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1 *Review*

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Tight junction proteins in gastrointestinal and liver disease

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

37 **Abstract**

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

52

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

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

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

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

100 The best studied cytoplasmic proteins of TJs are zona occludens (ZO) proteins. ZO-1,
101 -2, and -3 can interact with each other as well as with several transmembrane proteins
102 (OCLN, CLDNs, JAM-A) and F-actin (Figure 1). Cytoplasmic proteins of TJs have thus been
103 suggested to act as scaffolds linking TJs to the actin cytoskeleton and microtubules[12].

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

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

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

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

140

141 **TJ proteins and the GI tract**

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

143 The GI mucosal barrier plays an important role in the separation of the inside of the body
144 from the outside environment. TJs are present on the apical end of the lateral membrane
145 surface in epithelial cells and regulate paracellular transport and apicobasal cell polarity. The
146 expression of different TJs in the gut varies according to the gut's functional properties as
147 well as localization in villus or crypt (small bowel vs colon). The proteins can be localized
148 strictly at the apical cell-cell adhesion or extend to the lateral or basolateral surfaces[28, 29,
149 30, 31]. Moreover, expression and cellular distribution of the TJ proteins – such as the CLDN
150 family of proteins – is associated with regulation of differentiation of the intestinal
151 epithelium[32, 33, 34]: CLDN1 is mainly expressed at the apex of the epithelial cells with a
152 reticular pattern in the colon. CLDN2 is expressed in both villus and crypt cells of the small
153 intestine but restricted to undifferentiated crypt cells in the colon. CLDN3, -4, -7 and -8 are
154 predominantly expressed in the distal parts (colon, sigmoid and rectum) of the GI tract while
155 CLDN10 and -12 show an ubiquitous expression pattern throughout the GI tract.

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

165 upregulated CLDN2 expression in the mouse gut epithelium demonstrated a critical and
166 complex role of CLDN2 in intestinal homeostasis by regulating epithelial permeability,
167 inflammation and proliferation[38, 39]. Moreover, CLDN8 contributed to regulation of
168 paracellular Na⁺ permeability, protecting the leakage of Na⁺ into the intestinal lumen[40].
169 CLDN16 is responsible for the defective absorption of Ca²⁺ in the intestine causing primary
170 hypercalciuria[41]. Furthermore, the interdependence between CLDN proteins in regulating
171 intestinal homeostasis is well demonstrated by *in vivo* loss-of-function studies for CLDN15.
172 CLDN15 knockout mice grow normally despite having a mega-intestine[42]. However, a
173 double knockout of CLDN15 with CLDN2 chronically reduces the paracellular flow of Na⁺
174 from the intestinal submucosa into the lumen resulting in shunting of the nutrient absorption,
175 malnourishment and death [43]. Of note, both CLDN2 and -15 are paracellular transporters
176 of Na⁺[44]. Overall, these findings show a critical functional role of these proteins in
177 regulating intestinal homeostasis.

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

184

185 ***Functional role of TJ proteins during GI neoplastic transformations and growth***

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

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

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

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

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

233

234 ***TJ proteins and colorectal disease and cancer***

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

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

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

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

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

302

303

304

305 ***CLDNs and esophageal and gastric cancer***

306 Similar to CRC, TJ proteins are also regulated aberrantly in the esophageal and gastric
307 cancers and this abnormal expression correlates with specific clinicopathologic parameters.
308 In the esophageal adenocarcinoma (EA), CLDN expression has been tested as potential
309 biomarker for the transition of the Barrett's esophagus to the EA. Indeed, CLDN2, -3, -4 and
310 -7 are reported to have increased expression in EA compared to precancerous lesions and
311 normal esophageal squamous mucosa[85, 86]. JAMs, which comprise the integral parts of
312 TJs in the gastric epithelium, have been shown to promote proliferation, invasion, and inhibit
313 apoptosis. JAM-B was upregulated significantly in tumor samples compared with adjacent
314 normal tissues and was higher in high grade tumors than in the low grade and intermediate
315 grade tumors[87].

