World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i3.289

World J Gastroenterol 2010 January 21; 16(3): 289-295
ISSN 1007-9327 (print)

EDITORIAL

Hepatic tight junctions: From viral entry to cancer metastasis

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Author contributions: Lee NP collected data, prepared the figures and wrote the paper; Luk JM initiated the review, wrote, commented on and revised the paper.
Supported by A GRF Grant from the Research Grants Council of Hong Kong to Luk JM, No. 771607M
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Received: October 23, 2009 Revised: November 30, 2009
Accepted: December 7, 2009
Published online: January 21, 2010

Abstract

The tight junction (TJ) is a critical cellular component for maintenance of tissue integrity, cellular interactions and cell-cell communications, and physiologically functions as the "great wall" against external agents and the surrounding hostile environment. During the host-pathogen evolution, viruses somehow found the key to unlock the gate for their entry into cells and to exploit and exhaust the host cells. In the liver, an array of TJ molecules is localized along the bile canaliculi forming the blood-biliary barrier, where they play pivotal roles in paracellular permeability, bile secretion, and cell polarity. In pathology, certain hepatic TJ molecules mediate virus entry causing hepatitis infection; deregulation and functional abnormality of the TJ have also been implicated in triggering liver cancer development and metastasis. All these findings shed new insights on the understanding of hepatic TJs in the development of liver disease and provide new clues for potential intervention.

Key words: Tight junctions; Hepatocytes; Blood-biliary barrier; Bile canaliculi; Hepatitis; Liver steatosis; Liver neoplasms

Peer reviewers: Nikolaus Gassler, Professor, Institute of Pathology, University Hospital RWTH Aachen, Pauwelsstrasse 30, 52074 Aachen, Germany; Giammarco Fava, MD, Department of Gastroenterology, Università Politecnica delle Marche, Ancona, via Gervasoni 12, 60129 Ancona, Italy; Hidetsugu Saito, Assistant Professor, Department of Internal Medicine, Keio University, 35 Shinanomachi, Shinjuku-ku, Tokyo 1608582, Japan

Lee NP, Luk JM. Hepatic tight junctions: From viral entry to cancer metastasis. *World J Gastroenterol* 2010; 16(3): 289-295 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i3/289.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i3.289

INTRODUCTION

The liver is the core metabolic center in the mammalian body and is responsible for major physiological functions such as carbohydrate/amino acid/fatty acid metabolism, bile secretion, and detoxification. The cellular composition of the liver comprises specialized cell types - hepatocytes and nonparenchymal cells. In a normal adult liver, hepatocytes are epithelial cells and alone account for 60%-70% of the total cell mass. Hepatocytes adopt a polarized architecture, resulting in the formation of numerous cell plates in the liver. Because of the highly vascularized nature of the liver, this hepatic parenchyma is infiltrated by an extensive microcirculatory network[1,2]. In order to maintain this anatomical organization, hepatocytes are equipped basically with a vast variety of junctions, such as anchoring junctions, tight junctions (TJs), and gap junctions (GJs)[3-5]. These junctions are situated at the surface of the hepatocytes so as to mediate cell-cell contact and communication. This editorial focuses on the hepatocyte TJs - as the "cements" of the building block and the "door" for entry of hepatitis viruses. Deregulation of TJ expression and function dismantles

the architecture of the hepatic parenchyma and causes liver diseases and cancer.

TJ IN THE LIVER: FROM STRUCTURAL ARCHITECTURE TO SIGNALING NETWORK

In the liver, TJs can be found in 2 places, associating with either hepatocytes or bile duct epithelial cells (cholangiocytes). Those associated with the former cell type are alternatively called the blood-biliary barrier (BBB). Here we review the hepatocyte-associated TJ that concentrates at the specialized location surrounding the bile canaliculi. In addition to modulating paracellular passage of small molecules and ions, this BBB functions to keep bile in the bile canaliculi and apart from the blood circulation. TJ in the liver also segregates the apical surface from the basolateral surface of the hepatocytes, thereby maintaining cell polarity[4]. Having the same TJ components as in other epithelia and endothelia, the TJ in the liver is also composed of claudins, occludin, junctional adhesion molecules (JAMs), and others such as coxsackievirus and adenovirus receptor (CAR)[6-11].

