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Review

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Abbreviations: APC: adenomatous polyposis coli; CLDN: claudin; CRC: colorectal cancer; DAA: direct-acting antiviral; EA: esophageal carcinoma; ECL: extracellular loop; EGFR: epidermal growth factor receptor; EMT: epithelial-to-mesenchymal transition; Fab: antigen-binding fragment; GI: gastrointestinal; HCC: hepatocellular carcinoma; HCV: hepatitis C virus; IBD: inflammatory bowel disease; IgG: immunoglobulin G; IMAB: ideal monoclonal antibody; JAM: junctional adhesion molecule; mAb: monoclonal antibody; miR/miRNA: microRNA; MMP: matrix metalloprotease; TJ: tight junction; OCLN: occludin; SR-BI: scavenger receptor BI; TAMP: tight junction-associated marvel proteins UC: ulcerative colitis; ZO: zona occludens

Abstract

Over the past two decades a growing body of evidence has demonstrated an important role of tight junction (TJ) proteins in the physiology and disease biology of gastrointestinal (GI) and liver disease. On one side, TJ proteins exert their functional role as integral proteins of TJs in forming barriers in the gut and the liver. Furthermore, TJ proteins can also be expressed outside TJs where they play important functional roles in signaling, trafficking and regulation of gene expression. A hallmark of TJ proteins in disease biology is their functional role in epithelial-to-mesenchymal transition. A causative role of TJ proteins has been established in the pathogenesis of colorectal cancer and gastric cancer. Among the best characterized roles of TJ proteins in liver disease biology is their function as cell entry receptors for hepatitis C virus – one of the most common causes of hepatocellular carcinoma. At the same time TJ proteins are emerging as targets for novel therapeutic approaches for GI and liver disease. Here we review our current knowledge of the role of TJ proteins in the pathogenesis of GI and liver disease biology and discuss their potential as therapeutic targets.

Tight junctions (TJs) are intercellular adhesion complexes that are essential to the barrier function of epithelia and endothelia. They maintain cell polarity by limiting the movement of proteins within the plasma membrane and by regulating paracellular solute and water flux (for a recent review see[1]). Their functions are however not limited to these important structural gate and fence functions as TJs also act as signaling hubs[1, 2]. TJs are highly dynamic structures that constantly enable cells to adapt to their environment. Not surprisingly, perturbation of TJ protein expression or function and/or disruption of TJ integrity is associated with a variety of diseases, including skin, intestinal and lung diseases, and cancers[1, 3] (Table 1). Furthermore, pathogens have evolved strategies to overcome epithelial and endothelial barriers by using TJ components for their infection/invasion[1, 4]. TJs are composed of transmembrane proteins, including different claudins (CLDNs), tight junction-associated marvel proteins (TAMPs) such as occludin, junctional adhesion molecules (JAMs) as well as cytosolic proteins, which form what has been termed the junctional plaque and connect transmembrane components to the cytoskeleton[1] (Figure 1).

OCLN was the first identified integral membrane protein forming TJs. OCLN is a 65-kDa protein with 4 transmembrane domains. Posttranscriptional modification leads to several splice variants. OCLN contains a small intracellular loop and two extracellular loops (ECLs), ECL1 and ECL2, the latter being involved in homophilic interactions between OCLNs expressed on adjacent cells. The N- and C-terminal parts are both located within the cell. The C-terminal part is longer than the N-terminal part and its role in the modulation of TJ assembly, structure and function via posttranslational modifications of OCLN as well as for interaction with other TJ components and the cytoskeleton has been well studied[5] (Figure 1). OCLN contains a conserved four transmembrane marvel domain and is thus a member of the tight junction-associated marvel proteins (TAMPs) that also include tricellulin and marvelD3[6]. It has been shown that the three TAMPs have distinct but overlapping functions at the TJ. Tricellulin localizes at tricellular junctions formed by the corners of three epithelial cells while OCLN localizes at bicellular junctions. MarvelD3 has been reported to interact with tricellulin and OCLN, suggesting that it may be present at bi- and tricellular junctions[6].

The observation that TJs can form in the absence of OCLN[7] has led to the identification of CLDNs as integral TJ components. CLDNs form a family of 25-27 kDa proteins that in mammals comprises up to 27 members with high sequence homology. Like OCLN, CLDNs can also be subject to posttranslational and postranscriptional regulation. Their structure also resembles the one of OCLN, except for a shorter C-terminal part. The ECLs contribute through homophilic or heterophilic interactions with CLDNs or other integral membrane proteins located on adjacent cells to TJ formation[8]. The C-terminal part links the protein to intracellular TJ components and the actin cytoskeleton (Figure 1). Interestingly, CLDN expression patterns vary between different organs and cancers. CLDNs have thus been suggested as diagnostic markers and targets for cancer therapy[9, 10, 11].

Beside CLDNs and TAMPs that form TJ strands, additional transmembrane barrier proteins, including JAMs and related proteins, have been described (reviewed in[12, 13]. The best characterized JAM in the regulation of TJ barrier function is JAM-A, a member of the immunoglobulin (Ig) superfamily. The dimerization of two JAM-A molecules expressed on the same cell (cis-dimerization) contributes to the formation of a complex between transmembrane TJ proteins and cytoplasmic scaffold proteins[12] (Figure 1). Furthermore, JAM-A has been shown to act as a landmark for bicellular TJ formation[14] while lipolysis-stimulated lipoprotein receptor (LSR)/angulin-1, another member of the Ig superfamily of proteins, defines cell corners for tricellular TJ formation[15].

The best studied cytoplasmic proteins of TJs are zona occludens (ZO) proteins. ZO-1, -2, and -3 can interact with each other as well as with several transmembrane proteins (OCLN, CLDNs, JAM-A) and F-actin (Figure 1). Cytoplasmic proteins of TJs have thus been suggested to act as scaffolds linking TJs to the actin cytoskeleton and microtubules[12].

Importantly, TJ proteins have been reported to be also localized at sites outside TJs (non-junctional expression). Indeed, CLDN, OCLN and ZO proteins can be expressed at the basolateral membrane, in the cytoplasm and/or the nucleus where they have important functions in addition to those observed in TJs. For TJ protein expressed at the basolateral membrane these non-canonical functions include endosomal trafficking, signaling and

additional ion transport functions while TJ proteins in the nucleus have been shown to regulate gene transcription (reviewed in[16]). Noteworthy, non-junctional TJ proteins do not diffuse in a random manner throughout the membrane. Rather, by interacting with defined molecules within the membrane and/or their phosphorylation, non-junctional CLDNs have been shown to be stabilized in discrete domains within the basolateral membrane and contribute to cell adhesion through interactions with the extracellular matrix[17, 18]. Furthermore, CLDNs can regulate the expression/activity of matrix metalloproteases (MMPs) that contribute to matrix remodelling[19, 20, 21, 22]. These functions can contribute to epithelial-to-mesenchymal transition (EMT), a process by which polarized epithelial cells lose their contacts to neighbouring cells and enable them to migrate. EMT has been shown to play an important role in organogenesis (EMT type 1), homeostasis, inflammation and fibrosis (e.g. wound healing, fibrogenesis; EMT type 2) but can also promote tumorigenesis, invasion and metastasis (EMT type 3).

The role of TJ proteins in the physiology and disease biology of the GI system and the liver deserves special emphasis. The GI tract epithelium has to maintain a delicate however dynamic balance in allowing specific substances (food, ions and solutes) to pass through the epithelium while not allowing many others (e. g. pathogens) in order to maintain the delicate balance between immune tolerance and activation. These considerations are further enriched by the recent findings that a feed-forward loop may exist between the gut microbiota and mucosal barrier function in such regulatory schemes[23]. Moreover, studies have now also revealed non-canonical roles of specific TJ integral proteins in regulating cellular differentiation, proliferation and migration; cellular mechanisms implicated in normal repair/regeneration as well as the oncogenic growth during tumorigenesis[24, 25]. Accordingly, a causal role of TJ proteins in GI disease, including esophagitis, inflammatory bowel disease (IBD) and cancers has been demonstrated. Similar to the GI tract, the liver endothelial junctions are important for liver functions and TJ dysregulation has been observed in chronic liver disease and hepatocellular carcinoma (HCC). Interestingly, TJ proteins CLDN1 and OCLN have been shown to be essential entry factors for the hepatitis C

virus (HCV)[26, 27]. Here we review our current knowledge of the role of TJ proteins in GI and liver disease and discuss their potential as therapeutic targets focussing on GI cancer and viral infection of the liver.

