[34]. Studies have also shown that GJs promote orderly contraction of bile canaliculi by their function of intercellular communication and thus promote bile flow and secretion[71] (Fig. 5).

METHODS TO MEASURE BBLB

In Vitro

Cell culture models are excellent tools to study the structure–function of the BBlB in vitro[92]. Monolayers of intrahepatic bile duct epithelial cells develop a good barrier function when grown in cell culture[93]. Among various cell types HepG2, Hep3B, HepaRG, Wif-B, and Wif-9 are worth mentioning for exhibiting polarity and barrier properties. For analyzing bile canaliculi-associated junctions Wif-B cells are routinely used[25,40,87]. It is a hybrid cell line obtained by fusing nonpolarized rat hepatic cells with human fibroblasts and is one of the few hepatocyte cell lines that develop polarized surface domains, mimicking the developing liver. Upon culturing at low confluency, they initially adopt simple columnar polarity. Then over a 2-week period, columnar Wif-B cells initially become nonpolarized and proliferate and subsequently repolarize with hepatocyte polarity[94].

In Vivo

For analyzing BBlB in vivo there are several established as well as newly developed methods.

Ultrastructural Imaging. Transmission electron microscopy (TEM) is useful in analyzing ultrastructure of TJs, AJs, bile duct structures, and associated defects (Fig. 6)[95]. Similarly, scanning electron microscopy (SEM) allows the surface ultrastructure including bile duct structure, liver sinusoids, and arrangement of microvilli to be visualized (Fig. 6)[95,96].

Lanthanum Tracing. Lanthanum tracing experiments were one of the first assays that provided useful insight about the barrier property of hepatic TJ structure (Fig. 6)[97–99]. As these particles cannot pass through the TJ structures present at the apical side of hepatocyte and bile duct epithelium, lanthanum shows complete exclusion at the TJ structure. However, mutations causing impaired TJ structure leads to deposition of lanthanum particles in TJs. We have shown that loss of and -catenin allows a small amount of lanthanum to leak into the lax TJ, which ensues as a result of loss of key TJ proteins[34].

Permeability Assays. Due to integrity of intact TJs, several molecules are unable to penetrate and hence can be used as a measure of leakiness or permeability. Resistance of the BBlB to mannitol, insulin[32,100], urea, and Evans blue[34] has been established to measure such paracellular leakage and is useful to address the barrier function of TJs in hepatocytes.

Serum Biochemistry. Serum biochemical assays, if carefully interpreted, can also be useful surrogates for addressing the barrier function of hepatocytes and biliary epithelial cells. Whereas an increase in the levels of liver enzymes such as ALT, ALP, and AST indicates hepatobiliary injury[101], an increase in serum total and direct bilirubin may indicate defects in BBlB, especially if associated with absence of any overt injury and hence can suggest mixing of bile and bilirubin with blood. Likewise, an increase in serum bile acids can indicate a defect in BBlB.

Intravital Microscopy. Recently, we have developed quantitative liver intravital microscopy (qLIM) that uses multiphoton-excitation fluorescence microscopy to enable real-time assessment of BBlB integrity and kinetics of bile transport in the intact livers of live mice (Fig. 7). We use Texas Red (TXR)–dextran (red fluorescence) and carboxyfluorescein diacetate (CFDA)[102] to visualize the blood flow through liver sinusoids and bile flow through the bile canaliculi, respectively. CFDA in the blood can be internalized by hepatocytes, where it is hydrolyzed

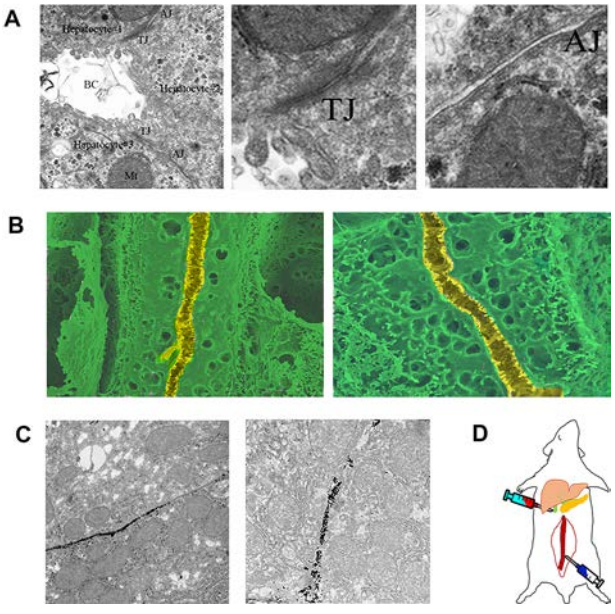

Figure 6. Detection of BBlB. Transmission electron micrograph of (A) wild-type mouse liver tissue exhibits organization of hepatocytes and bile canaliculi; location of (B) tight and (C) adherens junctions. Scanning electron micrograph of liver tissue shows the bile canaliculi and the presence of microvilli (finger-like projections shown in yellow) in it. (C) Transmission electron micrograph of liver sections shows intact TJs devoid of lanthanum hydroxide, while AJs are positive (black particles/dots). (D) Schematic of Evans blue extravasation assay.

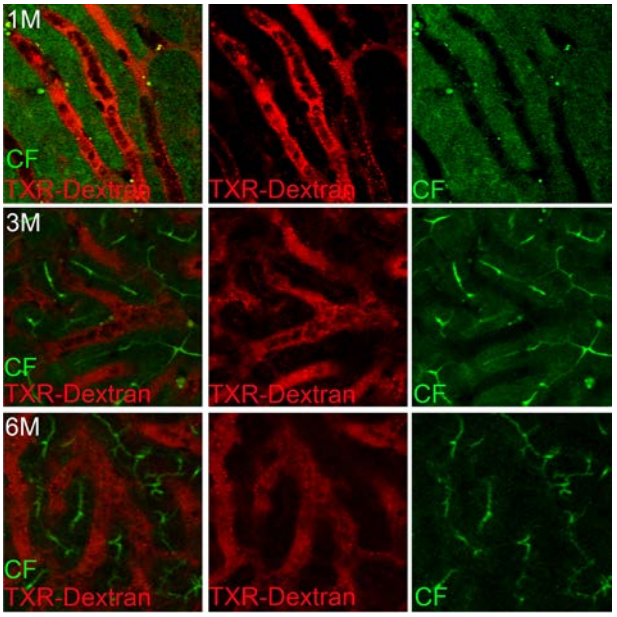

Figure 7. Intravital imaging as a method to measure the BBlB in liver. (A–C) Real-time localization of Texas Red–dextran (TXR–dextran) and carboxyfluorescein (CF) in blood and bile of liver of control mice shows exclusion of blood and bile flow at any given time point (1–6 min).

by esterase[102] into the fluorescent green metabolite carboxyfluorescein (CF), which is then excreted into the bile canaliculi evident as green fluorescence decorating these structures (Fig. 7). Recent study from our group shows that CF can be used as a surrogate to track bile transport across BBlB[103]. Under homeostatic condition, we found CF and vascular dye to be localized exclusively in bile duct and sinusoid, respectively. Interestingly, liver injury that leads to a breach in BBlB manifests as an aberrant localization of these dyes. Additionally, leakage of dyes seen as thin conduits in between hepatocytes, along with complete colocalization of the red and green dye seen as yellow, representing blood and bile, respectively, is also evident concurrently as an indication of the loss of BBlB[34,103] (Fig. 7).

BBLB IN LIVER PATHOPHYSIOLOGY

Liver Regeneration

Liver regeneration following partial hepatectomy leads to progressive loss of TJs approximately 20–40 h post-resection, followed by their reappearance[104]. Another study showed loss of TJs 24 h posthepatectomy and reappearance at day 6. These data suggest that loss of TJ and thus BBlB leads to hepatocyte death, and their reappearance accelerates tissue remodeling and cell proliferation in liver regeneration. Further in-depth study will be useful in understanding the role of TJ in liver regeneration including liver development as significant overlap is seen in regulation of these processes.