Claudins constitute the largest TJ family; 24 claudins have been found in mammals and at least 7 of these, namely claudin-1, -2, -3, -4, -5, -7, and -10, have been studied in the liver. Most claudins are small molecules having molecular weights of approximate 22-27 kDa. They are tetra-span molecules with the amino-terminus and carboxyl-terminus in the cell cytoplasm, and they possess 2 extracellular loops and one intracellular loop. For sealing the intercellular gap, claudin needs to interact with other claudins in the adjacent cell through its extracellular loops[12,13]. Occludin is the first studied TJ integral molecule. It has similar structural features to that of claudin, being a molecule with 4 transmembrane domains and utilizes a similar binding mechanism to that of claudin. However, it differs from claudin in its large molecular weight of 65 kDa[11,14]. Another variant of occludin, occludin 1B, has been identified and it differs from occludin in having an extended amino-terminus. Both of them are found in mouse livers[15].

JAM is a TJ molecule which has gained much attention recently. It is a single-pass membrane protein with its amino-terminus in the extracellular region and carboxyl-terminus in the cytoplasm. As the component of a barrier, a single JAM molecule needs to couple with another JAM in an adjacent cell. At least 4 members of JAM have been identified and JAM-1, JAM-2, and JAM-3 have been found in mouse livers[10,11,16].

CAR was known originally as a new class of viral receptor, belonging to the immunoglobulin-like family and further studies demonstrated that CAR also had TJ functions[17]. Of the 3 isoforms of CAR (i.e. CAR-1, CAR-2, and CAR-3), only CAR-2 has been positively identified in hepatocytes and both CAR-1 and CAR-2 are associated with cholangiocytes in mouse livers[7]. As in other organs, these integral TJ proteins interact with various scaffolding proteins to ensure structural integrity.

A plethora of adaptors and peripheral proteins are known to be present and associated with claudins, occludin, JAMs, and CARs. Adaptors such as zonula occludens-1 (ZO-1) act as a bridge linking the integral proteins to the underlying actin filament[18-20]. Cingulin, symplekin, and MAGI-1 (membrane-associated guanylate kinase inverted-1) are other peripheral proteins at TJs, and some of them have also been demonstrated in the liver[3,21-24]. By this way, a high degree of structural architecture is established at the TJ strand guarding the selective permeability barrier in the liver[25] (Figure 1).

Apart from its barrier functions, recent studies have also elucidated the other roles of the TJ as a core component in the signaling network, in particular for those junctional complexes concentrating at the BBB. Accumulating evidence suggests that the junction does not function alone on the plasma membrane, but different junctions can interact with each other either directly or indirectly. Studies performed in different systems demonstrated a disruption of one junction type could lead to loss or gain of function of another junction type, emphasizing the significance of inter-junctional crosstalk[26-28]. Epithelial cells, including hepatic cells, adopt this kind of junction-junction regulation. It is noted that enforced expression of connexin 32 into mouse hepatocytes derived from connexin 32-deficient mice results in TJ formation, accompanied by induced expression of occludin, claudin-1 and ZO-1, thereby leading to establishment of cell polarity[29]. In addition, using the same experiment setup induced the expressions of another junction protein MAGI-1 at the TJ in connexin 32 transfectants[30]. These findings unequivocally demonstrate the presence of a macrocomplex in the liver composed of at least a TJ and GJ[4]. Solid evidence from other studies also suggested the possible involvement of the TJ in manipulating other junctions such as the adherens junction (AJ). For instance, an abnormality of JAM-1 in hepatoma HepG2 cells induced the production of an AJ protein E-cadherin[31]. Our current understanding is that ZO-1 acts as the moderator in coordinating the cellular dynamics of various associated junctions and maintains the structural functionality of this multi-junctional network[32-34].

Several cellular proteins, such as protein kinases and phosphatases, are some of the major regulators of junctions. Since most of them have numerous substrates, events of phosphorylation or dephosphorylation can modulate the status of components related to certain junctions. The p38 mitogen-activated protein kinase (MAPK) is a serine/threonine kinase that phosphorylates a handful of substrates including those associated with TJs[35]. Treatment with SB203580, a p38 MAPK inhibitor, led to strengthening of the TJ with a concomitant increase in claudin-1 in rat livers after partial hepatectomy[36]. Other events triggered by cytokines and growth factors are also involved in the regulation of TJ dynamics[37,38]. For instance, incubation of rodent hepatocytes with a multifunctional cytokine oncostatin M triggered the