TJ proteins and the GI tract

The functional role of TJ proteins in the physiology of the GI system

The GI mucosal barrier plays an important role in the separation of the inside of the body from the outside environment. TJs are present on the apical end of the lateral membrane surface in epithelial cells and regulate paracellular transport and apicobasal cell polarity. The expression of different TJs in the gut varies according to the gut's functional properties as well as localization in villus or crypt (small bowel *vs* colon). The proteins can be localized strictly at the apical cell-cell adhesion or extend to the lateral or basolateral surfaces[28, 29, 30, 31]. Moreover, expression and cellular distribution of the TJ proteins – such as the CLDN family of proteins – is associated with regulation of differentiation of the intestinal epithelium[32, 33, 34]: CLDN1 is mainly expressed at the apex of the epithelial cells with a reticular pattern in the colon. CLDN2 is expressed in both villus and crypt cells of the small intestine but restricted to undifferentiated crypt cells in the colon. CLDN3, -4, -7 and -8 are predominantly expressed in the distal parts (colon, sigmoid and rectum) of the GI tract while CLDN10 and -12 show an ubiquitous expression pattern throughout the GI tract.

Loss- and gain-of-function studies in mice have revealed specific roles for a number of CLDNs in the TJ barrier function, selective ion permeability, as well as their related pathological phenotypes. For instance, knockout of CLDN7 in mice has severe intestinal defects including mucosal ulcerations, epithelial cell sloughing and inflammation, which leads to the death of the mice[35, 36]. Similarly, constitutive overexpression of CLDN1 in the mouse gut epithelium (to mimic upregulated CLDN1 expression in colon cancer) demonstrated a key role of CLDN1 in normal colonic epithelial homeostasis by regulating Notch-signaling[21], while in combination with APC (adenomatous polyposis coli) mutation (APC^{min} mice) CLDN1 overexpression induced colon tumorigenesis[37]. Similarly,

upregulated CLDN2 expression in the mouse gut epithelium demonstrated a critical and complex role of CLDN2 in intestinal homeostasis by regulating epithelial permeability, inflammation and proliferation[38, 39]. Moreover, CLDN8 contributed to regulation of paracellular Na^+ permeability, protecting the leakage of Na^+ into the intestinal lumen[40]. CLDN16 is responsible for the defective absorption of Ca^{2+} in the intestine causing primary hypercalciuria[41]. Furthermore, the interdependence between CLDN proteins in regulating intestinal homeostasis is well demonstrated by *in vivo* loss-of-function studies for CLDN15. CLDN15 knockout mice grow normally despite having a mega-intestine[42]. However, a double knockout of CLDN15 with CLDN2 chronically reduces the paracellular flow of Na^+ from the intestinal submucosa into the lumen resulting in shunting of the nutrient absorption, malnourishment and death [43]. Of note, both CLDN2 and -15 are paracellular transporters of Na+[44]. Overall, these findings show a critical functional role of these proteins in regulating intestinal homeostasis.

Furthermore, OCLN and JAMs have been shown to contribute to regulate intestinal homeostasis. For example, mice lacking JAM-A display an alteration of intestinal homeostasis as shown by perturbed regulation of epithelial permeability, inflammation, and proliferation, and significant alteration in CLDN protein expression[45]. Next generation gene editing technology such as CRISPR and fluorescent gene-reporter tags will enable to better understand the details of the function of TJ proteins in regulating GI physiology.

Functional role of TJ proteins during GI neoplastic transformations and growth

During neoplastic transformation in GI cancer, the expression and localization of TJ proteins is perturbed by several mechanisms occuring at the transcriptional, translational and post-transcriptional level (Figure 2). Signaling mechanisms that are known to promote neoplastic growth and cancer malignancy, including receptor tyrosine kinase signaling, inflammatory signaling cascades and non-coding RNAs, have been shown to perturb TJ protein expression and function. Perturbed TJ protein expression or function alters downstream signaling that targets cellular pathways relevant for epithelial homeostasis, invasion, chronic

inflammation and cancer (Zeb-1/E-cadherin, Wnt signaling, MMP9/Notch signaling and Src/PI3K/Akt signaling). Furthermore, disruption of TJs during infection or injury can result in increased permeability with translocation of bacteria and luminal antigen, which in turn increase IL-6/Stat3 signaling contributing to carcinogenesis (Figure 2). Delocalization of TJ proteins from their normal membrane-tethered expression appears to be common among inflammatory diseases and GI cancers. Aberrant cell signaling may contribute to this process. Notably, in Ras-overexpressing MDCK cells, CLDN1, OCLN, and ZO-1 were absent from the cell-cell contact sites however were present in the cell cytoplasm[46]. Inhibition of MEK1 activity recruited all three proteins to the cell membrane leading to a restoration of the TJ barrier function in these cells. In line with this, it has been shown that growth factor receptors, including EGF, HGF and IGF receptors, as well as proinflammatory and tumor promoting cytokines, including TNF-*α*, IFN-*γ*, IL-13, and IL-22, contribute to regulate CLDN expression[47, 48, 49, 50, 51]. It has also been reported that nonsteroidal anti-inflammatory drugs regulate CLDN expression in association with p38 MAPK activation in gastric epithelial cancer cells[52]. Furthermore, protein modifications by phosphorylation, sumoylation, palmitoylation[53, 54, 55] and endocytic recycling have emerged as potential mechanisms of regulating TJ protein function and expression [56, 57, 58].

Transcription factors known to be associated with cellular differentiation and EMT, including Snail, Cdx-2, HNF-*α*, and GATA-4[34, 59, 60], can bind to the promoter regions of specific CLDN genes to affect their expression in intestinal epithelial cells. In colon cancer cells, caudal homeobox proteins (Cdx1 & Cdx2) and GATA-4 in cooperation with the Wnt pathway are involved in CLDN1 promoter activation[34]. CLDN1 transcripts are regulated by Smad-4 (a known tumor suppressor) and HDAC inhibitors supporting a complex multiprotein regulatory scheme[61, 62]. Furthermore, transcription factor RUNX3, which is a gastric tumor suppressor, upregulates CLDN1 expression by binding to the promoter region of CLDN1 in gastric epithelial cells[63] and a similar regulatory scheme involving Cdx-proteins, GATA-4 and HNF-α has been demonstrated for CLDN2 and -4. Additionally, various epigenetic regulatory mechanisms likely also contribute to the transcriptional regulation of CLDN

expression (Table 2). Indeed, it has been shown that DNA hypermethylation associated with the downregulation of CLDN11 in gastric cancer cells[64] and CLDN7 in colon cancer cells[34]. Furthermore, loss of repressive histone methylations, including H3K27me3 and H4K20me3, is associated with the overexpression of CLDN4 in gastric cancer[65, 66].

Finally, microRNAs (miRNAs) post-transcriptionally regulate TJ formation and barrier function[67]. Indeed, different miRNAs have been shown to modulate CLDN1 expression (Table 2): targeting of CLDN1 mRNA by miR-29 has been shown to regulate intestinal permeability[68], while the regulation of CLDN1 mRNA by miR-155 plays an important role in promoting colorectal cancer (CRC) cell migration and invasion[69]. Moreover, the histone deacetylase has been shown to regulate CLDN1 mRNA stability in CRC cells through modulating the binding of the human antigen R and tristetraprolin to the 3′ UTR of CLDN1 mRNA[70].

TJ proteins and colorectal disease and cancer

Perturbation of the epithelial barrier function as well as TJ protein function and expression are hallmarks of GI disease including CRC. The breakdown of polarized epithelial barrier leads to the activation of specific signaling pathways as a response to injury. However, chronic activation of these signaling pathways can also promote cancer formation in premalignant epithelial tissues when TJs are chronically leaky. Furthermore, TJ proteins, especially CLDNs, have now been demonstrated to play an essential role in cell proliferation and neoplastic transformation during tumorigenic growth.

Understanding how these complex signaling networks are altered in cancer cells represents a major challenge for the success of anti-cancer therapies. Upregulation or aberrant tissue expression of CLDNs may contribute to neoplasia by altering TJ structure and function or affecting cell signaling pathways. As stated above, the loss of cell polarity, due to TJ deregulation can abrogate the normal check-points. Moreover, studies linking EMT with the acquisition of stem cell characteristics have demonstrated important role of CLDNs in regulating the EMT process and cancer progression[16, 71, 72, 73]. In addition to

regulating the barrier properties, TJs also serve as hubs for a multitude of signaling proteins including known tumor suppressor molecules like APC, PTEN (phosphatase and tensin homolog) and polarity proteins like Par-3[74, 75, 76]. Silencing of the expression and/or function of these proteins modulates CLDN expression and induces loss of polarity and EMT. Interestingly, genetic modulation of CLDN proteins in mice or cancer cells can similarly affect these signaling cascades, suggesting a feedback regulation.