Cholestasis

Loss of BBlB function has been shown to be the primary pathophysiology of cholestasis in children as well as adults. A significant body of evidence indicates that the pathogenesis of cholestatic diseases such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and progressive familial intrahepatic cholestasis (PFIC) involve disruption of TJs and biliary dysfunction. An association between cholestasis and impaired TJs was recognized almost two decades ago[105]. In PBC, the level of 7H6 (TJ-associated antigen) is reduced in bile duct epithelium, which was reversed by treatment with ursodeoxycholic acid[106]. On the other hand, in PSC, 7H6 disappeared from hepatocyte TJs[106], and disruption of hepatocyte TJs was confirmed in experimental models of cholestasis[107]. Claudin-1 deficiency is attributed as one of the primary defects in neonatal ichthyosis and sclerosing cholangitis syndrome[108]. Loss of TJ protein ZO-2 is associated with PFIC type 3[56]. Our recent study shows that loss of TJ proteins is linked to cholestatic liver disease induced in genetic mouse models or in models of diet-induced injury[34,103]. A few studies have also linked the dysregulation of TJs and bile canalicular structure with drug-induced acquired cholestasis[109,110]. Rifampicin induces cholestasis by regulating the expression of TJ proteins claudins, occludin, and ZOs[111]. Similar models of drug-induced cholestasis have shown the role of actin cytoskeletal proteins myosin and Rho kinases[109]. These findings implicate a role of impairment of TJ integrity in notable subsets of cholestatic diseases.

Viral Hepatitis

Hepatic TJs are important regulators of viral entry. HCV is one of the most common hepatic infections that frequently lead to hepatocellular carcinoma and is one of the most studied hepatic viruses in terms of mechanism of infection[112–114]. Studies have shown the role of claudin-1 in the HCV entry process, where claudin-1 is bound by HCV and acts as a coreceptor for HCV entry[28,115]. Among other claudins, claudin-6 and 9 are also involved in this process[116]. TJ protein occludin is essential for the post-binding step of the HCV entry[117]. TJ proteins also regulate other processes of HCV integration. Entry of HCV leads to increased production of claudin-1 and retention of occludin in the endoplasmic reticulum[115], which, in turn, promotes further virus entry. HCV infection is also associated with loss of TJ structures. Along with HCV, adenovirus and coxsackievirus use a similar mechanism to initiate viral infection[49]. These data shows the role of TJs in viral hepatitis.

Liver Tumors

Hepatocellular carcinoma (HCC) is currently the worldwide fourth most common cancer in men and the second leading cause of cancer-related death[118]. HCV, alcohol abuse, nonalcoholic steatohepatitis, various metabolic disorders, genetic predisposition, and aflatoxin ingestion can lead to HCC[119–122]. HCC is a disease of poor prognosis and lacks definitive cure. Most of the HCC cases are identified at an advanced stage, which excludes them from any surgical treatment. Therefore, the use of biomarkers can be useful in the early identification of the disease. The association of BBlB with HCC suggests its use as a potential biomarker, which could provide substantial advantages in the early detection of HCC. TJ proteins CAR, occludin, ZO-1, and synplakin are reduced in HCC tissue[123], whereas claudin-10 levels are upregulated in most HCC samples[124]. Additionally, focal and diffuse expression of claudin-5 was detected in hepatocellular carcinoma[123]. A rat model of HCC showed altered expression of TJ protein 7H6[125]. These findings highlight the role of loss of TJ barrier in liver cancer.

Apart from HCC, the incidence of gall bladder carcinoma is increasing worldwide. Claudins are also shown to be misexpressed in gall bladder carcimnoma[126,127]. Cholangiocarcinoma is a form of liver cancer that forms in cholangiocytes or bile duct epithelial cells[128–130]. Integrity

of cholangiocyte TJs appears to be compromised in cholangiocarcinoma. Claudin-7 is undetectable in normal hepatocytes, but it is present in cholangiocytes[126]. The level of claudin-7 is increased in cholangiocarcinoma, but not in HCC[126]. Therefore, claudin-7 may be used as a biomarker in distinguishing cholangiocarcinoma from HCC. Similarly, claudin-3, -4, and -7 levels are undetectable in fibrolamellar HCC, but high in cholangiocarcinomas[123]. Claudin-18 is absent in healthy liver, but it is known to be expressed in intrahepatic and extrahepatic carcinomas and papillary neoplasms[131]. Usually, claudin-4 is absent in healthy liver but overexpressed in cholangiocarcinoma, and its knockdown can reduce the expansion and metastasis of tumors[132]. Thus, expression of TJ proteins is altered in both HCC and cholangiocarcinoma, and loss of the BBlB may not only be involved in the carcinogenic transformation of cells but also a likely mechanism involved in tumor metastasis.

Nonalcoholic Fatty Liver Disease (NAFLD)

NAFLD is one of the most common chronic liver diseases characterized by increased fat accumulation in the liver without significant alcohol consumption[133–136]. NAFLD leads to increased fat accumulation, steatohepatitis, fibrosis, cirrhosis, and HCC[133–136]. The progression of this metabolic disorder depends on both genetic and environmental factors. Currently, due to lifestyle and obesity, the number of patients with NAFLD is rising, and lack of treatments makes it a substantive unmet medical need. Thus, a better understanding of disease progression is essential. A few studies have linked BBlB with NAFLD; TJ disruption including loss of TJ protein ZO-1 is found in NAFLD patients[137]. Similar results were obtained in a mouse model of NAFLD where both hepatic ZO-1 and ZO-2 levels were reduced[138]. Furthermore, the introduction of probiotics to these mice led to reappearance of ZO-1 and ZO-2 and amelioration of NAFLD disease symptoms[138], suggesting a direct link of BBlB with NAFLD progression. Other mouse models showed that loss of TJ protein occludin is also associated with NAFLD[33,139]. Taken together, these studies suggest that TJs play an essential role in maintaining the barrier function of the liver and provide protection from chronic liver injury such as NAFLD. Further studies elucidating the role of TJs in NAFLD are desirable.

Cirrhosis

Liver cirrhosis is a chronic liver injury leading to scarring of liver tissue[140,141]. A plethora of causes can lead to cirrhosis including alcoholic liver disease, NAFLD, and hepatitis C. Accumulation of bacterial endotoxin present in the outer leaflet of a Gram-negative bacterial membrane is primarily associated with liver cirrhosis[142–145]. Interestingly, increase in endotoxin levels led to hepatic TJ disruption and breach in the BBlB. TJ proteins claudin-1 and occludin levels were found to be low in cirrhosis patients[146]. In hepatitis C, loss of TJ structure leads to viral entry, which causes increased liver injury and cirrhosis[147]. Gut flora and bacterial translocation also play a vital role in cirrhosis[143]. Due to a leakage in the BBlB and leaky gut, bacterial translocation increases causing a rise in lipopolysaccharide level leading to steatohepatitis, increased injury, inflammation, and cirrhosis[148].

Liver Inflammation

The BBlB can also regulate the immune state of the liver or vice versa. Increased intestinal permeability due to the accumulation of toxic bile acids and dysregulated TJs causes an increase in proinflammatory and profibrogenic cytokines including tumor necrosis factor- (TNF-), interleukin-1 (IL-1), and interferon- (IFN-)[149]. TJ protein JAM-A promotes leukocyte trafficking and vascular inflammation[43]. JAM-B and JAM-C mediate stellate cell and endothelial cell interaction during hepatic fibrosis[45]. Activated T-lymphocytes produce TJ protein occludin, which regulates leukocyte trafficking and adhesion[150]. Together, these emerging data indicate a possible interdependence between inflammation and BBlB regulation in the liver.

Liver Transplantation

Liver transplantation is one of the most successful interventions for many end-stage liver diseases and chronic liver failure. However, transplant failure due to chronic hepatic allograft rejection is still a recurring problem. Altered biliary tract function is associated with graft failure. Studies have shown loss of biliary tract function after a transplant due to ischemia–reperfusion injury, which produces reactive oxygen species that alter BBlB function by causing misexpression of TJs and actin cytoskeletal proteins[151,152]. ZO-1 and claudin-1 are shown to be mislocalized after cold storage leading to bile duct injury following ischemia–reperfusion[153].

FACTORS CONTRIBUTING TO DISRUPTION AND MAINTENANCE OF BBLB

Studies on vertebrate models and patients with chronic liver injury have identified both BBlB disrupting and protecting/maintaining factors.