316 An increased expression of CLDN2 is also associated with gastric cancer
317 progression[88]. Additionally, expression of CLDN11 and -23 is downregulated in gastric
318 cancer[89] and miR-421 was implicated in regulating CLDN11 expression to promote the
319 proliferation, invasion and metastasis of gastric cancer cells[90] (Table 2). In contrast,
320 CLDN23 positive expression was associated with poor prognostic outcomes of gastric cancer
321 patients and may therefore serve as an independent predictor of patient survival. Similarly,
322 upregulated expression of CLDN4 in gastric cancer was associated with cancer progression
323 and poor prognosis[91]. Furthermore, CLDN4-expressing gastric adenocarcinoma AGS cells
324 were found to have increased MMP2 and -9 expression, indicating that CLDN-mediated
325 increased invasion may be mediated through the activation of MMPs[91]. Overall, these
326 results suggest that CLDN4 overexpression may promote gastric cancer metastasis through
327 the increased invasion of gastric cancer cells. Yet another CLDN family protein, CLDN18 is
328 significantly downregulated in gastric cancer tissues and cell lines, which was associated
329 with tumor size, location invasion, histologic type and tumor-node-metastasis stage. miR-
330 1303 was demonstrated to have putative binding sites in CLDN18 mRNA 3'-UTR and visibly
331 lower the expression of CLDN18[92] (Table 2). On the other hand, CLDN18.2 (isoform 2 of
332 claudin-18) was retained on malignant transformation and was expressed in a significant

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

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

339

340 **TJ proteins and the liver**

341 The liver plays an essential role in homeostasis by its metabolic and storage functions. It is
342 composed of different cell types, including two types of epithelial cells: hepatocytes and
343 cholangiocytes. Liver epithelial junctions are important for liver function. Hepatocytes are
344 liver parenchymal cells that exhibit a complex honeycomb morphology displaying at least two
345 basolateral membranes (facing the sinusoidal blood) and two intercellular apical membranes
346 (forming the bile canaliculi) separated by TJs. This peculiar architecture creates what has
347 been termed the blood-biliary barrier[94] and enables hepatocytes to perform distinct
348 secretory functions at the same time[95]. Hepatocytes produce and secrete bile into the bile
349 canaliculi from where it is transported via intrahepatic and extrahepatic bile ducts, formed by
350 cholangiocytes, to the gallbladder. Cholangiocytes contribute to modify the bile during its
351 transport to the latter. Hepatocyte polarity and bile duct TJs play a major role in the liver[96,
352 97] and thus defects in hepatocyte and/or cholangiocyte TJ integrity can result in
353 pathophysiological consequences.

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

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

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

372

373 ***CLDN1 and OCLN mediate hepatocyte entry of HCV***

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

382 First binding studies indicated that CLDN1 was unable to bind the HCV envelope
383 glycoprotein E2[26], suggesting that CLDN1 does not play the role of a primary receptor but
384 rather of a co-receptor, which contributes to (a) step(s) subsequent to viral binding[26]. This
385 was further confirmed in kinetic assays using anti-CLDN1 antibodies[113]. Several years
386 later it was shown that in contrast to soluble E2, HCV E1E2 complexes can interact with the
387 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]).

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

443 splice variants that exhibit an intracellular localization[136]. Of note, OCLN together with
444 CD81 define the HCV species specificity as HCV non-permissive mouse cells acquire HCV-
445 permissivity subsequent to human CD81 and OCLN expression both *in vitro* and *in vivo*[27,
446 137, 138, 139]. The species-specific determinants appear to be located within the second
447 extracellular loop of OCLN[27, 139].