Junctional proteins are known to play an important role or assist in cellular transformation when mislocalized from their normal membrane localization and could serve as oncogenic molecules. In this regard, the Wnt signaling pathway, essential for the differentiation of epithelial cells and imbalanced during intestinal epithelial oncogenic transformation is a major regulator of TJ protein expression[77]. For example, CLDN1 and CLDN2 proteins are known target genes of the Wnt/*β*-catenin signaling pathway with binding sites in the promoter of these genes[34, 59]. CLDN1 expression not only decreased significantly in response to the reduction of intracellular *β*-catenin by adenovirus-mediated transfer of wild-type APC into the APC-deficient colon cancer cells, but also two putative Tcf4 binding elements in the 5' flanking region of CLDN were confirmed to be responsible for activating its transcription[34]. Importantly, in the intestine CLDN1 is weakly expressed at the apical border of the lateral membrane of normal enterocytes but is strongly expressed at cell-cell boundaries as well as in the nucleus/cytoplasm of CRC cells. Many studies have demonstrated that the expression of CLDN1 at the mRNA and protein levels is increased in CRC tissue and correlates with tumor depth[78]. Additional studies using gene editing have further shown that an intricate interdependence between the Notch and Wnt-signaling upregulating CLDN1 expression to augment CRC progression[37] (Table 2). A role of the nuclear effectors of the Wnt signaling pathway is to bind directly to the CLDN2 promoter region and thereby enhance CLDN2 promoter activity. Also, a crosstalk between the Wnt signaling and Cdx related transcriptional activation machinery has been implicated in regulating CLDN2 promoter-activation[59]. Recent studies have also demonstrated that levels of CLDN1 and CLDN2 are elevated in IBD-associated carcinoma[39] (Table 2).

Kinugasa et al.[79] demonstrated increased staining for CLDN1 in both high-grade dysplasia and ulcerative colitis (UC)-associated CRC when compared with normal or UC samples. CLDN1 overexpression modulates Notch-signaling in an MMP9-dependent manner to modulate barrier properties and immune homeostasis to promote susceptibility to inflammation-induced colitis and cancer[21]. Here it is worth noting that the outcome from a series of studies have now provided ample evidence for a role of deregulated CLDN1 expression in promoting invasive and metastatic abilities of the colon cancer cells. Notably, CLDN1 expression was sufficient to induce metastatic abilities in a colon cancer cell line that normally does not metastasize well *in vivo*. In contrast, stable genetic inhibition of CLDN1 in a poorly differentiated, highly metastatic and CLDN1 high colon cancer cell significantly inhibited its metastatic abilities in a splenic model of CRC metastasis[78]. An increase in CLDN2 expression also participates in promoting colorectal carcinogenesis potentially dependent on the EGFR/ERK1/2 signaling[80, 81]. Here, overexpression of CLDN2 in CLDN2 deficient CRC cells resulted in increased cell proliferation, anchorage-independent growth and tumor growth[81]. A similar effect of the Wnt-/β-catenin signaling upon gene expression of yet another component of the TJ complex, ZO-1, in human colonic cancer cell lines with low endogenous *β*-catenin has been reported suggesting potential contribution to the loss of epithelial polarization in neoplastic cells[59] (Table 2). Decreased ZO-1 expression was noted in the human digestive tract[82]. Using tissue biopsy samples, Mees et al.[83] also found that CRC in human exhibits significantly elevated expression levels of CLDN1 and -4 compared with normal mucosa. However, CLDN expression in colon cancer tissues is differential and downregulation of CLDN7 and -8 has been reported in colorectal adenoma samples compared with the normal intestinal tissues[84]. Collectively, these studies suggest that these proteins may serve as potential biomarkers for CRC progression and therapy resistance.

CLDNs and esophageal and gastric cancer

Similar to CRC, TJ proteins are also regulated aberrantly in the esophageal and gastric cancers and this abnormal expression correlates with specific clinicopathologic parameters. In the esophageal adenocarcinoma (EA), CLDN expression has been tested as potential biomarker for the transition of the Barrett's esophagus to the EA. Indeed, CLDN2, -3, −4 and −7 are reported to have increased expression in EA compared to precancerous lesions and normal esophageal squamous mucosa[85, 86]. JAMs, which comprise the integral parts of TJs in the gastric epithelium, have been shown to promote proliferation, invasion, and inhibit apoptosis. JAM-B was upregulated significantly in tumor samples compared with adjacent normal tissues and was higher in high grade tumors than in the low grade and intermediate grade tumors[87].

An increased expression of CLDN2 is also associated with gastric cancer progression[88]. Additionally, expression of CLDN11 and -23 is downregulated in gastric cancer[89] and miR-421 was implicated in regulating CLDN11 expression to promote the proliferation, invasion and metastasis of gastric cancer cells[90] (Table 2). In contrast, CLDN23 positive expression was associated with poor prognostic outcomes of gastric cancer patients and may therefore serve as an independent predictor of patient survival. Similarly, upregulated expression of CLDN4 in gastric cancer was associated with cancer progression and poor prognosis[91]. Furthermore, CLDN4-expressing gastric adenocarcinoma AGS cells were found to have increased MMP2 and -9 expression, indicating that CLDN-mediated increased invasion may be mediated through the activation of MMPs[91]. Overall, these results suggest that CLDN4 overexpression may promote gastric cancer metastasis through the increased invasion of gastric cancer cells. Yet another CLDN family protein, CLDN18 is significantly downregulated in gastric cancer tissues and cell lines, which was associated with tumor size, location invasion, histologic type and tumor-node-metastasis stage. miR-1303 was demonstrated to have putative binding sites in CLDN18 mRNA 3'-UTR and visibly lower the expression of CLDN18[92] (Table 2). On the other hand, CLDN18.2 (isoform 2 of claudin-18) was retained on malignant transformation and was expressed in a significant

proportion of primary gastric cancers and its metastases[9]. The expression of CLDN7 has also been reported to have the potential of serving as an independent indicator of the poor prognosis in gastric cancer[93].

Taken together, TJ proteins are tissue-specific regulators of the epithelial and endothelial barrier function and EMT, which cumulatively perturb the epithelial and immune homeostasis leading to carcinogenesis in CRC as well as gastric and esophageal cancer.

TJ proteins and the liver

The liver plays an essential role in homeostasis by its metabolic and storage functions. It is composed of different cell types, including two types of epithelial cells: hepatocytes and cholangiocytes. Liver epithelial junctions are important for liver function. Hepatocytes are liver parenchymal cells that exhibit a complex honeycomb morphology displaying at least two basolateral membranes (facing the sinusoidal blood) and two intercellular apical membranes (forming the bile canaliculi) separated by TJs. This peculiar architecture creates what has been termed the blood-biliary barrier[94] and enables hepatocytes to perform distinct secretory functions at the same time[95]. Hepatocytes produce and secrete bile into the bile canaliculi from where it is transported via intrahepatic and extrahepatic bile ducts, formed by cholangiocytes, to the gallbladder. Cholangiocytes contribute to modify the bile during its transport to the latter. Hepatocyte polarity and bile duct TJs play a major role in the liver[96, 97] and thus defects in hepatocyte and/or cholangiocyte TJ integrity can result in pathophysiological consequences.

Several lines of evidence indicate that disruption or loss-of-function of TJs contributes to the pathogenesis of cholestatic diseases including primary biliary cholangitis and primary sclerosing cholangitis[98, 99]. Interestingly, mutations in CLDN1 are associated with neonatal ichthyosis and sclerosing cholangitis (NISCH) syndrome where deficient CLDN1 expression may contribute to paracellular bile leakage through deficient TJs[100]. Mutations in ZO-2 have been described in familiar hypercholanemia[101]. The loss-of-function of TJ

proteins is not lethal and the clinical manifestation is variable including very mild symptoms[102]. A comprehensive description of TJ protein alterations in biliary diseases is reviewed in reference[97].

Recent studies demonstrate that TJ protein expression is altered in HCC (primary liver cancer) and cholangiocarcinoma (biliary tract cancer). For example, CLDN1 has been shown to be up-regulated in advanced liver disease and HCC[103] and differential CLDN4 expression can help to distinguish these two forms of cancer at a molecular level[104]. Of note, TJ alteration in epithelia outside the liver can also contribute to liver disease. Indeed, dysfunction of the intestinal epithelial barrier - due to or unrelated to (aetiological factor(s) of) the underlying liver disease - has been associated with the pathogenesis of chronic liver disease and the development of complications in cirrhosis by favouring translocation of bacteria and bacterial products from the intestinal lumen into the systemic circulation[105].

CLDN1 and OCLN mediate hepatocyte entry of HCV

Ten years ago, expression cloning experiments uncovered CLDN1 to be required for HCV infection[26]. The role of OCLN in HCV infection was uncovered two years later using different approaches[27, 106, 107]. Subsequently several studies have characterized the underlying molecular mechanisms and highlighted the essential function played by these proteins in HCV entry and infection[4, 108, 109]. While over the past 20 years many host factors have been reported to contribute to the early steps of HCV infection[108, 110], CLDN1 and OCLN are regarded as two of the four major HCV host entry factors together with CD81[111] and scavenger receptor BI (SR-BI)[112] (Figure 3).