Factors Disrupting BBlB

Physical Damage. Physical damage of the bile duct epithelium by processes such as bile duct ligation (BDL) to induce extrahepatic bile duct obstruction[154] and estradiol treatment to induce intrahepatic bile duct obstruction, leads to disruption of hepatic barrier function in mice. Bile duct ligation causes sixfold increase in insulin permeability[155] and twofold increase in horseradish peroxidase

(HRP) secretion[156]. Further analysis showed discontinuous distribution of TJ protein occludin, claudin-1, -2, -3, -4, ZO-1, and 7H6 levels after BDL[33,98]. Similarly, estradiol treatment in mice causes loss of occludin, claudin-1, -2, -3, and ZO-1 in liver hepatocytes[98].

Chemical Damage. Use of chemical toxins and drugs such as chlordecane, lipopolysaccharides (LPS), and hydrogen peroxide significantly increases paracellular permeability both in vivo and in vitro. LPS treatment leads to reduced levels of occludin, ZO-1, and claudin-4, thus promoting paracellular permeability[26]. On the other hand, treatment with H_2O_2 promotes the loss of occludin, ZO-1, and claudin-3[157,158].

Bile Flow and Composition. Bile is a unique aqueous solution produced by hepatocytes and modified by the secretive and absorptive transport system of the cholangiocytes. Several aspects of bile including its flow and composition, which can, themselves, influence each other, can indirectly or directly impact the BBlB. Bile is composed of 95% water, electrolytes (sodium, bicarbonate, potassium, chloride, magnesium, etc.), amino acids (glutamic acid, cysteine), glutathione, bile acids, bilirubin, cholesterol, steroids, vitamins, enzymes, porphyrins, heavy metals, exogenous drugs, xenobiotics, and environmental toxins[159]. Although understudied, several components of the bile can affect the BBlB. For example, calcium is known to maintain the hepatocyte TJ seal[159]. Reduced excretion of biliary glutathione has been linked with increased junctional permeability[160,161]. Similarly, loss of biliary bicarbonate transport causes higher paracellular permeability by altering the TJ structures[162]. Reduced bicarbonate transport is also associated with primary biliary cholangitis[163]. Intermittent obstruction of biliary flow due to porphyrin plugs such as after administration of a diet containing 3,5-diethoxycarbonyl-1.4-dihydrocollidine (DDC) to mice can lead to disruption of BBlB[103].

Bile acids are the end product of cholesterol metabolism, and impairment in their synthesis leads to accumulation of cholesterol and byproducts, which may promote toxicity and progressive liver injury[164–167]. Primary bile acids—cholic acid (CA) and chenodeoxycholic acid (CDCA)—are synthesized in the liver, whereas secondary bile acids—lithocholic acid (LCA), deoxyxholic acid (DCA), and ursodeoxyxholic acid (UDCA)—are produced due to bacterial action in the colon[168]. A few studies have explored how bile acid components can affect the BBlB. In vitro experiments using Caco-2 cells showed that selective bile acids, namely, CA, DCA, and CDCA, but not UDCA, can increase intestinal permeability via EGF receptor autophosphorylation, occludin dephosphorylation, and rearrangement of the TJ[81]. Increased level of acidic bile salts are also shown to cause enhanced permeability[169]. Long-term treatment with CDCA and LCA had different effect on the TJ and barrier function. Whereas CDCA dramatically altered barrier function, LCA did not have any notable change[149]. Biliary nonmicellar-bound bile acids like UDCA can cause damage to cell membranes, leading to BBlB disruption[170]. Cholangiocytes are more susceptible to bile acid toxicity than hepatocytes, which can lead to loss of barrier function and associated hepatopathologies including cholestasis. More research is needed to further our understanding of the role of components of bile in the regulation of BBlB.

Aging. Compromised BBlB function has been associated with aging. Age-related increased paracellular permeability of hepatocytes has been reported in mice[27]. Expression of claudins is also regulated by aging. Significant loss of claudin-3 and -4 and enrichment of claudin-1 was found in the liver of aged female and male mice[27], whereas claudin-5 levels were elevated in older female mice. No significant changes were found in other claudins including claudin-2 and -7, suggesting specific regulation of claudins[27]. However, the role of these claudins in age-associated liver damage remains poorly understood.

Chronic Liver Disease. Chronic liver disease is the 12th leading cause of death in the US[171]. It results in over 70,000 deaths every year in the US[172], and the current treatment is limited to supportive therapy[173–177]. The cellular and molecular basis underlying the histopathological manifestation of liver injury has remained elusive. Recently, we have shown that diet-induced chronic liver injury [using choline-deficient ethionine (CDE)-supplemented and DDC diet] is associated with the loss of BBlB function and integrity[103]. Remarkably, while a breach in BBlB was associated with injury initiation and progression, rescue of injury was linked with amelioration of barrier suggesting a direct link between the two events[103]. Further, we discovered that loss of TJ adhesion molecules (claudins) and bile transporters is linked to physical disruption of BBlB and mixing of blood with bile. Our findings suggest that preventing the loss of claudins or bile transporters could be a useful approach to prevent or delay liver failure in chronic liver disease. Our findings also highlighted that the feasibility of such therapy should be tested in future studies using mouse models of chronic liver injury.

Acute Liver Injury. Acetoaminophen (APAP)-induced liver failure is the most common cause of acute liver failure in humans, and APAP can be used to study drug-induced acute liver injury in mice[178,179]. APAP can cause hepatocyte TJ disruption even at a subtoxic dose (5 mM), leading to mislocalization of ZO-1 and ZO-2[178]. Acute liver failure, itself, is associated with actin cytoskeletal disruption, loss of 1-integrin activity, and cellular adhesion[178].

Factors Protecting or Maintaining BBlB

EGF Treatment. In vitro studies suggest that treating hepatocytes with growth factors promotes barrier selectivity. Treating cells with epidermal growth factor (EGF) prior to H_2O_2 administration protects them from barrier disruption[157]. However, these findings need to be confirmed in vivo using mouse models of liver injury.

Probiotics. Administration of a multispecies probiotic preparation led to amelioration of NAFLD phenotypes in mice[180]. Loss of TJ proteins was reversed in the liver by probiotic administration, which led to rescue of liver injury through recovery of lipid metabolism and liver function and amelioration of liver steatosis[180–182].

FXR Analogs. Farsenoid X receptor (FXR) is the primary regulator of bile acid homeostasis[183,184]. It also regulates lipid, cholesterol, and glucose metabolism[185–187]. There is therapeutic potential for FXR in diseases such as metabolic syndrome, diabetes, gallstone disease, hypertriglyceridemia, and steatohepatitis[186,187]. Several studies have linked FXR with epithelial barrier function. Activation of FXR signaling pathway promotes epithelial permeability in irritable bowel syndrome (IBS)[188]. FXR also causes reduced reperfusion-based injury[189]. FXR-mediated bile acid homeostasis is pivotal for metabolic functions of the liver. Mutation in FXR is associated with cholestasis including PFIC[190]. FXR agonist obeticholic acid prevents bacterial translocation to the liver and thus prevents cholestasis in rats by stabilizing TJ proteins claudin-1, -2, and occludin[188]. Future studies aimed at finding a direct interaction of FXR with TJ molecules in liver are necessary to analyze its role in hepatic barrier formation.

REGULATION OF BBLB BY SIGNALING PATHWAYS

Although not much is known about signaling pathways governing BBlB regulation, TJs appear to be highly dynamic structures that undergo disintegration and reassembly in response to various external stimuli (physiological or pathological) present in the hepatocyte microenvironment. The TJ protein complex itself can activate intracellular signaling pathways directly by engaging signaling proteins or growth receptors, or indirectly by capturing transcription factors at the plasma membrane. Much of our knowledge in TJ regulation by signaling networks comes from studies done in the kidneys, intestines, and brain[191,192]. Recent research has found exciting evidence of liver TJs promoting signaling networks. TJ protein claudin-2 is a transcriptional target of the Wnt pathway[193]. Similarly, TJ protein ZOs are involved in cellular signaling pathways including TGF- , Jun, and Fos[191,194].

