Gap junctions in liver disease: Implications for pathogenesis and therapy

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Abbreviations: GJ, gap junction; Cx, connexin; NO, nitric oxide; ACLF, acute on chronic liver failure; HCC, hepatocellular carcinoma; LPS, lipopolysaccharide; KLF2, Kruppel-like factor 2; HE, hepatic encephalopathy; NASH, nonalcoholic steatohepatitis

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Abstract

In the normal liver, cells interact closely through gap junctions. By providing a pathway for the trafficking of low molecular mass molecules, these channels contribute to tissue homeostasis and maintenance of hepatic function. Thus, dysfunction of gap junctions is related to a wide variety of liver processes such as differentiation, cell death, inflammation and fibrosis. In fact, dysfunctional gap junctions have been implicated, for more than a decade, in cholestatic disease, hepatic cancer and cirrhosis. Additionally, in recent years there is increasing body of evidence that these channels are also involved in other relevant and prevalent liver pathological processes such as nonalcoholic fatty liver disease, acute liver injury and portal hypertension. In parallel to these new clinical implications the available data describe controversial observations, which requires a compelling overview for a better understanding of the functional complexity of these pores. This paper will review the most recent knowledge concerning gap junction dysfunction, with special focus on the role of these channels in the pathogenesis of relevant clinical entities to gap junction dysfunction and describe potential therapeutic targets that are amenable to modification by drugs.

Highlights

- Gap junctions and hemichannels participate in a variety of liver diseases
- Connexins form gap junctions and hemichannels, which have different expression patterns depending of the type of liver disease
- In general, connexins aim to protect the liver from injury in response to several insults
- Function of gap junctions and hemichannels are amenable of modification with drugs, making them attractive therapeutic targets

Gap junctions, Hemichannels and Connexins: Molecular characteristics and function

Cell-to-cell communication is of extreme importance in tissue homeostasis, which is maintained by transmission of regulatory signals(1) (Figure 1). Intercellular communication via gap junctions (GJ) represents one of the most important routes of rapid signaling between cells. GJ channels span two plasma membranes and consist of two hemichannels (connexons), one belonging to each cell. Each hemichannel is formed by six connexin (Cx) subunits and is permeable to small molecules up to 1-1.5kD(1). They serve to provide electrical and chemical conductance as well as metabolic assistance(2, 3). GJ communication is modulated by many factors such as cytokines, growth factors and nitric oxide (NO) making them susceptible to change during cell stress and injury(4, 5).

Cxs consist of 4 transmembrane helices (M1-M4). The N- and C- terminal ends are intracellular. The primary sequence of the intracellular loop is not well conserved, while the C-terminal sequence varies a lot between Cxs with Cx26 being ~20 amino acids and Cx43 being 150 amino acids long(6). More than 20 Cxs have been identified with different molecular weights and their expression patterns vary between cell types and tissues. Many different Cxs have been observed in the liver. Endothelial cells, Kupffer and stellate cells mainly express Cx43, hepatocytes express Cx32 and to a less extent Cx26, while liver vascular cells express Cx37 and Cx40 (Figure 2)(2, 7-9).

Across Cxs isoforms, there is a wide variation in conductance (most hemichannels have a fixed negative charge in the pore therefore cation selective) and permeability characteristics that have likely evolved according to the requirements of the tissue in which they are expressed. Moreover, their plasticity allows them to compensate for the loss or down regulation of other Cxs as revealed by several knock-out models(1). Furthermore, in these models, disturbed cell development has been observed suggesting that GJs and hemichannels play an important role in processes such as migration, differentiation and proliferation(3).

Cxs can also exist as functional hemichannels allowing the exchange of ions between the intra and the extracellular milieu(2, 4). Under normal physiological conditions, hemichannels are

either closed(10) or in a flickering state(11). Maintaining controlled gating to allow entry or exit of molecules from the cell is very important to preserve normal cellular integrity and function. Hemichannels are therefore, constantly under the control of factors such as membrane potential, pH, post-translational modification (phosphorylation, ubiquitination, S-nitrosylation), mechanical stimulation and intracellular/extracellular calcium(3, 12, 13). Facilitated opening of hemichannels has been shown to correlate with cell death in cerebral ischemia resulting in loss of osmoregulation, excitotoxicity and spread of inflammation(14). Although hemichannels and pannexins (structurally similar to Cx proteins) are also of great interest in liver disease, their role in liver disease will not be discussed in any detail in this review (for an extended review on liver pannexins see(15)).