448 Beside cell-free HCV entry, CLDN1 and OCLN have also been shown to be important
449 for HCV cell-to-cell transmission (Figure 3), a major mode of viral dissemination that enables
450 the virus to avoid the host's immune surveillance and to establish chronic infection[140, 141,
451 142, 143]. The exact localization at the plasma membrane of this process as well as the
452 form(s) of CLDN1 that contribute(s) to HCV cell-to-cell transmission remain unknown. The
453 importance of CLDN1 and OCLN for the pathogenesis of HCV infection *in vivo* has been
454 confirmed by observations of liver tissues from HCV-infected liver transplant patients. HCV
455 recurrence was associated with an increase in CLDN1 and OCLN expression levels in
456 hepatocytes over time after transplantation[144]. This is in line with findings indicating
457 increased CLDN1 and OCLN expression levels in HCV-infected livers[122, 145, 146]. In
458 contrast, in cell-based studies HCV infection was shown to downregulate CLDN1 and OCLN
459 expression to prevent superinfection[106]. Differences in TJ protein expression upon HCV
460 infection may thus exist depending on the analyzed samples.

461

462 **CLDNs and HCC**

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

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

482 CLDN1 likely contributes to proliferation, motility and invasion by modulating cellular
483 signaling. Overexpression of CLDN1 increases the migration and invasiveness of human
484 hepatoma cells as well as normal liver cells through expression of MMP2 via the c-Abl-PKC
485 pathway[20] (Figure 4). Furthermore, increased CLDN1 expression has been associated with
486 mitochondrial dysfunction and invasiveness of hepatoma cells, and reactive oxygen species-
487 mediated activation of heat shock factor 1 (HSF1) was demonstrated to increase CLDN1
488 expression in these cells[156, 157] (Table 2). These data are in line with reports indicating
489 that CLDN1 enhances cell growth, migration and/or invasiveness of other cancer cell types
490 such as oral squamous cell carcinoma cells, CRC cells, ovarian cancer-initiating cells or
491 melanoma cells[69, 158, 159, 160, 161]. Taken together, these data suggest that by
492 promoting cell migration and increasing the invasive behaviour of cancer cells, CLDN1 can
493 contribute to cancer spread. Of note, CLDN1 has been shown to promote EMT in normal
494 liver cells and HCC cells that thereby acquire an invasive phenotype[162]. This process is
495 mediated by the c-Abl-Ras-Raf-1-ERK pathway and involves the transcription factors Slug
496 and Zeb1[162] (Figure 4). Confirming the functional role of these signaling pathways, an
497 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

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

533

534 ***CLDN1 and OCLN - targets for cure of HCV infection***

535 CLDN1 was the first TJ protein to be explored as a therapeutic target for HCV infection using
536 anti-CLDN1 antibodies directed against its extracellular domain(s) (Table 3). Such antibodies
537 could be used to prevent liver graft infection in HCV-positive transplant recipients and as a
538 promising alternative for patients who fail current anti-HCV therapies[166]. The first
539 antibodies directed against human CLDN1 and blocking HCV infection were generated by
540 genetic immunization in rats[113, 119]. They recognize a conformational-dependent epitope
541 within the ECL1 and prevent CD81-CLDN1 co-receptor association at the basolateral
542 membrane[113, 119]. They are characterized by pan-genotypic inhibition of the infection by
543 all major HCV genotypes by blocking both cell-free virus entry and viral cell-to-cell
544 transmission[119, 143]. Of note, studies in human liver-chimeric mice demonstrated that the
545 lead antibody OM-7D3-B3 was not only able to prevent acute *de novo* infection with HCV
546 (i.e. the anticipated effect of an entry inhibitor) but also to cure already established chronic
547 HCV infection without detectable side or off-target effects[120]. These results highlighted the
548 importance of viral dissemination for maintenance of chronic HCV infection. It is of interest to
549 note that this antibody interfered with MAPK signalling suggesting an important role in this
550 pathway potentially also contributing to its antiviral effect[120]. The therapeutic potential of
551 this antibody is further underscored by the fact that it acts in synergy with HCV direct-acting
552 antivirals (DAAs), the current state-of-the-art antiviral therapy, to clear viral infection and is
553 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