First binding studies indicated that CLDN1 was unable to bind the HCV envelope glycoprotein E2[26], suggesting that CLDN1 does not play the role of a primary receptor but rather of a co-receptor, which contributes to (a) step(s) subsequent to viral binding[26]. This was further confirmed in kinetic assays using anti-CLDN1 antibodies[113]. Several years later it was shown that in contrast to soluble E2, HCV E1E2 complexes can interact with the CLDN1 ECL1 and that this interaction is involved in viral fusion[114]. Based on the

388 Coxsackie B virus cell entry model, it was suggested that HCV may first interact with host
389 factors on the basolateral surface of hepatocytes and then move to the TJ co-entry factor(s),
390 e.g. through CD81-lateral membrane movements[26, 115]. Elegant fluorescence resonance
391 energy transfer studies showed that CLDN1 interacts with CD81 to promote viral
392 internalization[116, 117, 118]. Interestingly, no cellular function for CD81-CLDN1 interaction
393 has been reported so far and disruption of these complexes by defined anti-CLDN1
394 antibodies prevents HCV infection without affecting TJ integrity or any detectable adverse
395 effect[113, 119, 120]. Indeed, several lines of evidence support a model in which the non-
396 junctional form of CLDN1 rather than CLDN1 localized within TJ mediates HCV entry[117,
397 121]. The major pool of CLDN1 is expressed at TJs of hepatocytes and polarized hepatoma
398 cells but a minor fraction is also located at the basal membranes of these cells[117, 122]. Of
399 note, CD81-CLDN1 co-receptor association could only be detected at the basal membranes
400 but not in TJ-associated pools of CLDN1 and CD81[117]. Furthermore, the ECL1 appears to
401 be the critical part of the protein for HCV entry while the intracellular C-terminal part of
402 CLDN1 that plays an important role for its interaction with intracellular TJ components is not
403 required for this process[26, 121]. Interestingly, CLDN6 and CLDN9 - but not other members
404 of the CLDN family of proteins - have been shown to be able to promote HCV entry into
405 CLDN-deficient 293T-derived cell lines[123, 124]. This is most likely due to their ability to
406 form co-receptor associations with CD81 like CLDN1[118]. It is of interest to note that in
407 experimental model systems using a liver tumor cell lines some HCV genotypes have been
408 reported to be able to use either CLDN1 or CLDN6[125] through mutation in the HCV E1
409 envelope protein[126]. Whether CLDN6 or 9 can replace CLDN1 in the liver of HCV-infected
410 patients remains questionable since CLDN6 and CLDN9 expression is very low or absent in
411 human liver tissues[124, 125, 127]. Furthermore, treatment of HCV infection in human liver
412 chimeric mice with a monoclonal CLDN1-specific antibody did not reveal any detectable
413 escape (for a detailed review of the role of CLDN6 and CLDN9 in HCV entry please
414 see[128]).

Like CLDN1, OCLN does not appear to play the role of a primary HCV attachment receptor but rather is required for late postbinding event(s) during the HCV entry process[107, 129] (Figure 3). Nevertheless, HCV might interact with OCLN during viral entry and/or in infected cells. Indeed, imaging studies evidenced a co-localization between OCLN and HCV E2 in the endoplasmic reticulum of hepatoma cells[130]. Furthermore, it was shown that an anti-E2 antibody could immunoprecipitate OCLN while GST-OCLN could pull down E2[129, 130]. The OCLN ECL2 appears to be important for this interaction with HCV E2 as well as for HCV entry[129]. However, the OCLN ECL2 was unable to pull down E2, suggesting that either this interaction might not be direct or not be visualized in the utilized experimental design[129]. Experiments using OCLN engineered to be recognized by anti-FLAG antibodies are in favour of a HCV-OCLN interaction as different clones displaying the FLAG epitope at different locations within ECL1 or ECL2 exhibited HCV isolate-dependent host factor activity[131]. Using kinetic assays in polarized cells, this study also showed that OCLN plays a role subsequent to SR-BI, CD81 and CLDN1[131]. These results were recently confirmed in kinetic assays using anti-OCLN mAbs directed against either the ECL1 or ECL2 of OCLN[132]. How and where HCV interacts with OCLN as well as what pool(s) of OCLN is(are) involved in this process remain to be further characterized. Although in liver sections OCLN has been located at apical surfaces of hepatocytes[117], a minor pool of this protein is expressed on the basolateral surface of hepatocytes. Indeed, OCLN is known to traffic through the basolateral membrane towards TJs[133] and its subcellular localization appears to be dependent on its phosphorylation status: phosphorylated forms of OCLN mainly are found in TJs of epithelial cells, while less phosphorylated forms are localized at the basolateral membrane and in the cytosol[134]. This is in line with a recent report showing that tumor-associated calcium signal transducer 2 (TACSTD2) regulates HCV entry by leading to the phosphorylation of CLDN1 and OCLN and regulates their subcellular localization[135]. The importance of the subcellular localization of OCLN for HCV entry is also underscored by the fact that only OCLN and its splice variant OCLN-ex7ext that both localize to the plasma membrane are able to promote HCV entry in contrast to other OCLN

splice variants that exhibit an intracellular localization[136]. Of note, OCLN together with CD81 define the HCV species specificity as HCV non-permissive mouse cells acquire HCV-permissivity subsequent to human CD81 and OCLN expression both *in vitro* and *in vivo*[27, 137, 138, 139]. The species-specific determinants appear to be located within the second extracellular loop of OCLN[27, 139].

Beside cell-free HCV entry, CLDN1 and OCLN have also been shown to be important for HCV cell-to-cell transmission (Figure 3), a major mode of viral dissemination that enables the virus to avoid the host's immune surveillance and to establish chronic infection[140, 141, 142, 143]. The exact localization at the plasma membrane of this process as well as the form(s) of CLDN1 that contribute(s) to HCV cell-to-cell transmission remain unknown. The importance of CLDN1 and OCLN for the pathogenesis of HCV infection *in vivo* has been confirmed by observations of liver tissues from HCV-infected liver transplant patients. HCV recurrence was associated with an increase in CLDN1 and OCLN expression levels in hepatocytes over time after transplantation[144]. This is in line with findings indicating increased CLDN1 and OCLN expression levels in HCV-infected livers[122, 145, 146]. In contrast, in cell-based studies HCV infection was shown to downregulate CLDN1 and OCLN expression to prevent superinfection[106]. Differences in TJ protein expression upon HCV infection may thus exist depending on the analyzed samples.

CLDNs and HCC

The expression of several TJ proteins has been reported to be perturbed in liver tissue from HCC patients. Many studies have shown different expression levels of the individual CLDNs and OCLN and CLDN1 are being investigated as biomarkers for liver disease progression[103, 122, 147, 148, 149, 150, 151]. From these studies, it appears that expression of CLDNs is associated with more severe disease and/or bad prognosis in HCC patients (Table 2): epigenetic silencing of CLDN14 was significantly associated with advanced tumor state and tumor aggressiveness[152]; CLDN11 downregulation by miR-99 has been associated with metastasis of HCC[153]; and CLDN3 downregulation has been

suggested to promote EMT via Wnt-β-catenin signaling[154]. However, more data are needed to decipher the role of these TJ proteins in the pathogenesis of HCC. Several studies have shown an increase in CLDN1 expression on basolateral and apical hepatocyte membranes in cirrhotic livers and HCC compared to normal livers[103, 122, 149]. Interestingly, this increase was observed in tissues from both HCV-positive and -negative patients, although it appeared to be stronger in tissues from HCV-infected patients, as well as in HCC that developed on either cirrhotic or non-cirrhotic livers[103] and in paediatric HCC[149]. In advanced HCC down-regulation of CLDN1 has been observed[147, 151] which may correspond to the de-differentiation of cancer cells. Of note, a greater cytoplasmic localization of CLDN1 was observed in some HCC in line with reports indicating that CLDN localization has a causal role in cellular transformation[78, 155].