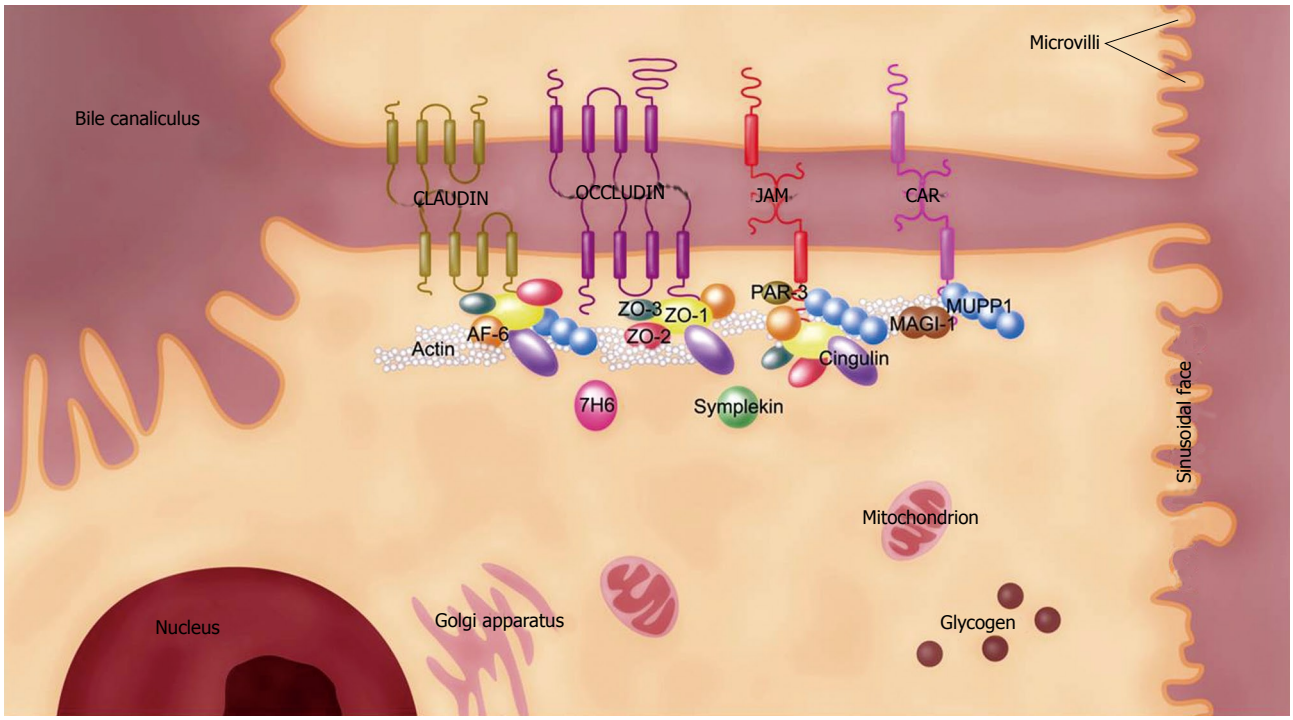

Figure 1 Molecular structure of the TJ in the mammalian liver. The TJ in the liver is associated with hepatocytes and bile duct cells. TJ location around the bile canaliculus between 2 adjacent hepatocytes is shown. For simplicity, the molecular structure depicted in this figure represents the TJ molecules found in the mammalian liver. Claudin, occludin, JAM, and CAR are 4 core units for constituting TJ by uniting a panel of peripheral proteins like ZO-1 to form multiprotein complexes. TJ molecules display differential localizations in the mammalian liver, such that some of them like human symplekin and mouse CAR-2 are associated with both hepatocytes and bile duct cells while others, such as mouse CAR-1, are only found in the latter cell type. CAR: Coxsackievirus and adenovirus receptor; JAM: Junctional adhesion molecule; MAGI-1: Membrane-associated guanylate kinase inverted-1; MUPP1: Multiple PDZ domain protein-1; PAR-3: Partitioning defective 3 homolog; TJ: Tight junction; ZO: Zonula occludens.

production of claudin-2 and subsequently strengthened the TJ barrier[39]. Further, the transforming growth factor-β (TGF-β) could reduce the production of claudin-1 and weaken the barrier function in rat hepatocytes[40].