TJ proteins also undergo rapid changes in expression, subcellular redistribution, and posttranslational modifications during physiologic and pathologic changes, which, in turn, affect protein–protein interactions.[192] TJ proteins occludin, ZO1 and 2 are phosphorylated at multiple sites, which promote their activity, thus providing TJ stability[195–198]. Kinases such as PKC are involved in the assembly of TJs[199], whereas c-Src disrupts TJs in the intestine and renal epithelia[200]. Other kinases including MLCK[109,157], Rho kinase[109,201], protein kinase A, and AMP kinase are also reported to promote TJ integrity. Kinases were also found to promote BBlB by rescuing the breach in barrier caused by LPS treatment[26]. Knockdown of kinase c-Src rescued BBlB loss caused by LPS treatment. Among the phosphatases, protein phosphatase 1 (PP1)[202] and protein phosphatase 2A PP2A[203,204] are shown to promote TJ stability. Studies done in rats exhibited increased activity of TJ protein ZO-2 following phosphorylation[33]. Signaling pathways such as Wnt[193,205] and TGF- [206,207] are increasingly linked with BBlB regulation. However, future studies are necessary to provide additional information on these regulatory pathways.

CONCLUDING REMARKS

During the last few years, a surge of interest in the structure and function of BBlB has been seen, generated from both a basic biological and a clinical perspective. From a clinical perspective, understanding the fundamental biology behind the mechanisms of BBlB formation and regulation is crucial for the appropriate management and development of new therapies for a number of liver diseases, such as cholestasis, viral hepatitis, NAFLD, and cirrhosis. What is needed, however, is a more vigorous effort to apply the knowledge gained in experimental work to solve clinical problems, such as identifying the potential role of breach in BBlB in cholestasis and other disease models. Another important avenue is the potential use of TJ proteins as targets for gene therapies. Finally, would the restoration of BBlB function in the hepatocytes correct functional defects, thus causing rescue of the associated cholestatic disease phenotypes? An important gap in our knowledge is the lack of understanding of the factors that determine the selective permeability of the BBlB and whether it is a true barrier with selective to no permeability. Moreover, more knowledge needs to be gained on the role of BBlB in liver development, regeneration, and cancer. Major advances in the genetic manipulation of mice enable the development of appropriate animal models to study unresolved issues of biological and clinical significance. Knocking down key TJ molecules in the liver of mice may result in useful insights into many of the unanswered questions about the role of BBlB in hepatic pathophysiology. Overall, understanding the structure and physiology of the BBlB will lead to new therapies for liver diseases.

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REFERENCES

1. Bradbury M. Why a blood-brain barrier? Trends Neurosci. 1979;2(C):36–38.
2. Wang L, Boyer JL. The maintenance and generation of membrane polarity in hepatocytes. Hepatology 2004; 39(4):892–9.
3. Kojima T, Yamamoto T, Murata M, Chiba H, Kokai Y, Sawada N. Regulation of the blood-biliary barrier: Interaction between gap and tight junctions in hepatocytes. Med Electron Microsc. 2003;36(3):157–64.
4. Schlegel N, Meir M, Heupel W-M, Holthöfer B, Leube RE, Waschke J. Desmoglein 2-mediated adhesion is required for intestinal epithelial barrier integrity. Am J Physiol Gastrointest Liver Physiol. 2010;298(5):G774–83.
5. Rao RK, Samak G. Bile duct epithelial tight junctions and barrier function. Tissue Barriers 2013;1(4):e25718.
6. Kojima T, Murata M, Yamamoto T, Lan M, Imamura M, Son S, Takano K, Yamaguchi H, Ito T, Tanaka S, Chiba H, Hirata K, Sawada N. Tight junction protein and signal transduction pathway in hepatocytes. Histol Histopathol. 2009;24(3):1463–72.
7. LaRusso NF. Morphology, physiology, and biochemistry of biliary epithelia. Toxicol Pathol. 1996;24(1):84–9.
8. Roberts SK, Yano M, Ueno Y, et al. Cholangiocytes express the aquaporin CHIP and transport water via a channel-mediated mechanism. Proc Natl Acad Sci USA 1994;91(26):13009–13.
9. Garcia-Hernandez V, Quiros M, Nusrat A. Intestinal epithelial claudins: Expression and regulation in homeostasis and inflammation. Ann NY Acad Sci. 2017;1397(1):66–79.
10. Cording J, Berg J, Käding N, et al. In tight junctions, claudins regulate the interactions between occludin, tricellulin and marvelD3, which, inversely, modulate claudin oligomerization. J Cell Sci. 2013;126(Pt 2):554–64.
11. Hartsock A, Nelson WJ. Adherens and tight junctions: Structure, function and connections to the actin cytoskeleton. Biochim Biophys Acta 2008;1778(3):660–9.
12. Bauer H, Zweimueller-Mayer J, Steinbacher P, Lametschwandtner A, Bauer HC. The dual role of zonula occludens (ZO) proteins. J Biomed Biotechnol. 2010; 2010:402593.
13. Saitou M, Furuse M, Sasaki H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol Biol Cell 2000;11(12):4131–42.
14. Huang S, Guo W, Zhou Z, Li J, Yang F, Wang J. Altered expression levels of occludin, claudin-1 and myosin light chain kinase in the common bile duct of pediatric patients with pancreaticobiliary maljunction. BMC Gastroenterol. 2016;16(1):7.
15. Traweger A, Fang D, Liu YC, et al. The tight junction-specific protein occludin is a functional target of the E3 ubiquitin-protein ligase itch. J Biol Chem. 2002;277(12): 10201–8.
16. Kolosov D, Donini A, Kelly SP. Claudin-31 contributes to corticosteroid-induced alterations in the barrier properties of the gill epithelium. Mol Cell Endocrinol. 2017;439:457–66.
17. Lal-Nag M, Morin PJ. The claudins. Genome Biol. 2009; 10(8):235.
18. Morita K, Furuse M, Fujimoto K, Tsukita S. Claudin multigene family encoding four-transmembrane domain protein components of tight junction strands. Proc Natl Acad Sci USA 1999;96(2):511–6.
19. Angelow S, Ahlstrom R, Yu ASL. Biology of claudins. Am J Physiol Renal Physiol. 2008;295(4):F867–76.
20. Marchiando AM, Graham WV, Turner JR. Epithelial barriers in homeostasis and disease. Annu Rev Pathol Mech Dis. 2010;5:119–44.
21. Van Itallie CM, Anderson JM. Claudin interactions in and out of the tight junction. Tissue Barriers 2013;1(3): e25247.
22. Angelow S, Yu AS. Claudins and paracellular transport: An update. Curr Opin Nephrol Hypertens. 2007;16:459–64.
23. Hadj-Rabia S, Baala L, Vabres P, et al. Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: A tight junction disease. Gastroenterology 2004;127(5):1386–90.
24. Matsumoto K, Imasato M, Yamazaki Y, et al. Claudin 2 deficiency reduces bile flow and increases susceptibility to cholesterol gallstone disease in mice. Gastroenterology 2014;147(5):1134–45.
25. Furuse M, Fujita K, Hiiragi T, Fujimoto K, Tsukita S. Claudin-1 and -2: Novel integral membrane proteins localizing at tight junctions with no sequence similarity to occludin. J Cell Bio1. 1998;141(7):1539.
26. Sheth P, Delos Santos N, Seth A, LaRusso NF, Rao RK. Lipopolysaccharide disrupts tight junctions in cholangiocyte monolayers by a c-Src-, TLR4-, and LBP-dependent mechanism. AJP Gastrointest Liver Physiol. 2007;293(1):G308–18.
27. D'Souza T, Sherman-Baust CA, Poosala S, Mullin JM, Morin PJ. Age-related changes of claudin expression in mouse liver, kidney, and pancreas. J Gerontol A Biol Sci Med Sci. 2009;64A(11):1146–53.
28. Benedicto I, Molina-Jiménez F, Barreiro O, et al. Hepatitis C virus envelope components alter localization of hepatocyte tight junction-associated proteins and promote occludin retention in the endoplasmic reticulum. Hepatology 2008;48(4):1044–53.
29. Yeh TH, Krauland L, Singh V, et al. Liver-specific -catenin knockout mice have bile canalicular abnormalities, bile secretory defect, and intrahepatic cholestasis. Hepatology 2010;52(4):1410–9.
30. Saitou M, Furuse M, Sasaki H, et al. Complex phenotype of mice lacking occludin, a component of tight junction strands. Mol Biol Cell 2000;11(12):4131–42.
31. Tsukita S, Furuse M. Occludin and claudins in tight-junction strands: Leading or supporting players? Trends Cell Biol. 1999;9(7):268–73.
32. Al-Sadi R, Khatib K, Guo S, Ye D, Youssef M, Ma T. Occludin regulates macromolecule flux across the intestinal epithelial tight junction barrier. Am J Physiol Gastrointest Liver Physiol. 2011;300(6):G1054–64.
33. Maly IP, Landmann L. Bile duct ligation in the rat causes upregulation of ZO-2 and decreased colocalization of claudins with ZO-1 and occludin. Histochem Cell Biol. 2008;129(3):289–99.
34. Pradhan-Sundd T, Zhou L, Vats R, et al. Dual catenin loss in murine liver causes tight junctional deregulation and