Connexin and gap junction alterations in disease

Cx protein mutations are associated with various diseases such as hearing loss, which is linked to Cx26 and Cx30; atrial fibrillation is associated with a Cx40 mutation(16, 17). Additionally, under other pathological conditions such as focal ischemia, opening of GJs serves a protective role to save their compromised neighbors by providing essential molecules to areas of high demand(18). On the other hand, maintaining GJ communication in severely injured or diseased tissue areas allows the spread of toxic substances propagating and worsening cell injury(19). Figure 3 shows the diseases associated with congenital or acquired Cx involvement. It is of note that none of the described mutations affect the liver.

During cardiac ischemia, a decrease in GJ coupling is observed, which results in slowing of conduction of electrical impulses and a higher risk of arrhythmias(13, 20). In Huntington's disease an increase in the expression of 5 Cxs was observed in the astrocytes in the brain suggesting an adaptive protective response(21). Cerebral ischemia results in uncoupling of astrocytes due to a decrease in GJ function, which prevents astrocytes from being able to redistribute ions and neurotransmitters resulting in "cell swelling"(22). In cirrhosis and acute on chronic liver failure (ACLF)(23), studies indicated increased expression of hepatic Cx43, which

was related to the severity of inflammation. This was suggested to be an adaptive response of the liver for protection through better intercellular communication.

The results on Cx and GJ alteration during various pathological states are controversial (reviewed by(2)). However, the ability of GJ proteins to participate in different physiological and pathological states makes them attractive therapeutic targets in different diseases(24). This paper will therefore review the recent knowledge concerning the role of GJ in the pathogenesis of liver diseases.

Modulators of GJ function and targeting in diseases other than the liver

Opposing approaches aiming at increasing or decreasing GJ function have been explored to treat different diseases. To improve GJ function in heart diseases, GJ openers, such as synthetic peptide rotigaptide(25) and danegaptide(26) showed reduced burden of arrhythmias and myocardial infarct size. However, a recently published phase II study did not confirm the early results(27). Other enhancers of GJ function such as ACT1, a peptide that mimics the carboxyl terminus of Cx43 was evaluated in cutaneous ulcers(28) and arrhythmias. The results showed wound re-epithelialization and reduced inducible arrhythmias following ventricular injury respectively(29). On the other hand, where blocking the intercellular communication is the goal, strategies targeting specific Cxs with antisense oligonucleotide and mimetic peptides are available(24). They have been shown to reduce inflammation and improve neuronal survival after cerebral(30) and retinal ischemia(31). They were also shown to promote wound healing(32, 33).

In addition to the direct beneficial effects of simply potentiating or blocking the channel, the Cx targeting drugs may be used as adjuvants potentiating the effects of other known therapeutic agents. This is of particular interest in hepatocellular carcinoma (HCC) where GJ may favor the delivery of cytotoxic drugs to tumor cells. In this regard, studies have shown that GJ mimetics facilitate the spread of the drug for a better effect of therapy(34). Quinolone, a GJ opener was recently shown to enhance cisplatin-induced cytotoxicity(35) supporting the rationale for

combination therapies that include GJ openers in the treatment of various cancers such as colon cancer(36), prostate cancer(37) and breast cancer(38). In addition, inhibition of GJ may reduce toxic effects of drugs by preventing the propagation of inflammatory or death stimulus to neighboring cells(39). Given these potentially opposite effects of modulating GJ function, clinical application in a given disease needs to be carefully considered.

Acute liver injury and inflammation

GJ and Cxs are involved in circumstances where homeostatic regulation is of relevance such as during inflammation and cell death. Available data indicate that Cx26, Cx32 and Cx43 can contribute to acute liver injury and inflammation related to drugs, lipopolysaccharide (LPS) and ischemia-reperfusion injury. Given that several immune cells including monocytes, macrophages and Kupffer´s cells express Cx43(40) and are known to be involved in autoimmune liver diseases, the role of GJ in specific autoimmune liver diseases should be explored(41).