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

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

591

592 **Conclusions and future perspectives**

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

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622

623 **Conflict of interests**

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

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1262

1263

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- β 1 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- κ B, 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	-			

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

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

Inflammation		[230]
Inflammatory bowel disease: Morbus Crohn	↓	

Vision loss		[251]
Diabetic eye disease: diabetic retinopathy	↑	

1267

1268

1269 **Table 2. Perturbation of tight junction protein expression in gastrointestinal and liver**
 1270 **disease.** Examples of perturbed CLDN and ZO protein expression in gastrointestinal and
 1271 liver disease as well as the underlying mechanism are shown. CLDN: claudin, miR: micro-
 1272 RNA, TJ: tight junction, ZO: zona occludens

1273

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 ↑	β-catenin activation	[39]
	CLDN2 ↑		
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]

1274

1275 **Table 3. Preclinical and clinical development of antibodies directed against tight**
 1276 **junction proteins.** The respective target, names of monoclonal antibodies (that have at least
 1277 reached preclinical stage of development), clinical indication, and stage of development are
 1278 shown. CLDN: claudin, HCV: hepatitis C virus, OCLN: occludin

1279

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]

1280

1281

1282

1283 **Figures legends**

1284 **Figure 1. Schematic representation of the expression and function of the major tight**
1285 **junction proteins addressed in this review.** This simplified cartoon only displays the
1286 localization and interactions of the major tight junction (TJ) protein families that are
1287 addressed in this review. TJs are composed of transmembrane proteins, including different
1288 claudins (CLDNs), tight junction-associated marvel proteins (TAMPs, e.g. OCLN), junctional
1289 adhesion molecules (JAMs e.g. JAM-A) as well as cytosolic proteins (e.g. ZO-1, -2 and -3),
1290 which connect transmembrane components to the cytoskeleton (actin filaments,
1291 microtubules). For a more detailed description please refer to reference[1].

1292 **Figure 2. Schematic representation of differential regulation of tight junction proteins**
1293 **and associated signaling in gastrointestinal cancer.** Signaling and molecular
1294 mechanisms that are known to promote neoplastic growth and cancer malignancy include
1295 the receptor tyrosine kinase signaling, inflammatory signaling cascades and non-coding
1296 RNAs that perturb tight junction (TJs) expression and function. TJ perturbation alters
1297 downstream signaling that target important cellular events in epithelial homeostasis,
1298 invasion, chronic inflammation and cancer (Zeb-1/E-cadherin, Wnt signaling, MMP9/Notch
1299 signaling and Src/PI3K/Akt signaling). Furthermore, disruption of TJs can result in increased
1300 permeability to promote translocation of bacteria and luminal antigens, which then activate
1301 IL-6/Stat3 signaling to induce carcinogenic processes.

1302 **Figure 3. Functional roles of CLDN1 as hepatitis C virus entry factor.** CLDN1 is one of
1303 the four main hepatitis C virus (HCV) host factors (i.e. SR-BI, CD81, CLDN1 and OCLN)
1304 essential for the early steps of HCV infection. Several other host factors (e.g. highly
1305 sulphated heparan sulfate (HS), low-density lipoprotein receptor (LDLR), epidermal growth
1306 factor receptor (EGFR), integrin beta 1 (ITGB1), transferrin receptor 1 (TfR1) and Niemann
1307 Pick C1 like 1 (NPC1L1)) contribute to viral binding and entry. EGFR-mediated signaling
1308 leads to the formation of a CD81-CLDN1 co-receptor complex that ultimately leads to viral
1309 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-PKC δ 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