CLDN1 likely contributes to proliferation, motility and invasion by modulating cellular signaling. Overexpression of CLDN1 increases the migration and invasiveness of human hepatoma cells as well as normal liver cells through expression of MMP2 via the c-Abl-PKC pathway[20] (Figure 4). Furthermore, increased CLDN1 expression has been associated with mitochondrial dysfunction and invasiveness of hepatoma cells, and reactive oxygen species-mediated activation of heat shock factor 1 (HSF1) was demonstrated to increase CLDN1 expression in these cells[156, 157] (Table 2). These data are in line with reports indicating that CLDN1 enhances cell growth, migration and/or invasiveness of other cancer cell types such as oral squamous cell carcinoma cells, CRC cells, ovarian cancer-initiating cells or melanoma cells[69, 158, 159, 160, 161]. Taken together, these data suggest that by promoting cell migration and increasing the invasive behaviour of cancer cells, CLDN1 can contribute to cancer spread. Of note, CLDN1 has been shown to promote EMT in normal liver cells and HCC cells that thereby acquire an invasive phenotype[162]. This process is mediated by the c-Abl-Ras-Raf-1-ERK pathway and involves the transcription factors Slug and Zeb1[162] (Figure 4). Confirming the functional role of these signaling pathways, an CLDN1-specific antibody inhibits the HCV-induced increase in ERK1/2 phosphorylation in

498 human liver tissue [120]. Further studies are needed to understand the detailed role of
499 CLDNs in pathogenesis of liver disease and cancer.
500
501 **Targeting TJ proteins for therapeutic approaches in the gut and the liver**
502 ***CLDNs as targets for CRC***
503 While the field related to the role and regulation of TJ proteins in GI pathologies and
504 oncogenic growth has taken a significant leap forward, therapeutic application of this
505 knowledge is now emerging. Significant progress has been made at several fronts including
506 the development of prognostic biomarkers, imaging and targeting. In this regard, CLDN
507 proteins are currently investigated as potential biomarkers for disease progression and
508 therapy resistance. A recent study has shown that serum levels of CLDN1 and CLDN7 may
509 be a useful tool in the differential diagnosis of CRC[163]. Furthermore, a progressive
510 increase in CLDN1 expression in colon cancer and the recent findings that infra-red imaging
511 using CLDN1-targeted conjugated peptide can enhance the ability of conventional
512 colonoscopy for detecting human colonic adenomas strongly supports the potential impact of
513 CLDN1 as a biomarker[164]. CRC has been found to arise from missed polypoid and flat
514 precancerous lesions which are more difficult to visualize by colonoscopy and the new
515 CLDN1 targeted fluorescent peptides may be used to improve screening of high-risk patients
516 with multiple polyps, inflammatory bowel disease, Lynch syndrome, or a family history of
517 CRC.
518 Aiming to develop targeted therapies, several antibodies against the extracellular
519 domain of CLDNs have been developed. Their therapeutic effects for cancer and metastasis
520 are summarized in Table 3. Ideal monoclonal antibodies (IMAB) specific to the proteins
521 expressed only on the tumor and hence avoiding potential off-target effects are actively being
522 developed. Currently, monoclonal antibodies (mAbs) have been generated against CLDN1,-
523 2, -3, -4, -6, and -18.2. The antibody against CLDN18.2 (claudiximab) is in clinical
524 development for gastric cancer[9]. Interestingly, claudiximab significantly extends median
525 survival when added to standard chemotherapy (13.2 vs 8.4 months) in patients with

advanced gastric cancer[9]. Importantly, this target is not present in any healthy tissues except the lining of the stomach, thereby minimizing treatment side effects. In addition, recent studies have investigated the anti-tumor effect of anti-CLDN1 and anti-CLDN2 mAbs using cancer cell models[11, 165] (Table 3). Importantly, anti-CLDN mAbs have been shown to be safe and no relevant off-targets have been reported. Their marked therapeutic effects combined with excellent safety profiles are highly encouraging for their development in clinical applications.

CLDN1 and OCLN - targets for cure of HCV infection

CLDN1 was the first TJ protein to be explored as a therapeutic target for HCV infection using anti-CLDN1 antibodies directed against its extracellular domain(s) (Table 3). Such antibodies could be used to prevent liver graft infection in HCV-positive transplant recipients and as a promising alternative for patients who fail current anti-HCV therapies[166]. The first antibodies directed against human CLDN1 and blocking HCV infection were generated by genetic immunization in rats[113, 119]. They recognize a conformational-dependent epitope within the ECL1 and prevent CD81-CLDN1 co-receptor association at the basolateral membrane[113, 119]. They are characterized by pan-genotypic inhibition of the infection by all major HCV genotypes by blocking both cell-free virus entry and viral cell-to-cell transmission[119, 143]. Of note, studies in human liver-chimeric mice demonstrated that the lead antibody OM-7D3-B3 was not only able to prevent acute *de novo* infection with HCV (i.e. the anticipated effect of an entry inhibitor) but also to cure already established chronic HCV infection without detectable side or off-target effects[120]. These results highlighted the importance of viral dissemination for maintenance of chronic HCV infection. It is of interest to note that this antibody interfered with MAPK signalling suggesting an important role in this pathway potentially also contributing to its antiviral effect[120]. The therapeutic potential of this antibody is further underscored by the fact that it acts in synergy with HCV direct-acting antivirals (DAAs), the current state-of-the-art antiviral therapy, to clear viral infection and is also active on viral variants escaping DAAs[143, 167]. This antibody has recently been

554 successfully humanized (IgG4) for further clinical development[168]. Subsequently, other
555 CLDN1-specific antibodies inhibiting HCV infection have been reported: clones 3A2 and 7A5,
556 generated in mice and recognizing the human CLDN1 ECL2 can prevent HCV infection of
557 human liver-chimeric mice[169] while several antigen-binding fragments (Fab) and single
558 chain antibody fragments selected using phage display were demonstrated to inhibit HCV
559 infection *in vitro* when converted into human IgG1 or IgG4[170, 171]. Administration of
560 CLDN1-specific antibodies has been shown to be very safe in various animal and human-cell
561 based models without any adverse effects on the liver or other organs such as the gut or
562 skin[120, 168, 172]. This is most likely to the mechanism of action of CLDN1-specific
563 antibodies targeting the non-junctional expressed CLDN1 on the hepatocyte basolateral
564 membrane without affecting TJ barrier function as shown in several TJ model systems[120,
565 168, 172].

566 Antibodies directed against OCLN have been more difficult to generate than anti-
567 CLDN mAbs but very recently five mAbs with anti-HCV activity were described by two
568 different groups[132, 173]. The mouse mAb (67-2) - raised against a linear peptide within
569 OCLN ECL2 - was shown to recognize an epitope present in the ECL2 of both human and
570 mouse OCLN[173]. Interestingly, this mAb hardly inhibited the infection of human hepatoma
571 Huh7.5.1 cell monolayers with HCV when applied to the apical membrane of cells while it
572 was able to efficiently prevent HCV infection when applied to the basolateral membrane of
573 cells using a double-chamber culture system or a 3D culture model[173]. Of note, in line with
574 the hypothesis that mAb 67-2 interacts with OCLN monomers expressed on the basolateral
575 membrane, this mAb had no effect on TJ function in Eph4 cells[173]. Four rat mAbs -
576 generated by genetic immunization and directed against either the ECL1 or ECL2 of OCLN –
577 inhibited the entry of HCV into human hepatoma cells without affecting TJ barrier function of
578 polarized cells. Since the mAb directed against ECL2 appeared to be more potent in
579 inhibiting HCV infection than mAbs directed against ECL1 and in line with previous studies
580 using OCLN mutants/chimeras having demonstrated the essential function of ECL2 for HCV
581 infection[27, 129, 174], the authors hypothesized that the mAb directed against ECL2 may

block an essential function of OCLN in the HCV entry process while mAbs directed against ECL1 may block HCV infection through steric hindrance. Two of those mAbs targeting either ECL1 or ECL2 (1-3 and 37-5) were shown to inhibit HCV infection in human liver chimeric mice without apparent side effects highlighting the possibility to target OCLN *in vivo*[132].

The positioning of CLDN1- and OCLN-specific antibodies in the widening arsenal of anti-HCV therapies is most likely for patients with multi-resistance to DAAs or in organ transplantation including HCV-positive donors where prevention of *de novo* infection may be preferable to cure of an established HCV infection. They may offer also perspectives to further shorten therapy regimens when combined with DAAs[175, 176].

Conclusions and future perspectives

Research in the last two decades has demonstrated an important role of TJ proteins in the physiology and disease biology in GI and liver disease. TJ proteins exert their functional role as integral proteins of TJs in forming barriers in the gut and the liver. Furthermore, TJ proteins are expressed non-junctionally where they play important roles in signaling, trafficking and regulation of gene expression outside the TJs. A hallmark of TJ proteins in disease biology is their role in EMT, which is relevant for organogenesis and differentiation (EMT type 1), inflammation and fibrosis (EMT type 2) and cancer metastasis/invasion (EMT type 3). A causative role of TJ proteins has been established in the pathogenesis of CRC and gastric cancer. Among the best characterized role of TJ proteins in liver disease biology is their function as cell entry receptors for HCV – one of the most common causes of HCC. At the same time TJ proteins are emerging as targets for novel therapeutic approaches for GI and liver disease: these include treatment of CRC and gastric cancer as well as antiviral therapy for chronic HCV infection complementing DAAs. Further studies are needed to study their role in chronic inflammation, fibrosis and their role as drivers for carcinogenesis. The understanding of these mechanisms offers new perspectives for novel therapeutic approaches for key unmet medical needs in the gut including CRC and gastric cancer as well as chronic liver disease and HCC.

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Conflict of interests

TFB is a co-inventor of a patent/patent application of CLDN1-specific antibodies for prevention and treatment of HCV infection. TFB and MBZ are co-inventors on patent applications for anti-claudin 1 monoclonal antibodies for the prevention and treatment of liver disease and HCC.