TJ AND LIVER-RELATED DISEASES

Hepatitis

Hepatitis is an infection of the liver caused commonly by hepatitis viruses such as hepatitis B and C. At least 7 hepatitis viruses are known today and new species are being identified[41]. Hepatitis C virus (HCV) is the best studied for its ability to bind to TJ molecules on hepatocytes, and these molecules act as co-receptors for HCV entry[42]. The concept of junction proteins mediating viral entry is not restricted to the hepatitis virus, but is apparent for several other viruses including the adenovirus and coxsackievirus[43]. For the scenario of HCV infection, occludin and claudin-1 have been determined to be 2 key molecules for HCV entry[44,45]. Some reports also demonstrated other molecules including CD81 and human scavenger receptor class B member 1 (SR-BI) as co-receptors for HCV entry[46,47] and the expression levels of some of these receptors define the viral entry rate[48]. Cooperation between these receptors and TJ molecules is essential for viral entry into hepatocytes. Several studies provided further evidence indicating that the events occurred in the hepatocytes after HCV infection. Hepatoma HuH-7 cells, having genomic replicons of HCV, could alter TJ dynamics, such that a disarrangement of TJ components was found and retention of occludin in the endoplasmic reticulum was noted[49]. Internalization of HCV is accompanied by an induced synthesis of fatty acid synthase, which is an enzyme responsible for fatty acid synthesis, and this event is associated with the production of claudin-1, but not CD81, in hepatoma HuH-7 cells[50]. This can partly explain why HCV infection frequently leads to steatosis, a fatty liver-related disease. In addition to fatty acid synthase, protein kinase A has an important role in HCV infection, since an aberration in protein kinase A function in hepatoma HuH-7 cells led to a disorganization of claudin-1 and reduced the infection susceptibility[51]. Together, these findings suggest an explicit role of TJ molecules, especially claudin-1 and occludin, in mediating HCV infection (Figure 2).

Liver cancer

Liver cancer or hepatocellular carcinoma (HCC) is one of the most aggressive liver malignancies worldwide. The development of HCC is a complex process that is not totally established even after several decades of research. In most cases, HCC results from a pre-neoplastic inflammation of the diseased liver and is the end stage of a progressive worsening of liver conditions originating from hepatitis or cirrhotic livers, encompassing 3 phases

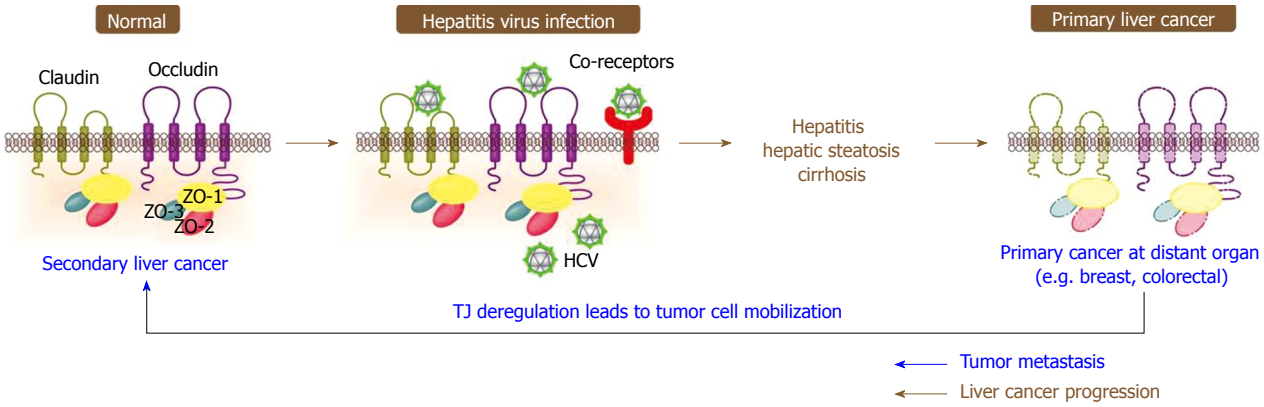

Figure 2 The TJ at different stages of liver cancer progression and distant liver metastasis. During viral infection, HCV binds onto several TJ molecules (claudin-1 and occludin) and co-receptors (CD81 and SR-BI) on hepatocytes before its internalization. This event leads to hepatic steatosis and/or cirrhosis before the subsequent development of primary liver cancer that is associated with TJ deregulation. For metastatic liver cancer originating from the intrahepatic site or distant organs such as breast and colorectum, there is an initial loss of TJ molecules at the primary tumor site and a subsequent gain of these molecules in the liver with tumor cell colonization. HCV: Hepatitis C virus; SR-BI: Scavenger receptor class B member-I.