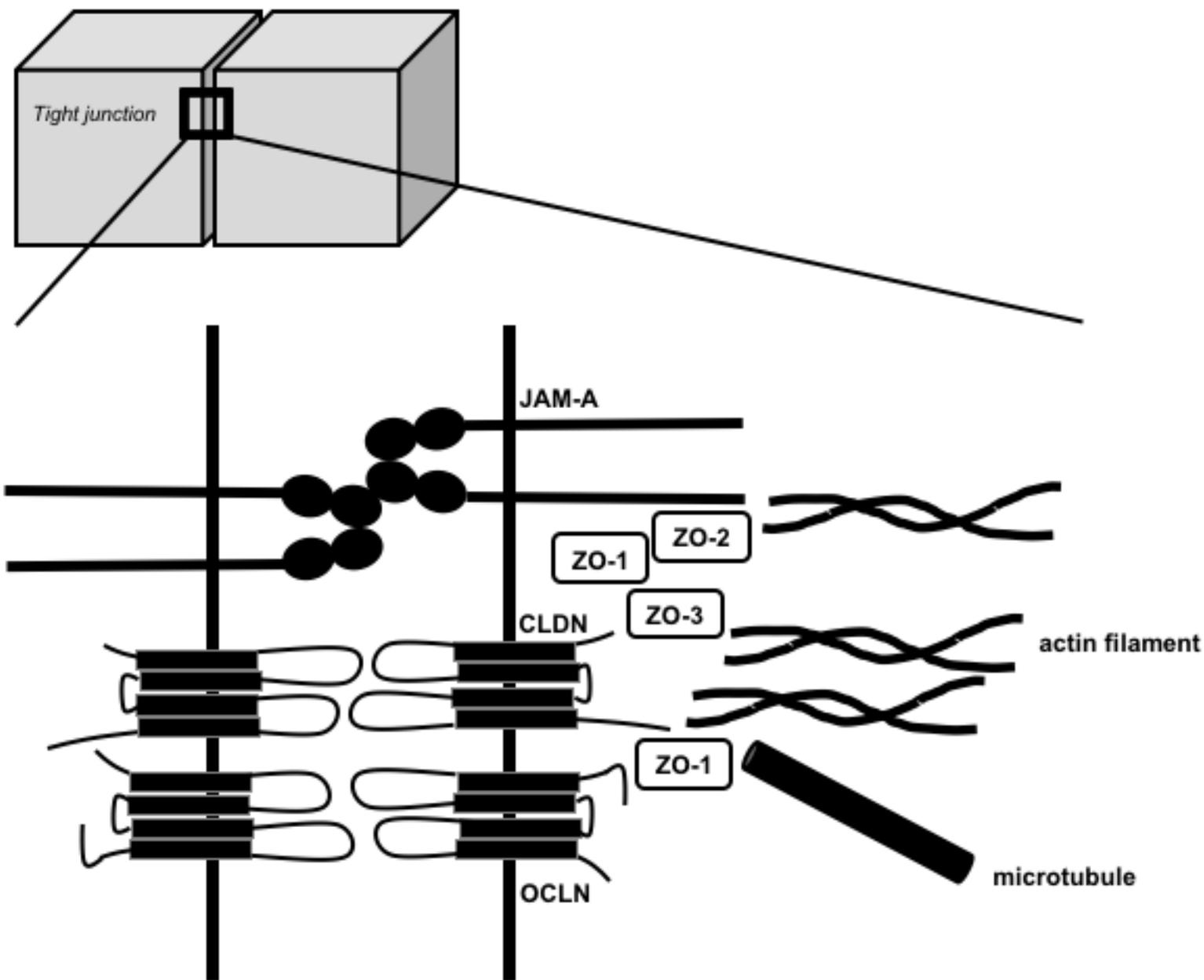


Figure 2