progressive intrahepatic cholestasis. Hepatology 2017; 67(6):2320–37.
35. Raleigh DR, Boe DM, Yu D, et al. Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. J Cell Biol. 2011;193(3):565–82.
36. Woodfin A, Voisin M-B, Beyrau M, et al. The junctional adhesion molecule JAM-C regulates polarized transendothelial migration of neutrophils in vivo. Nat Immunol. 2011;12(8):761–9.
37. Paris L, Tonutti L, Vannini C, Bazzoni G. Structural organization of the tight junctions. Biochim Biophys Acta 2007;1778(3):646–59.
38. Mandell KJ, Parkos CA. The JAM family of proteins. Adv Drug Deliv Rev. 2005;57(6):857–67.
39. Sugano Y, Takeuchi M, Hirata A, et al. Junctional adhesion molecule-A, JAM-A, is a novel cell-surface marker for long-term repopulating hematopoietic stem cells. Blood 2008;111(3):1167–72.
40. Braiterman LT, Heffernan S, Nyasae L, et al. JAM-A is both essential and inhibitory to development of hepatic polarity in WIF-B cells. Am J Physiol Gastrointest Liver Physiol. 2008;294(2):G576–88.
41. Ebnet K. The junctional adhesion molecule (JAM) family members JAM-2 and JAM-3 associate with the cell polarity protein PAR-3: A possible role for JAMs in endothelial cell polarity. J Cell Sci. 2003;116(19):3879–91.
42. Laukoetter MG, Nava P, Lee WY, et al. JAM-A regulates permeability and inflammation in the intestine in vivo. J Exp Med. 2007;204(13):3067–76.
43. Chavakis T, Preissner KT, Santoso S. Leukocyte transendothelial migration: JAMs add new pieces to the puzzle. Thromb Haemost. 2003;89(1):13–7.
44. Zhang D, Xu C, Manwani D, Frenette PS. Neutrophils, platelets, and inflammatory pathways at the nexus of sickle cell disease pathophysiology. Blood J. 2016;127(7): 801–10.
45. Hintermann E, Bayer M, Ehser J, et al. Murine junctional adhesion molecules JAM-B and JAM-C mediate endothelial and stellate cell interactions during hepatic fibrosis. Cell Adhes Migr. 2016;10(4):419–33.
46. Coyne CB, Bergelson JM. CAR: A virus receptor within the tight junction. Adv Drug Deliv Rev. 2005;57(6): 869–82.
47. Raschperger E, Neve EPA, Wernerson A, Hultenby K, Pettersson RF, Majumdar A. The coxsackie and adenovirus receptor (CAR) is required for renal epithelial differentiation within the zebrafish pronephros. Dev Biol. 2008;313(1):455–64.
48. Raschperger E, Thyberg J, Pettersson S, Philipson L, Fuxe J, Pettersson RF. The coxsackie- and adenovirus receptor (CAR) is an in vivo marker for epithelial tight junctions, with a potential role in regulating permeability and tissue homeostasis. Exp Cell Res. 2006;312(9):1566–80.
49. Cohen CJ, Shieh JTC, Pickles RJ, Okegawa T, Hsieh J-T, Bergelson JM. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. Proc Natl Acad Sci USA 2001;98(26):15191–6.
50. Itoh M, Morita K, Tsukita S. Characterization of ZO-2 as a MAGUK family member associated with tight as well as adherens junctions with a binding affinity to occludin and catenin. J Biol Chem. 1999;274(9):5981–6.
51. Stevenson BR, Siliciano JD, Mooseker MS, Goodenough DA. Identification of ZO-1: A high molecular weight polypeptide associated with the tight junction (Zonula Occludens) in a variety of epithelia. J Cell Biol. 1986; 103(3):755–66.
52. Anerson JM, Stevenson BR, Jesaitis LA, Goodenough DA, Mooseker MS. Characterization of ZO-1, a protein component of the tight junction from mouse liver and Madin-Darby canine kidney cells. J Cell Biol. 1988; 106(4):1141–9.
53. Itoh M, Nagafuchi A, Yonemura S, Kitani-Yasuda T, Tsukita S, Tsukita S. The 220-kD protein colocalizing with cadherins in non-epithelial cells is identical to ZO-1, a tight junction-associated protein in epithelial cells: cDNA cloning and immunoelectron microscopy. J Cell Biol. 1993;121(3):491–502.
54. Anderson JM, Glade JL, Stevenson BR, Boyer JL, Mooseker MS. Hepatic immunohistochemical localization of the tight junction protein ZO-1 in rat models of cholestasis. Am J Pathol. 1989;134(5):1055–62.
55. Austinat M, Dunsch R, Wittekind C, Tannapfel A, Gebhardt R, Gaunitz F. Correlation between beta-catenin mutations and expression of Wnt-signaling target genes in hepatocellular carcinoma. Mol Cancer 2008;7:21.
56. Sambrotta M, Strautnieks S, Papouli E, et al. Mutations in TJP2 cause progressive cholestatic liver disease. Nat Genet. 2014;46(4):326–8.
57. Carlton VEH, Harris BZ, Puffenberger EG, et al. Complex inheritance of familial hypercholanemia with associated mutations in TJP2 and BAAT. Nat Genet. 2003;34(1): 91–6.
58. Sambrotta M, Thompson RJ. Mutations in TJP2, encoding zona occludens 2, and liver disease. Tissue Barriers 2015;3(3):e1026537.
59. Umeda K, Ikenouchi J, Katahira-Tayama S, et al. ZO-1 and ZO-2 independently determine where claudins are polymerized in tight-junction strand formation. Cell 2006; 126(4):741–54.
60. Doctor RB, Nichols M. 3. The actin cytoskeleton in liver function. In: Bitter E, editor. Principles in medical biology, volume 15. The liver in biology and disease. Oxford, UK: Elsevier, 2004. p. 49–79.
61. Cui X, Zhang X, Yin Q, et al. F-actin cytoskeleton reorganization is associated with hepatic stellate cell activation. Mol Med Rep. 2014;9(5):1641–7.
62. Yamada S, Pokutta S, Drees F, Weis WI, Nelson WJ. Deconstructing the cadherin-catenin-actin complex. Cell 2005;123(5):889–901.
63. D'Souza-Schorey C. Disassembling adherens junctions: Breaking up is hard to do. Trends Cell Biol. 2005;15(1): 19–26.
64. Hinck L, Näthke IS, Papkoff J, Nelson WJ. Dynamics of cadherin/catenin complex formation: Novel protein interactions and pathways of complex assembly. J Cell Biol. 1994;125(6):1327–40.
65. Herr KJ, Tsang YHN, Ong JWE, et al. Loss of a-catenin elicits a cholestatic response and impairs liver regeneration. Sci Rep. 2014;4:6835.
66. Behari J, Yeh T-H, Krauland L, et al. Liver-specific -catenin knockout mice exhibit defective bile acid and cholesterol homeostasis and increased susceptibility to diet-induced steatohepatitis. Am J Pathol. 2010;176(2): 744–53.
67. Wickline ED, Du Y, Stolz DB, Kahn M, Monga SPS. -Catenin at adherens junctions: Mechanism and biologic implications in hepatocellular cancer after -catenin knockdown. Neoplasia 2013;15(4):421–34.