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1264 **Table 1. Role of TJ proteins in disease.** Examples of TJ protein-disease associations and
1265 the underlying mechanisms are shown. CLDN: claudin, JAM: junctional adhesion molecule,
1266 TJ: tight junction, ZO: zona occludens

TJ protein	Disease	TJ protein expression	Described mechanism	References
JAM-A	**Cancer**		PI3K/MAPK signaling, Notch signaling, TGF-β1 signaling	[177, 178, 179, 180, 181, 182, 183, 184, 185]
	Brain	↑		
	Breast	↑↓		
	Gastric	↑		
	Lung	↑		
	Endometrial	↓		
	Pancreatic	↓		
	Hereditary diseases			[186]
	Cystic fibrosis	↓		
	Viral infection			[187]
	Retroviral infection (hydrocephalus, encephalitis)	↑		
JAM-C	**Cancer**		LRP5/AKT/β-catenin/CCND1 signaling, PI3K/MAPK signaling	[188, 189, 190, 191, 192, 193, 194, 195]
	Fibrosarcoma	↑		
	Lung	↑		
	Melanoma	↑		
	Ovarian	↑		
Coxsackie virus and adenovirus receptor (CAR)	**Cancer**		MyD88/IRAK-4/NF-κB, ERK1/2 signaling, estrogen signaling	[196, 197, 198, 199, 200, 201, 202, 203, 204]
	Breast	↑		
	Endometrial	↑		
	Lung	↑		
	Oral	↑		
	Ovarian	↑		
	Thyroid	↑		
CLDN1	**Cancer**		Reactive oxygen species-mediated activation of heat shock factor 1 (HSF1)	[34, 78, 156, 157, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221]
	Breast	↑↓		
	Cervical	↑		
	Colorectal	↑↓		
	Gastric	↑		
	Liver	↑		
	Oral	↑		
	Ovarian	↑		
	Prostate	-		
	Thyroid neoplasma	↑		
	Hereditary disease			
	Cystic fibrosis	-		

				[186]
CLDN2	**Cancer**		EGFR/MEK/ERK signaling	[212, 221,
	Breast	↑↓	PI3K signaling	222, 223,
	Colorectal	↑		224, 225,
	Lung	↑		226, 227,
	Skin	↑		228, 229]
	Prostate	↓		
	Inflammation			
	Inflammatory bowel disease:			[230, 231]
	Morbus Crohn	↑		
	Collagenous colitis	↑		
CLDN3	**Cancer**		EGFR/MEK/ERK signaling	[19, 217, 232,
	Breast	↑	PI3K/Akt signaling	233, 234,
	Colorectal	↑	Wnt signaling	235, 236,
	Endometrial	↑↓	Stat3	237, 238]
	Gastric	↑		
	Kidney	↑		
	Lung	↑		
	Ovarian	↑		
	Prostate	-		
	Uterine	↑		
	Inflammation			
	Inflammatory bowel disease:			[230, 239]
	Morbus Crohn	↓		
	Bacterial toxins			
	Clostridium perfringens enterotoxin	↓		[240]
CLDN4	**Cancer**		ERK signaling	[19, 91, 217,
	Breast	↑	AMPK signaling	232, 235,
	Endometrial	↑		241, 242,
	Gastric	↑↓		243]
	Kidney	↑		
	Lung	↑		
	Nasopharyngeal	↑		
	Ovarian	↑		
	Pancreatic	↑		
	Uterine	↑		
	Inflammation			
	Collagenous colitis	↓		[231]
	Hereditary diseases			
	Cystic fibrosis	↓		[186]
	Bacterial toxins			

	Clostridium perfringens enterotoxin	↓		[240]
CLDN7	**Cancer** Breast Cervical Colon Gastric Liver Lung Nasopharyngeal Ovarian Pancreatic Prostate Thyroid neoplasma	 ↓ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑ ↑	ERK/MAPK signaling Wnt signaling Integrin/FAK signaling	[211, 217, 218, 232, 241, 243, 244]
	Inflammation Crohn's disease Ulcerative colitis Celiac disease	 - ↓ -		[239]
CLDN11	**Cancer** Gastric	 ↑	TGF-β, ERK, p38 signaling	[88]
CLDN16	**Cancer** Breast Ovarian Renal	 ↑ ↑ ↑		[245, 246, 247]
	Hereditary diseases Familial hypomagnesemia	 Mutation		[248]
CLDN20	**Cancer** Breast	 ↑		[249]
OCLN	**Cancer** Thyroid neoplasma	 Diverse expression	PI3K signaling MAPK signaling	[218, 221]
	Inflammation Crohn's disease Ulcerative collitis Celiac Disease	 ↓ ↓ ↓		[231, 239, 250]
	Hereditary diseases Cystic fibrosis	 ↑		[186]
	Vision loss Diabetic eye disease: diabetic retinopathy	 ↑		[251]
ZO-1	**Cancer** Breast	 ↓	PI3K signaling MAPK signaling	[207, 221]

	Inflammation Inflammatory bowel disease: Morbus Crohn	↓	[230]
	Vision loss Diabetic eye disease: diabetic retinopathy	↑	[251]

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Table 2. Perturbation of tight junction protein expression in gastrointestinal and liver disease. Examples of perturbed CLDN and ZO protein expression in gastrointestinal and liver disease as well as the underlying mechanism are shown. CLDN: claudin, miR: micro-RNA, TJ: tight junction, ZO: zona occludens

Disease/Cell type	TJ protein expression	Described mechanism	References
Gastric cancer	CLDN4 ↑	Loss of repressive histone methylation (H3K27m3, H4K20m3)	[65, 66]
	CLDN11 ↓	DNA hypermethylation miR-421	[64] [90]
	CLDN18 ↓	miR-1303	[92]
Intestinal bowel disease-associated carcinoma	CLDN1 ↑ CLDN2 ↑	β-catenin activation	[39]
Colorectal cancer	CLDN1 ↑	miR-155 Histone deacetylase-mediated binding of human antigen R and tristetraprolin to CLDN1 mRNA Increased Notch and Wnt signaling	[37, 59, 69, 70]
	CLDN2 ↑	Increased Notch and Wnt signaling	[37, 59]
	CLDN7 ↓	DNA hypermethylation	[34]
	ZO-1 ↑	β-catenin activation	[59]
Hepatocellular carcinoma	CLDN1 ↑	Reactive oxygen species-mediated activation of heat shock factor 1 (HSF1)	[156, 157]
	CLDN11 ↓	miR-99	[153]

Table 3. Preclinical and clinical development of antibodies directed against tight junction proteins. The respective target, names of monoclonal antibodies (that have at least reached preclinical stage of development), clinical indication, and stage of development are shown. CLDN: claudin, HCV: hepatitis C virus, OCLN: occludin

Targets	Monoclonal antibodies	Clinical indication	Stage of development	References
CLDN1	OM-7D3-B3 and H3L3	HCV infection	Preclinical	[120] [168]
	3A2	HCV infection	Preclinical	[169]
	6F6	Colorectal cancer	Preclinical	[11]
CLDN2	xi-1A2	Cancer	Preclinical	[165]
CLDN3 and CLDN4	KM3907	Cancer	Preclinical	[252]
	5A5	Cancer	Preclinical	[253]
CLDN4	KM3900	Pancreatic and ovarian cancers	Preclinical	[254]
CLDN6	IMAB027	Ovarian cancer	Phase I/II	NCT02054351
CLDN18.2	IMAB362 (claudiximab)	Gastroesophageal cancer	Phase II	NCT01630083
OCLN	1-3 and 37-5	HCV infection	Preclinical	[132]

Figures legends

Figure 1. Schematic representation of the expression and function of the major tight junction proteins addressed in this review. This simplified cartoon only displays the localization and interactions of the major tight junction (TJ) protein families that are addressed in this review. TJs are composed of transmembrane proteins, including different claudins (CLDNs), tight junction-associated marvel proteins (TAMPs, e.g. OCLN), junctional adhesion molecules (JAMs e.g. JAM-A) as well as cytosolic proteins (e.g. ZO-1, -2 and -3), which connect transmembrane components to the cytoskeleton (actin filaments, microtubules). For a more detailed description please refer to reference[1].

Figure 2. Schematic representation of differential regulation of tight junction proteins and associated signaling in gastrointestinal cancer. Signaling and molecular mechanisms that are known to promote neoplastic growth and cancer malignancy include the receptor tyrosine kinase signaling, inflammatory signaling cascades and non-coding RNAs that perturb tight junction (TJs) expression and function. TJ perturbation alters downstream signaling that target important cellular events in epithelial homeostasis, invasion, chronic inflammation and cancer (Zeb-1/E-cadherin, Wnt signaling, MMP9/Notch signaling and Src/PI3K/Akt signaling). Furthermore, disruption of TJs can result in increased permeability to promote translocation of bacteria and luminal antigens, which then activate IL-6/Stat3 signaling to induce carcinogenic processes.