Table 1 Clinical significance of TJ molecules in human HCC

Molecules	Expression in HCC[1]	Clinical correlations	Ref.
CAR	Reduced	Poor tumor differentiation	[64]
Claudin-1	Reduced	Poor tumor differentiation, tumor invasion, poor survival	[63]
Claudin-10	Higher	Tumor recurrence	[67]
Occludin	Reduced	-	[9]
Symplekin	Reduced	Poor tumor differentiation	[3]
ZO-1	Reduced	-	[9]

[1]Relative expression of TJ molecules in HCC tissues compared to normal or adjacent non-tumor tissues. TJ: Tight junction; HCC: Hepatocellular carcinoma; CAR: Coxsackievirus and adenovirus receptor.

of development - molecular, preclinical, and clinical[52]. At the molecular level, this malignant transformation of the liver is accompanied by a stepwise change in genetic and proteomic information, which can be readily revealed using laboratory technologies including gene microarray and gel- or non-gel-based proteomics profiling, coupled with mass spectrometry[53-55]. As a result of rapid developments in molecular and profiling techniques, the gene, protein, and microRNA data related to HCC are gradually being decoded[56]. A handful of molecules, such as heat shock proteins and cadherins, and different pathways, such as Wnt and TGF-β pathways, have been determined to be HCC-related[56-60]. The newly derived information assists the construction of the molecular network of HCC and enhances our knowledge of this cancer.

Primary tumors in the liver

Tumor nodules originating in the liver are generally termed as primary tumors. During hepatocarcinogenesis, the liver usually undergoes several phases of transition from pre-neoplasia, dysplasia, to neoplasia[61]. Several TJ molecules are regarded as HCC biomarkers[55,62]. An endogenous expression of certain claudins is found in the normal adult liver and their expression is attenuated when HCC develops. It is noted that claudin-1 has reduced expression in cancerous liver when compared to its healthy counterpart[63]. A general reduction in the levels of occludin, ZO-1, and CAR has been found in HCC when compared to normal liver[9,64]. Also, there is a gradual decrease in the level of 7H6 TJ-associated antigen in rats during hepatocarcinogenesis[65]. Apart from these molecules, other TJ molecules, such as JAM and cingulin, are present in hepatic cells[31,66], but not all of these are associated with HCC. Those claudins with a high expression in normal liver have a role in liver physiology by maintaining a functional TJ barrier, whereas those claudins with elevated expression in the cancerous liver are likely to be involved in tumor formation. Sometimes these biomarkers not only indicate the advent of tumors, but can also be prognostic in nature (Table 1). A loss of claudin-1 expression in resected HCC indicates poor differentiation and high invasiveness of the tumor, and is associated with poor outcomes of patients[63]. Similarly for other TJ molecules, a reduced CAR expression in resected livers is correlated with poor differentiation of HCC[64]. However, Cheung *et al*[67] linked the high expression of claudin-10 in HCC with the high incidence of postoperative tumor recurrence in patients.

With regard to biological significance, these biomarkers usually demonstrate gain- or loss-of-function in HCC. For instance, the expression of claudin-1 is associated preferentially with the fetal cell type of human hepatoblastoma, but not the highly proliferating embryonal cell type, with expression of proliferating cell nuclear antigen (PCNA) and Ki-67, suggesting its expression is negatively correlated with rapid cell growth and division[68]. This anti-proliferative behavior of hepatic claudin-1 is further supported by a study showing a loss of claudin-1 associated with tumor aggressiveness[63]. An overexpression of claudin-10 is linked to poor outcome of HCC patients after hepatic resection. To prove the tumorigenic features of this molecule, an overexpression experiment was performed in claudin-10-deficient Hep3B hepatoma cells and an induction of tumor phenotypes was observed. In the reciprocal experiment

using RNA interference (RNAi) to silence claudin-10 in HLE hepatoma cells with a high level of claudin-10, an alleviation of tumorigenic potential accompanied by reduced cell invasion was found[69]. By these approaches, molecules can be studied for their tumorigenic properties. Besides their intrinsic tumorigenic properties being the subject of intense interest in research, TJ molecules have also been studied with regard to their associated pathways, some of which have been unfolded successfully. Borlak *et al*[70] utilized an epidermal growth factor-induced HCC mouse model showing a positive effect of this growth factor in inducing claudin-7 in small HCCs. Also, vascular endothelial growth factor (VEGF)-treated HepG2 hepatoma cells had disruptive TJs accompanied by reduced occludin expression, suggesting VEGF as one factor triggering the spread of tumor cells into the normal liver parenchyma[71]. All these findings implicate the direct involvement of TJ molecules in the presentation of tumor phenotypes and the tumor-related signaling pathways, suggesting their interference may counteract the process of HCC. Therefore, TJ molecules may be another class of therapeutic target for HCC.