68. Reed MA, Zhou C, Muller CJ, Burgin TP, Tour JM. Conductance of a molecular junction. Science 1997;278: 252–4.
69. Zampighi G, Corless JM, Robertson JD. On gap junction structure. J Cell Biol. 1980;86(1):190–8.
70. Vinken M, Doktorova T, Decrock E, Leybaert L, Vanhaecke T, Rogiers V. Gap junctional intercellular communication as a target for liver toxicity and carcinogenicity. Crit Rev Biochem Mol Biol. 2009;44(4):201–22.
71. Temme A, Stümpel F, Söhl G, et al. Dilated bile canaliculi and attenuated decrease of nerve-dependent bile secretion in connexin32-deficient mouse liver. Pflugers Arch. 2001;442(6):961–6.
72. Patel SJ, Milwid JM, King KR, et al. Gap junction inhibition prevents drug-induced liver toxicity and fulminant hepatic failure. Nat Biotechnol. 2012;30(2):179–83.
73. Green KJ, Getsios S, Troyanovsky S, Godsel LM. Intercellular junction assembly, dynamics, and homeostasis. Cold Spring Harb Perspect Biol. 2010;2(2):a000125.
74. Wickline ED, Awuah PK, Behari J, Ross M, Stolz DB, Monga SPS. Hepatocyte -catenin compensates for conditionally deleted -catenin at adherens junctions. J Hepatol. 2011;55(6):1256–62.
75. Arrese M, Ananthananarayanan M, Suchy FJ. Hepatobiliary transport: Molecular mechanisms of development and cholestasis. Pediatr Res. 1998;44(2):141–7.
76. Arrese M, Trauner M. Molecular aspects of bile formation and cholestasis. Trends Mol Med. 2003;9(12):558–64.
77. Dawson PA, Lan T, Rao A. Bile acid transporters. J Lipid Res. 2009;50(12):2340–57.
78. Alrefai WA, Gill RK. Bile acid transporters: Structure, function, regulation and pathophysiological implications. Pharm Res. 2007;10:1803–23.
79. Attili AF, Angelico M, Cantafora A, Alvaro D, Capocaccia L. Bile acid-induced liver toxicity: Relation to the hydrophobic-hydrophilic balance of bile acids. Med Hypotheses 1986;19(1):57–69.
80. Perez MJ, Britz O. Bile-acid-induced cell injury and protection. World J Gastroenterol. 2009;15(14):1677–89.
81. Raimondi F, Santoro P, Barone MV, et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. Am J Physiol Liver Physiol. 2008;294(4):G906–13.
82. Montesano R, Friend DS, Perrelet A, Orci L. In vivo assembly of tight junctions in fetal rat liver. J Cell Biol. 1975;67(2 Pt.1):310–9.
83. Luzzatto A. Hepatocyte differentiation during early fetal development in the rat. Cell Tissue Res. 1981;215(1): 133–42.
84. Wood RL. An electron microscope study of developing bile canaliculi in the rat. Anat Rec. 1965;151(4):507–29.
85. Gissen P, Arias IM. Structural and functional hepatocyte polarity and liver disease. J Hepatol. 2015;63:1023–37.
86. Treyer A, Müsch A. Hepatocyte polarity. Compr Physiol. 2013;3(1):243–87.
87. Son S, Kojima T, Decaens C, et al. Knockdown of tight junction protein claudin-2 prevents bile canalicular formation in WIF-B9 cells. Histochem Cell Biol. 2009; 131(3):411–24.
88. Gissen P, Arias IM. Structural and functional hepatocyte polarity and liver disease. J Hepatol. 2015;63(4): 1023–37.
89. Boyer JL. Bile formation and secretion. Compr Physiol. 2013;3(3):1035–78.
90. Meier PJ, Stieger B. Molecular mechanisms in bile formation. News Physiol Sci. 2000;15:89–93.
91. Quinn M, McMillin M, Galindo C, Frampton G, Pae HY, DeMorrow S. Bile acids permeabilize the blood brain barrier after bile duct ligation in rats via Rac1-dependent mechanisms. Dig Liver Dis. 2014;46(6):527–34.
92. Decaens C, Durand M, Grosse B, Cassio D. Which in vitro models could be best used to study hepatocyte polarity? Biol Cell. 2008;100(7):387–98.
93. Okamoto H, Ishii M, Mano Y, et al. Confluent monolayers of bile duct epithelial cells with tight junctions. Hepatology 1995;22(1):153–9.
94. Ihrke G, Neufeld EB, Meads T, et al. WIF-B cells: An in vitro model for studies of hepatocyte polarity. J Cell Biol. 1993;123(6 II):1761–75.
95. Goldblatt PJ, Gunning WT. Ultrastructure of the liver and biliary tract in health and disease. Ann Clin Lab Sci. 14(2):159–67.
96. Grisham JW, Nopanitaya W, Compagno J, Nägel AE. Scanning electron microscopy of normal rat liver: The surface structure of its cells and tissue components. Am J Anat. 1975;144:295–21.
97. Whittembury G, Rawlins FA. Evidence of a paracellular pathway for ion flow in the kidney proximal tubule: Electromicroscopic demonstration of lanthanum precipitate in the tight junction. Pflugers Arch. 1971;330(4):302–9.
98. Takakuwa Y, Kokai Y, Sasaki KI, et al. Bile canalicular barrier function and expression of tight-junctional molecules in rat hepatocytes during common bile duct ligation. Cell Tissue Res. 2002;307(2):181–9.
99. McCarter SD, Johnson DL, Kitt KN, Donohue C, Adams A, Wilson JM. Regulation of tight junction assembly and epithelial polarity by a resident protein of apical endosomes. Traffic 2010;11(6):856–66.
100. Higashi H, Brüstle O, Daley GQ, et al. Endothelial adherens junctions control tight junctions by VE-cadherin-mediated upregulation of claudin-5. Cell 2009;8(4):1–2.
101. Giannini EG, Testa R, Savarino V. Liver enzyme alteration: A guide for clinicians. CMAJ 2005;172(3):367–79.
102. Liu Y, Chen H-C, Yang S-M, et al. Visualization of hepatobiliary excretory function by intravital multiphoton microscopy. J Biomed Opt. 2011;12:014014.
103. Pradhan-Sundd T, Vats R, Russell JM, et al. Dysregulated bile transporters and impaired tight junctions during chronic liver injury in mice. Gastroenterology 2018; 155(4):1218–32.
104. Takaki Y, Hirai S, Manabe N, et al. Dynamic changes in protein components of the tight junction during liver regeneration. Cell Tissue Res. 2001;305(3):399–409.
105. Anderson JM. Leaky junctions and cholestasis: A tight correlation. Gastroenterology 1996;110(5):1662–5.
106. Sakisaka S, Kawaguchi T, Taniguchi E, et al. Alterations in tight junctions differ between primary biliary cirrhosis and primary sclerosing cholangitis. Hepatology 2001; 33(6):1460–8.
107. Landmann L. Cholestasis-induced alterations of the trans- and paracellular pathways in rat hepatocytes. Histochem Cell Biol. 1995;103(1):3–9.
108. Paganelli M, Stephenne X, Gilis A, et al. Neonatal ichthyosis and sclerosing cholangitis syndrome: Extremely variable liver disease severity from claudin-1 deficiency. J Pediatr Gastroenterol Nutr. 2011;53(3):350–4.
109. Sharanek A, Burban A, Burbank M, et al. Rho-kinase/myosin light chain kinase pathway plays a key role in the