Figure 3. Functional roles of CLDN1 as hepatitis C virus entry factor. CLDN1 is one of the four main hepatitis C virus (HCV) host factors (i.e. SR-BI, CD81, CLDN1 and OCLN) essential for the early steps of HCV infection. Several other host factors (e.g. highly sulphated heparan sulfate (HS), low-density lipoprotein receptor (LDLR), epidermal growth factor receptor (EGFR), integrin beta 1 (ITGB1), transferrin receptor 1 (TfR1) and Niemann Pick C1 like 1 (NPC1L1)) contribute to viral binding and entry. EGFR-mediated signaling leads to the formation of a CD81-CLDN1 co-receptor complex that ultimately leads to viral internalization[116, 118]. HCV infection induces CLDN1-depend signaling via the ERK1/2

1310 pathway[120]. HCV infection increases CLDN1 expression[122, 145]. TJ proteins involved in
1311 the HCV entry process are depicted in black, non-TJ host entry factors are depicted in white.
1312
1313 **Figure 4. Functional role of CLDN1 in signal transduction and EMT in liver disease.** In
1314 transformed liver cells, CLDN1 over-expression activates the c-Abl-PKCδ pathway to
1315 increase cellular migration and invasion via MMP2 activation[20] as well as the c-Abl-Ras-
1316 Raf-ERK pathway to promote EMT via the transcription factors Slug and Zeb1[162].

Figure 1

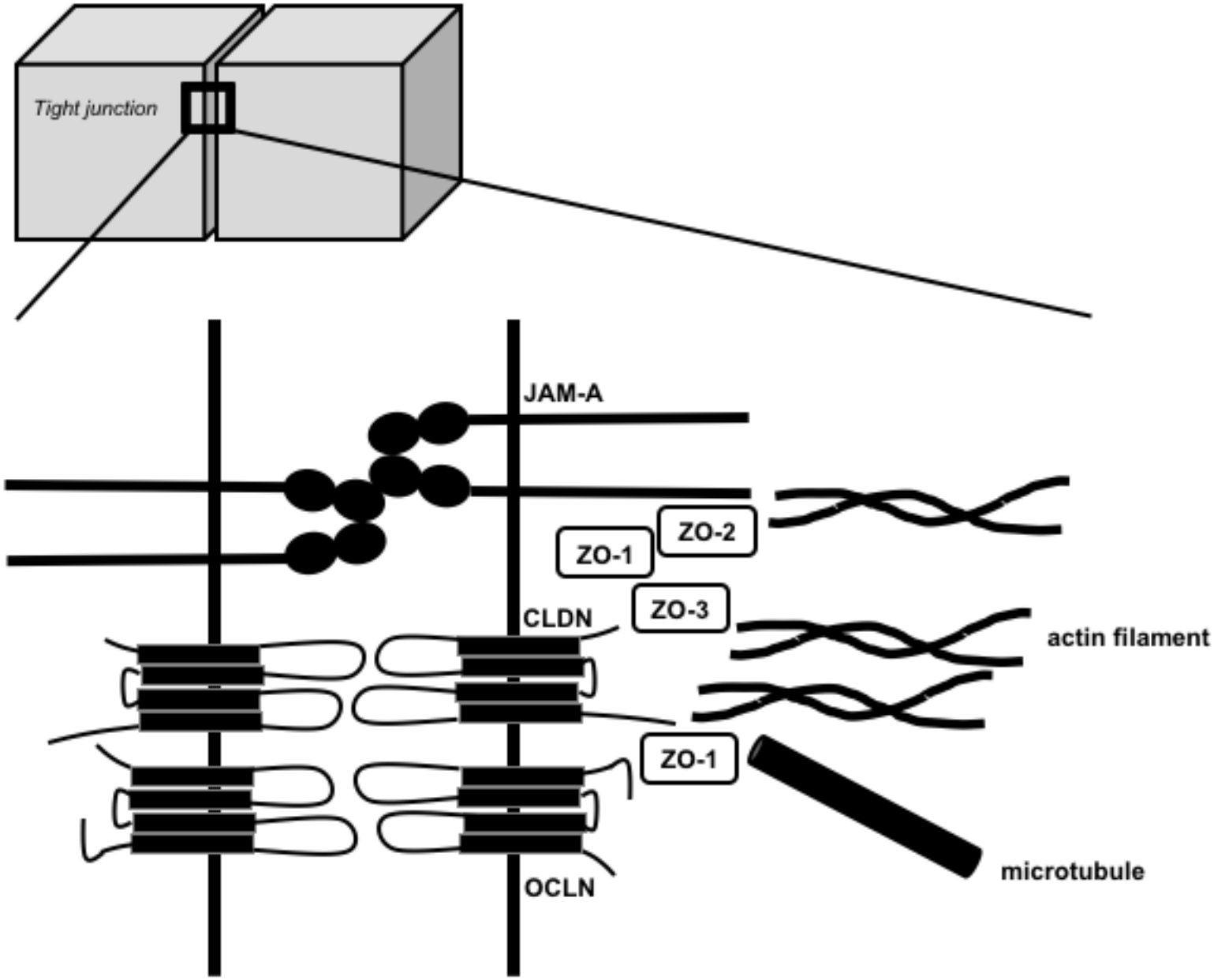

Tight junction
JAM-A
ZO-2
ZO-1
ZO-3
CLDN
actin filament
ZO-1
microtubule
OCLN

Figure 2

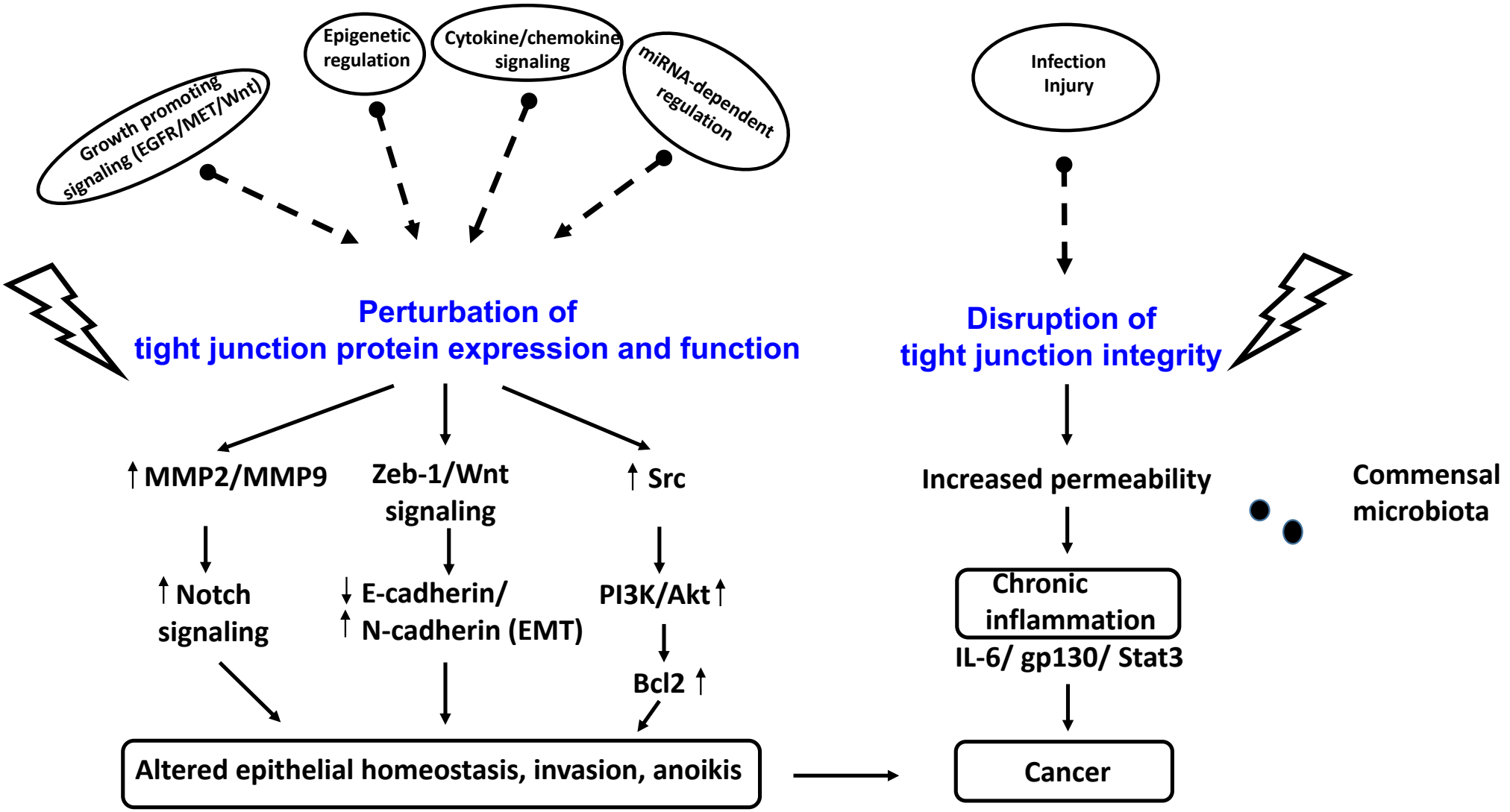

Growth promoting signaling (EGFR/MET/Wnt)
Epigenetic regulation
Cytokine/chemokine signaling
miRNA-dependent regulation
Infection Injury
Perturbation of tight junction protein expression and function
Disruption of tight junction integrity
↑ MMP2/MMP9
Zeb-1/Wnt signaling
↑ Src
Increased permeability
Commensal microbiota
↑ Notch signaling
↓ E-cadherin/ ↑ N-cadherin (EMT)
PI3K/Akt ↑
Chronic inflammation
IL-6/ gp130/ Stat3
Bcl2 ↑
Altered epithelial homeostasis, invasion, anoikis
Cancer