Metastatic tumors in liver

Metastatic tumors initiated elsewhere in the body may spread to and colonize the liver. Angiogenesis is a prerequisite process for tumor metastasis, enabling the migration of tumor cells through the circulatory system of the body from one site to the other[72]. A number of factors such as growth factors and chemokines are important in triggering this event[73,74]. For tumor cells to metastasize, loss of TJ function is usually observed in cancer cells prior to this process[75]. Of the TJ molecules, claudin-7, ZO-1, and other emerging ones are associated with tumor metastasis. Thus, a decrease of claudin-7 in tumor tissues is associated with tumor metastasis in patients with breast carcinoma[76]. This finding is further validated by a separate gene microarray study, in which breast cancer metastasis to the liver is associated with a reduced expression of a panel of TJ molecules including claudin-4 and ZO-1, in addition to claudin-7[77]. For lung cancer, overexpression of claudin-1 in human lung adenocarcinoma cells reduced the metastatic potentials of tumor cells[78]. Apart from these 2 cancer types, elimination of claudin-7 is frequently observed in colorectal cancer, and is clinically related to the event of tumor cell invasion into the blood circulation and the eventual development of tumor masses in the liver[79]. In addition, malfunction of ZO-1 is observed after its phosphorylation, which also induces the migration of colorectal tumor cells into the liver[80]. Interestingly, there is a restoration of the expression of TJ molecules in tumor cells after metastasis to the liver from a distant organ. It is noted that a re-expression of ZO-1 is observed in colorectal cancer cells after metastasis to the liver when compared to those developing metastatic potential at the primary cancer site[80]. Similar re-expression of claudins such as claudin-1 and claudin-4 in liver-residing colorectal cancer cells is reported in another study examining colorectal tumor metastasis[81]. Therefore, it is clear that loss of TJ function is a key factor triggering the induction of metastatic potential in tumor cells to other sites including the liver, while a restoration of its function is needed for tumor cells to colonize in the liver.

CONCLUSION

In the liver, TJ is found to be associated with bile duct cells and hepatocytes. As hepatocytes are the most predominant cell type in the liver and the most studied in liver diseases, we focused our discussion on the role of hepatocyte-associated TJ molecules in hepatitis infection and liver cancer. TJs found in hepatocytes are also known as the BBB, keeping the bile in the bile canaliculi away from the blood circulation. Emerging evidence has further demonstrated the direct involvement of TJ molecules as co-receptors for HCV. On the other hand, accumulating evidence support sthe notion that deregulation of TJ molecules is frequently associated with increased incidence of HCC and poor prognosis of patients, signifying their putative use as biomarkers for diagnosis and prognosis of this liver malignancy. Molecules with induced expression in HCC are predicted to be tumor-inducing, while those with reduced expression are likely to be anti-tumor molecules. Proof-of-principle studies by means of RNAi or overexpression should enhance our knowledge of the roles of specific TJ molecules in liver diseases. Based on previous findings of TJ molecules as viral receptors, it is highly possible that a blocking peptide or antibody can be developed to prevent the binding of the viral particles to the TJ receptors on hepatocytes. This can open a new study area for therapeutic targeting of the TJ. Those TJ molecules with tumor-inducing properties are potential therapeutic targets for HCC. More in-depth studies should be performed to find antagonists to inhibit the functions of these molecules or to block their specific signaling pathways. With regard to advancement of HCC diagnosis, novel TJ molecules may be useful as HCC biomarkers. Further studies should be performed to investigate whether these biomarkers are eligible as supplemental criteria to diagnose HCC in patients with low levels of serum alpha-fetoprotein who constitute up to one-third of HCC cases. Therefore, study of the TJ in the liver can increase the clinical usefulness of this molecule in liver diseases.

ACKNOWLEDGMENTS

We would like to thank Professor Ronnie Poon for his insightful comments and advice on HCC clinical medicine. We would like to acknowledge Mr. Kar-wai Leung for preparing the illustrations.

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