impairment of bile canaliculi dynamics induced by cholestatic drugs. Sci Rep. 2016;6:24709.
110. Fickert P, Wagner M. Biliary bile acids in hepatobiliary injury—What is the link? J Hepatol. 2017;67(3):619–31.
111. Chen X, Zhang C, Wang H, et al. Altered integrity and decreased expression of hepatocyte tight junctions in rifampicin-induced cholestasis in mice. Toxicol Appl Pharmacol. 2009;240(1):26–36.
112. Webster DP, Klenerman P, Dusheiko GM. Hepatitis C. Lancet 2015;14:62401–6.
113. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis. 2005; 5(9):558–67.
114. Manns MP, Buti M, Gane E, et al. Hepatitis C virus infection. Nat Rev Dis Prim. 2017;2;3:17006.
115. Liu S, Yang W, Shen L, Turner JR, Coyne CB, Wang T. Tight junction proteins claudin-1 and occludin control hepatitis C virus entry and are downregulated during infection to prevent superinfection. J Virol. 2009;83(4): 2011–4.
116. Findley MK, Koval M. Regulation and roles for claudin-family tight junction proteins. IUBMB Life 2009;61(4): 431–7.
117. Benedicto I, Molina-Jimenez F, Bartosch B, et al. The tight junction-associated protein occludin is required for a postbinding step in hepatitis C virus entry and infection. J Virol. 2009;83(16):8012–20.
118. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.
119. Mittal S, El-Serag HB. Epidemiology of hepatocellular carcinoma: Consider the population. J Clin Gastroenterol. 2013;47(Suppl 0):S2–6.
120. Balogh J, Victor D, Asham EH, et al. Hepatocellular carcinoma: A review. J Hepatocell Carcinoma 2016;3:41–53.
121. Forner A, Llovet JM, Bruix J, Bruix J, Forner A, Llovet JM. Hepatocellular carcinoma. Lancet 2012;391:1301–14.
122. Llovet JM, Burroughs A, Bruix J. Hepatocellular carcinoma. Lancet 2003;362:1907–17.
123. Patonai A, Erdélyi-Belle B, Korompay A, et al. Claudins and tricellulin in fibrolamellar hepatocellular carcinoma. Virchows Arch. 2011;458(6):679–88.
124. Cheung ST, Leung KL, Ip YC, et al. Claudin-10 expression level is associated with recurrence of primary hepatocellular carcinoma. Clin Cancer Res. 2005;11(2 Pt 1): 551–6.
125. Zhong Y, Enomoto K, Tobioka H, Konishi Y, Satoh M, Mori M. Sequential decrease in tight junctions as revealed by 7H6 tight junction-associated protein during rat hepatocarcinogenesis. Jpn J Cancer Res. 1994;85(4):351–6.
126. Jakab C, Kiss A, Schaff Z, et al. Claudin-7 protein differentiates canine cholangiocarcinoma from hepatocellular carcinoma. Histol Histopathol. 2010;25(7):857–64.
127. Németh Z, Szász AM, Tátrai P, et al. Claudin-1, -2, -3, -4, -7, -8, and -10 protein expression in biliary tract cancers. J Histochem Cytochem. 2009;57(2):113–21.
128. Razumilava N, Gores GJ. Cholangiocarcinoma. Lancet 2014;383:2168–79.
129. Khan SA, Thomas HC, Davidson BR, Taylor-Robinson SD. Cholangiocarcinoma. Lancet 2005;366(9493):1303–14.
130. Blechacz BRA, Gores GJ. Cholangiocarcinoma. Clin Liver Dis. 2008;12(1):131–50.
131. Takasawa K, Takasawa A, Osanai M, et al. Claudin-18 coupled with EGFR/ERK signaling contributes to the malignant potentials of bile duct cancer. Cancer Lett. 2017;403:66–73.
132. Bunthot S, Obchoei S, Kraiklang R, Pirojkul C, Wongkham S, Wongkham C. Overexpression of claudin-4 in cholangiocarcinoma tissues and its possible role in tumor metastasis. Asian Pacific J Cancer Prev. 2012;13(Suppl 1): 71–6.
133. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med. 2002;346(16):1221–31.
134. Trasher T, Abelmalek M. Non-alcoholic fatty liver disease. N C Med J. 2016;77(3):216–9.
135. Sattar N, Forrest E, Preiss D. Non-alcoholic fatty liver disease. BMJ 2014;349.
136. Byrne CD, Targher G. NAFLD: A multisystem disease. J Hepatol. 2015;62:S47–64.
137. Miele L, Valenza V, La Torre G, et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology 2009;49(6): 1877–87.
138. Zhang M, Wang X-Q, Zhou Y-K, et al. Effects of oral Lactobacillus plantarum on hepatocyte tight junction structure and function in rats with obstructive jaundice. Mol Biol Rep. 2010;37(6):2989–99.
139. Sawada N. Tight junction-related human diseases. Pathol Int. 2013;63(1):1–12.
140. Tsochatzis EA, Bosch J, Burroughs AK. Liver cirrhosis. Lancet 2014;383:1749–61.
141. Li MK, Crawford JM. The pathology of cholestasis. Semin Liver Dis. 2004;24(1):21–42.
142. Sakisaka S, Koga H, Sasatomi K, Mimura Y, Kawaguchi T, Tanikawa K. Biliary secretion of endotoxin and pathogenesis of primary biliary cirrhosis. Yale J Biol Med. 1997;70(4):403–8.
143. Wiest R, Lawson M, Geuking M. Pathological bacterial translocation in liver cirrhosis. J Hepatol. 2014;60(1): 197–209.
144. Tandon P, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. Semin Liver Dis. 2008;28(1):26–42.
145. Zhou WC, Zhang QB, Qiao L. Pathogenesis of liver cirrhosis. World J Gastroenterol. 2014;20(23):7312–24.
146. Assimakopoulos SF, Tsamandas AC, Tsiaoussis GI, et al. Altered intestinal tight junctions' expression in patients with liver cirrhosis: A pathogenetic mechanism of intestinal hyperpermeability. Eur J Clin Invest. 2012;42(4): 439–46.
147. Lee NP, Luk JM. Hepatic tight junctions: From viral entry to cancer metastasis. World J Gastroenterol. 2010; 16(3):289–95.
148. Ilan Y. Leaky gut and the liver: A role for bacterial translocation in nonalcoholic steatohepatitis. World J Gastroenterol. 2012;18(21):2609.
149. Sarathy J, Detloff SJ, Ao M, et al. The Yin and Yang of bile acid action on tight junctions in a model colonic epithelium. Physiol Rep. 2017;5(10):e13294.
150. Alexander JS, Dayton T, Davis C, et al. Activated T-lymphocytes express occludin, a component of tight junctions. Inflammation 1998;22(6):573–82.
151. Molitoris BA, Falk SA, Dahl RH. Ischemia-induced loss of epithelial polarity. Role of the tight junction. J Clin Invest. 1989;84(4):1334–9.
152. Benkoël L, Dodero F, Hardwigsen J, et al. Effect of ischemia-reperfusion on bile canalicular F-actin microfilaments in hepatocytes of human liver allograft: Image