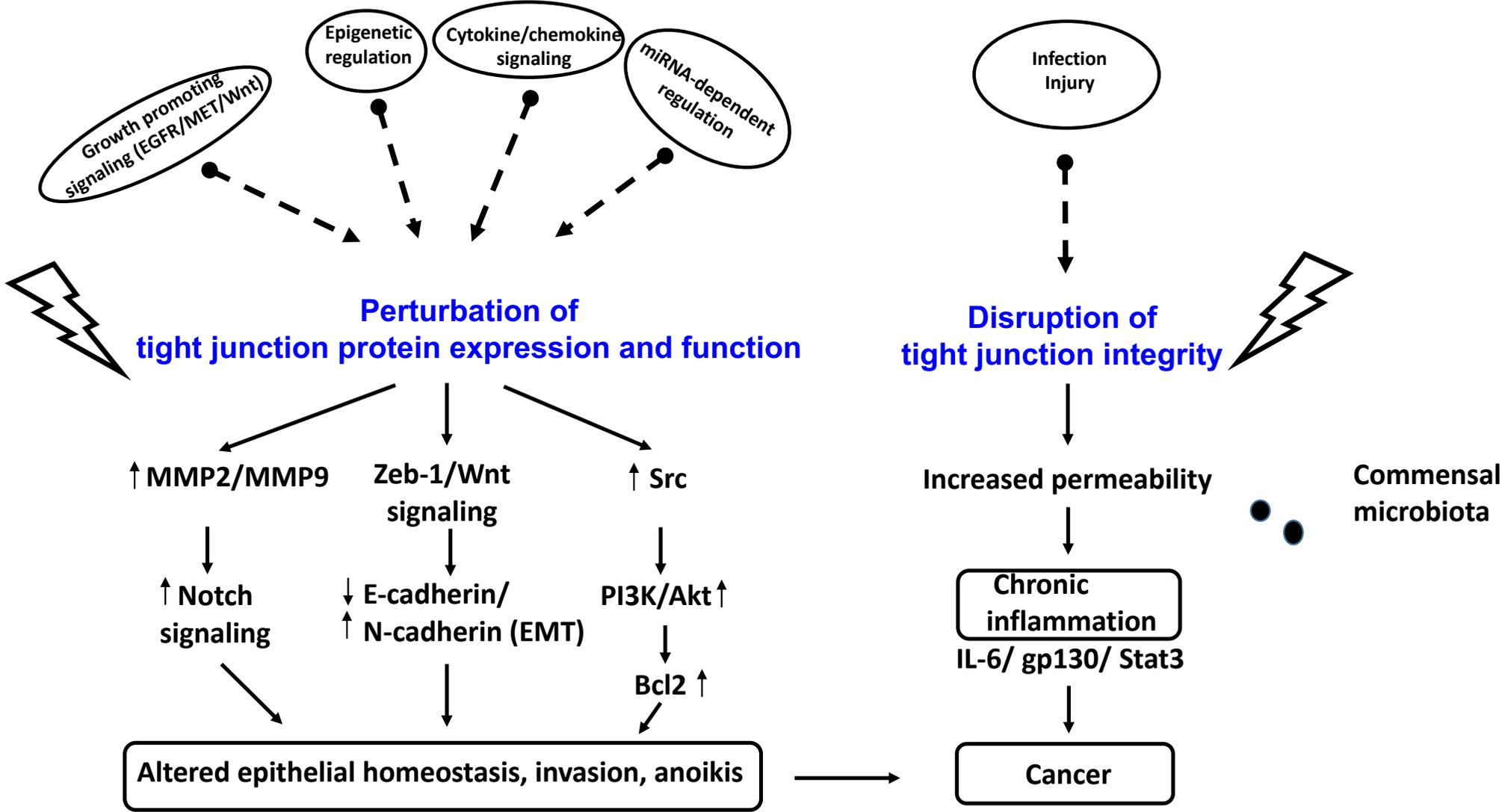


Figure 3