Figure 3

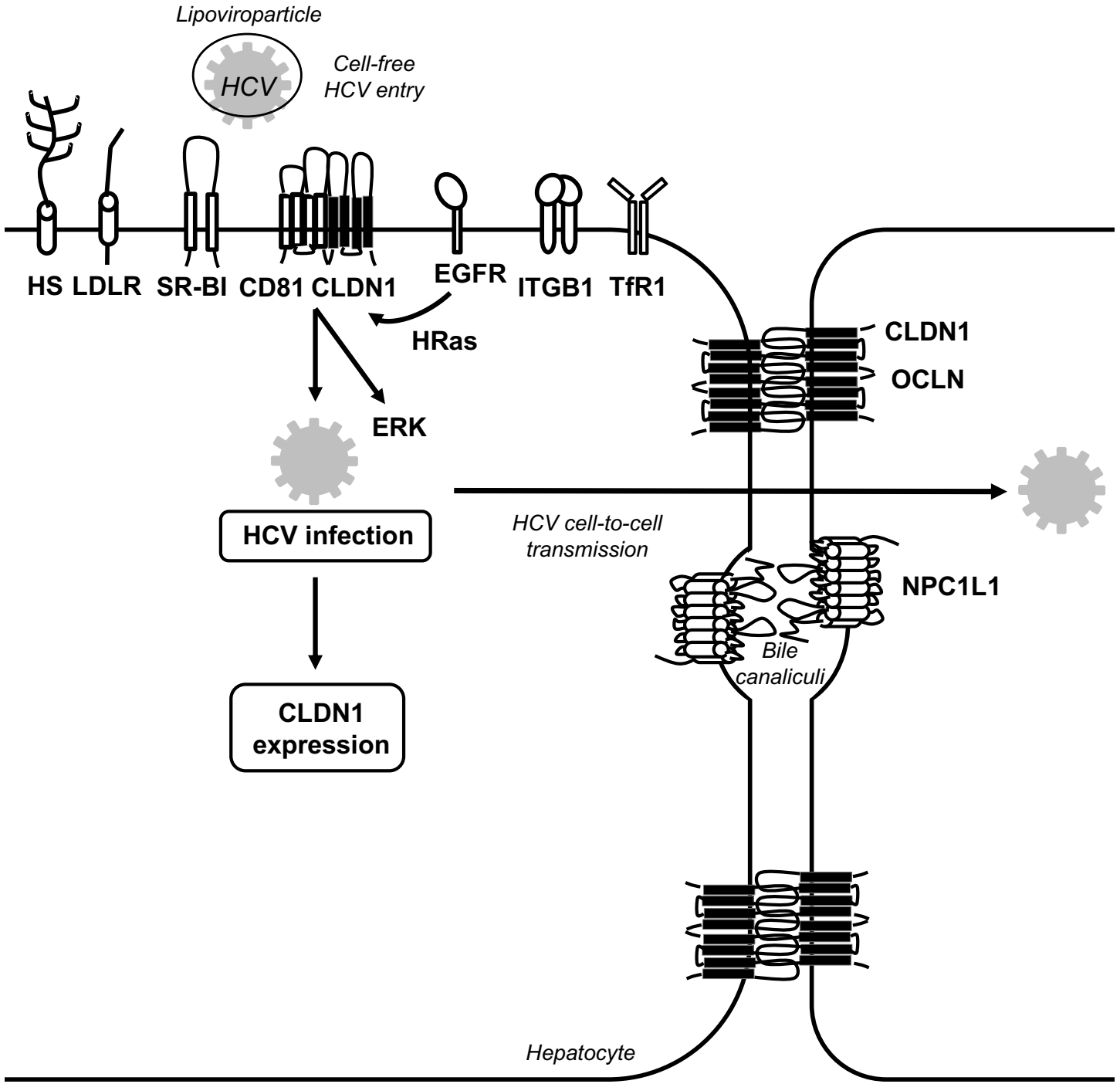
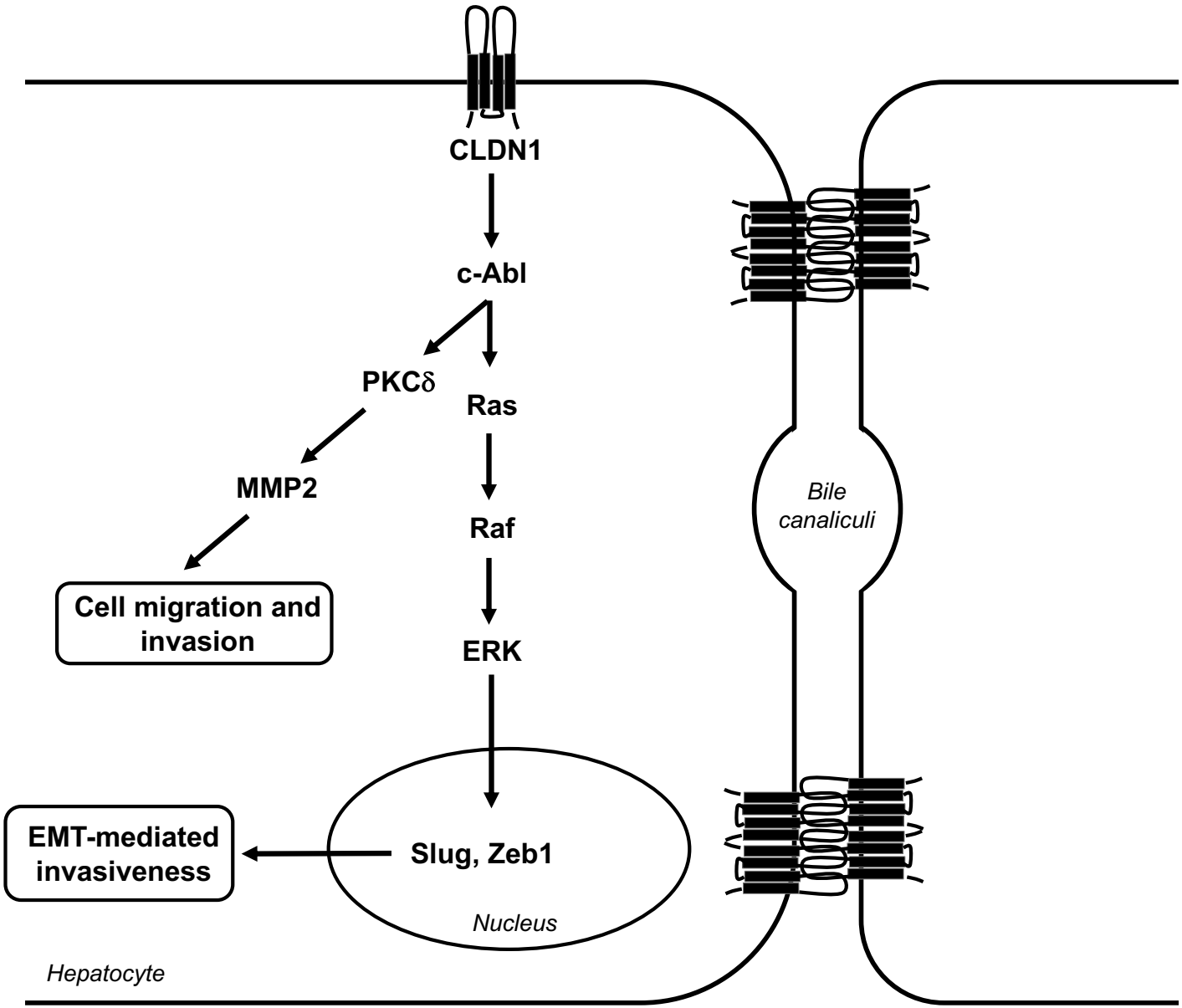

Lipoviroparticle
HCV
Cell-free
HCV entry
HS
LDLR
SR-BI
CD81
CLDN1
EGFR
ITGB1
TfR1
HRas
ERK
HCV infection
CLDN1
expression
CLDN1
OCLN
HCV cell-to-cell
transmission
NPC1L1
Bile
canaliculi
Hepatocyte

Figure 4


CLDN1
c-Abl
PKCδ
Ras
MMP2
Raf
ERK
Cell migration and invasion
EMT-mediated invasiveness
Slug, Zeb1
Nucleus
Bile canaliculi
Hepatocyte