analysis by confocal laser scanning microscopy. Dig Dis Sci. 2001;46(8):1663–7.
153. Brunner SM, Junger H, Ruemmele P, et al. Bile duct damage after cold storage of deceased donor livers predicts biliary complications after liver transplantation. J Hepatol. 2013;58(6):1133–9.
154. Metz J, Aoki A, Merlo M, Forssmann WG. Morphological alterations and functional changes of interhepatocellular junctions induced by bile duct ligation. Cell Tissue Res. 1977;182(3):299–310.
155. Accatino L, Contreras A, Fernandez S, Quintana C. The effect of complete biliary obstruction on bile flow and bile acid excretion: Postcholestatic choleresis in the rat. J Lab Clin Med. 1979;93(5):706–17.
156. Rahner C, Stieger B, Landmann L. Structure-function correlation of tight junctional impairment after intrahepatic and extrahepatic cholestasis in rat liver. Gastroenterology 1996;110(5):1564–78.
157. Guntaka SR, Samak G, Seth A, Larusso NF, Rao R. Epidermal growth factor protects the apical junctional complexes from hydrogen peroxide in bile duct epithelium. Lab Invest. 2011;91(9):1396–1409.
158. Sheth P, Samak G, Shull JA, Seth A, Rao R. Protein phosphatase 2A plays a role in hydrogen peroxide-induced disruption of tight junctions in Caco-2 cell monolayers. Biochem J. 2009;421(1):59–70.
159. Boyer JL. Bile formation and secretion. Compr Physiol. 2013;3(3):1035–78.
160. Ballatori N, Truong AT. Altered hepatic junctional permeability, bile acid excretion and glutathione efflux during oxidant challenge. J Pharmacol Exp Ther. 1989; 251(3):1069–75.
161. Ballatori N, Truong AT. Glutathione as a primary osmotic driving force in hepatic bile formation. Am J Physiol. 1992;263:G617–24.
162. Juric M, Xiao F, Amasheh S, et al. Increased epithelial permeability is the primary cause for bicarbonate loss in inflamed murine colon. Inflamm Bowel Dis. 2013; 19(5):904–11.
163. Prieto J, Garcia N, Marti-Climent JM, Penuelas I, Richter JA, Medina JF. Assessment of biliary bicarbonate secretion in humans by positron emission tomography. Gastroenterology 1999;117(1):167–72.
164. Wang R, Sheps JA, Ling V. ABC Transporters, bile acids, and inflammatory stress in liver cancer. Curr Pharm Biotechnol. 2011;12(4):636–46.
165. Krishnamurthy N, Kurzrock R. Targeting the Wnt/beta-catenin pathway in cancer: Update on effectors and inhibitors. Cancer Treat Rev. 2017;62:50–60.
166. Woolbright BL, Jaeschke H. Novel insight into mechanisms of cholestatic liver injury. World J Gastroenterol. 2012;18(36):4985–93.
167. Woodhead JL, Yan K, Siler SQ, et al. Exploring BSEP inhibition-mediated toxicity with a mechanistic model of drug-induced liver injury. Front Pharmacol. 2014; 5:240.
168. Hofmann AF. Bile acids: The good, the bad, and the ugly. News Physiol Sci. 1999;14:24–9.
169. Chen X, Oshima T, Tomita T, et al. Acidic bile salts modulate the squamous epithelial barrier function by modulating tight junction proteins. Am J Physiol Gastrointest Liver Physiol. 2011;301(2):G203–9.
170. Fickert P, Zollner G, Fuchsbichler A, et al. Ursodeoxycholic acid aggravates bile infarcts in bile duct-ligated and Mdr2 knockout mice via disruption of cholangioles. Gastroenterology 2002;123(4):1238–51.
171. Xu J, Murphy SL, Kochanek KD, Bastian BA. Deaths: Final data for 2013. Natl Vital Stat Rep. 2016;64(2):1–119.
172. Vong S, Bell BP. Chronic liver disease mortality in the United States, 1990–1998. Hepatology 2004;39(2): 476–83.
173. Takami T, Terai S, Sakaida I. Stem cell therapy in chronic liver disease. Curr Opin Gastroenterol. 2012;28(3): 203–8.
174. Nicolas CT, Wang Y, Nyberg SL. Cell therapy in chronic liver disease. Curr Opin Gastroenterol. 2016; 32(3):189–94.
175. Arora G, Keeffe EB. Management of chronic liver failure until liver transplantation. Med Clin North Am. 2008;92(4):839–60.
176. Fox RK. When to consider liver transplant during the management of chronic liver disease. Med Clin North Am. 2014;98(1):153–68.
177. Solà E, Ginès P. Chronic kidney disease: A major concern in liver transplantation in the XXI century. J Hepatol. 2014;61(2):196–7.
178. Gamal W, Treskes P, Samuel K, et al. Low-dose acetaminophen induces early disruption of cell-cell tight junctions in human hepatic cells and mouse liver. Sci Rep. 2017;7:37541.
179. Mossanen JC, Tacke F. Acetaminophen-induced acute liver injury in mice. Lab Anim. 2015;49(1 Suppl):30–6.
180. Briskey D, Heritage M, Jaskowski L-A, et al. Probiotics modify tight-junction proteins in an animal model of nonalcoholic fatty liver disease. Ther Adv Gastroenterol. 2016;9(4):463–72.
181. Zhao H, Zhao C, Dong Y, et al. Inhibition of miR122a by Lactobacillus rhamnosus GG culture supernatant increases intestinal occludin expression and protects mice from alcoholic liver disease. Toxicol Lett. 2015;234(3): 194–200.
182. Xue L, He J, Gao N, et al. Probiotics may delay the progression of nonalcoholic fatty liver disease by restoring the gut microbiota structure and improving intestinal endotoxemia. Sci Rep. 2017;7:1–13.
183. Goodwin B, Jones SA, Price RR, et al. A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. Mol Cell. 2000;6(3):517–26.
184. Claudel T, Staels B, Kuipers F. The farnesoid X receptor: A molecular link between bile acid and lipid and glucose metabolism. Arterioscler Thromb Vasc Biol. 2005; 25(10):2020–30.
185. Eloranta JJ, Kullak-Ublick GA. The role of FXR in disorders of bile acid homeostasis. Physiology (Bethesda) 2008;23(5):286–95.
186. Wang YD, Chen WD, Moore DD, Huang W. FXR: A metabolic regulator and cell protector. Cell Res. 2008;18(11):1087–95.
187. Neuschwander-Tetri BA. Targeting the FXR nuclear receptor to treat liver disease. Gastroenterology 2015; 148(4):704–6.
188. Verbeke L, Farre R, Verbinnen B, et al. The FXR agonist obeticholic acid prevents gut barrier dysfunction and bacterial translocation in cholestatic rats. Am J Pathol. 2015;185(2):409–19.
189. Ceulemans LJ, Verbeke L, Decuypere J-P, et al. Farnesoid X receptor activation attenuates intestinal ischemia reperfusion injury in rats. PLoS One 2017;12(1):e0169331.

190. Gomez-Ospina N, Potter CJ, Xiao R, et al. Mutations in the nuclear bile acid receptor FXR cause progressive familial intrahepatic cholestasis. Nat Commun. 2016;7:10713.
191. Tsukita S, Furuse M, Itoh M. Structural and signalling molecules come together at tight junctions. Curr Opin Cell Biol. 1999;11(5):628–33.
192. Matter K, Balda MS. Signalling to and from tight junctions. Nat Rev Mol Cell Biol. 2003;4(3):225–36.
193. Behari J, Yeh T-H, Krauland L, et al. Liver-specific beta-catenin knockout mice exhibit defective bile acid and cholesterol homeostasis and increased susceptibility to diet-induced steatohepatitis. Am J Pathol. 2010; 176(2):744–53.
194. Kim SK. Tight junctions, membrane-associated guanylate kinases and cell signaling. Curr Opin Cell Biol. 1995;7(5):641–9.
195. Kale G, Naren AP, Sheth P, Rao RK. Tyrosine phosphorylation of occludin attenuates its interactions with ZO-1, ZO-2, and ZO-3. Biochem Biophys Res Commun. 2003;302(2):324–9.
196. Rao R. Occludin phosphorylation in regulation of epithelial tight junctions. Ann NY Acad Sci. 2009;1165:62–8.
197. Raleigh DR, Boe DM, Yu D, et al. Occludin S408 phosphorylation regulates tight junction protein interactions and barrier function. J Cell Biol. 2011;193(3):565–82.
198. Wong V. Phosphorylation of occludin correlates with occludin localization and function at the tight junction. Am J Physiol. 1997;273(6 Pt 1):C1859–67.
199. Suzuki T, Elias BC, Seth A, et al. PKC regulates occludin phosphorylation and epithelial tight junction integrity. Proc Natl Acad Sci USA 2009;106(1):61–6.
200. Basuroy S, Sheth P, Kuppuswamy D, Balasubramanian S, Ray RM, Rao RK. Expression of kinase-inactive c-Src delays oxidative stress-induced disassembly and accelerates calcium-mediated reassembly of tight junctions in the Caco-2 cell monolayer. J Biol Chem. 2003;278(14): 11916–24.
201. Walsh SV, Hopkins AM, Chen J, Narumiya S, Parkos CA, Nusrat A. Rho kinase regulates tight junction function and is necessary for tight junction assembly in polarized intestinal epithelia. Gastroenterology 2001;121(3):566–79.
202. Seth A, Sheth P, Elias BC, Rao R. Protein phosphatases 2A and 1 interact with occludin and negatively regulate the assembly of tight junctions in the CACO-2 cell monolayer. J Biol Chem. 2007;282(15):11487–98.
203. Jia H, Liu Y, Yan W, Jia J. PP4 and PP2A regulate Hedgehog signaling by controlling Smo and Ci phosphorylation. Development 2009;136:307–16.
204. Wang N, Leung HT, Mazalouskas MD, Watkins GR, Gomez RJ, Wadzinski BE. Essential roles of the tap42–regulated protein phosphatase 2a (PP2A) family in wing imaginal disc development of drosophila melanogaster. PLoS One 2012;7(6): e38569.
205. Pradhan-Sundd T, Zhou L, Vats R, et al. Dual catenin loss in murine liver causes tight junctional deregulation and progressive intrahepatic cholestasis. Hepatology 2018; 67(6):2320–37.
206. Shen L. Role of epithelial cells in initiation and propagation of intestinal inflammation. Eliminating the static: Tight junction dynamics exposed. AJP Gastrointest Liver Physiol. 2006;290(4):G577–82.
207. Ozdamar B, Bose R, Barrios-Rodiles M, Wang HR, Zhang Y, Wrana JL. Regulation of the polarity protein Par6 by TGF receptors controls epithelial cell plasticity. Science 2005;307(5715):1603–9.