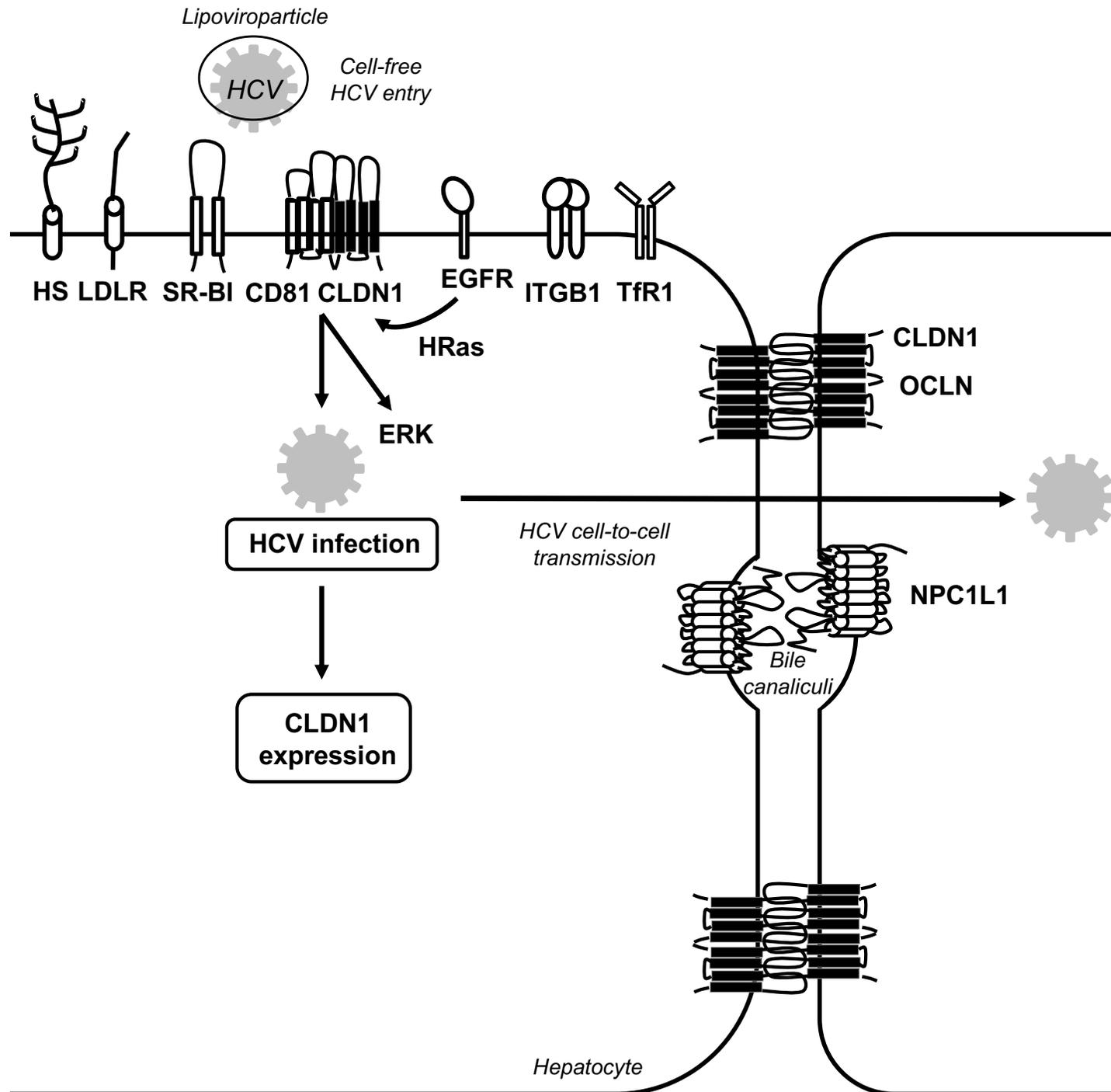


Figure 4