Acute liver injury

For better understanding of the role of GJ in drug-induced liver injury, studies in cells and animal models have been conducted modifying Cx expression by gene therapy or drugs (Table 1). The observation that HeLa cells transfected with herpes simplex virus induced the killing of a neighboring cell through the diffusion of toxic phosphorylated ganciclovir molecules after enhancement of GJ(42), provided the rationale to explore the role of GJ in acute liver injury. Acute administration of carbon tetrachloride and dimethynitrosamine, which induce acute liver injury, resulted in reduced expression of Cx32(43) due to transcriptional downregulation(44). Additionally, Cxs were mislocalised from the cell surface to the cytoplasm. Cx32 depleted animals, exhibited less severe liver injury after acute administration of D-galactosamine, carbon tetrachloride, thioacetamide and acetaminophen(45, 46). The severity of liver injury increased to that in wild type animals following restoration of Cx32 by gene transfection(47). The potential role of GJ in contributing to cell death is further supported by studies in cultured hepatocytes, where suppression of Cx26 and Cx32 reduced the synchronization of cell death after

administration of acetaminophen(48). Taken together, these data suggest that the reduction in Cx32 during acute liver injury is likely to be an adaptive response aimed to protect healthy cells from the propagation of toxins or messengers associated with cell death originating from injured cells. These data have been translated into potential novel therapeutics targeting blockade of Cx26 and Cx32 using 2-aminoethoxydiphenyl-borate (2-APB). Administration of 2-aminoethoxydiphenyl-borate(49) before, concurrently or after inducing acute liver injury was protective(50).

In contrast to the decreased expression of Cx26 and Cx32 during acute liver injury, Cx43 expression increases(44, 46). This unexpected increase suggests that Cx43 may play a role in propagating death signals(51). Accordingly, in liver cell cultures, a progressive increase in Cx43 mRNA and protein expression was observed during apoptosis(52). It is possible that Cx43 mediates propagation of cell death through caspase-3, a relevant factor in the apoptotic cascade, as they co-localize when apoptosis is induced. In support of this hypothesis, inhibition of Cx43 resulted in downregulation of caspase-3(46). Overall, these data suggest that blockade of Cx26 and Cx32, and counteracting Cx43 over expression may represent potential therapeutic targets to reduce acute toxic liver injury. Despite this, there are data contradicting the protective role of Cx26 and 32 and the deleterious role of Cx43 in acute liver injury suggesting that the situation may be more complex than is apparent. Complete deletion of Cx32 was shown to worsen acute liver injury(53) and another recent study has suggested that the increase in Cx43 may well be an adaptive response as knocking out Cx43 was associated with worse liver injury(44). It is possible that these radically different observations may be due to differences in the animal species used, type of blocker/deletion and the route and dose of administration of toxins (Table 1).

Altogether, by means of targeted disruption of Cx genes or drug manipulation, these results argue towards a crucial role of Cxs in the propagation of acute liver injury irrespective of the type of hepatotoxin. However, the exact contribution of Cx remains unknown, as GJ may provoke a positive or negative effect on the severity of injury. The complexity lies in the fact that

the cell death or survival response mediated by GJs may be determined by the transfer of molecules that can pass through them(18).

Lipopolysaccharide-induced liver injury

There is compelling evidence in experimental animal models that administration of LPS, which induces an inflammatory response results in decreased expression of Cx26 and Cx32(54-58) (Table 2). This reduction in levels of Cx26 and Cx32 protein expression in hepatocytes was related to inflammation(55, 59). However, a downregulation of these Cxs at the level of gene expression by a post-transcriptional mechanism has also been postulated(56). In the setting of experimental cirrhosis, the administration of LPS resulted in a further reduction in both Cx26 and Cx32(23). This argues in favor of a protective role of these Cxs, which shuts down intercellular communication and propagation of inflammation.

On the other hand, an increased expression of Cx43 has been shown in stellate cells, macrophages, endothelial cells and also in leukocytes in response to LPS(7, 60). This increase in Cx43 expression was also associated with increased activity of Cx indicated by a higher dye coupling suggesting that Cx43 may play role in liver inflammation(61). Interestingly, inhibiting Cx43 in rats treated with LPS using mimetic peptides was associated with increased hepatocellular necrosis, suggesting that the increased hepatic Cx43 expression is most likely an adaptive protective response(23).

Liver ischemia and reperfusion

GJ channels and Cxs have a role in ischemia-reperfusion injury of the heart(62), brain(19) and vascular tissues(63-66). The proposed mechanism is the initiation of an injury-signaling cascade that is propagated through GJ, affecting cellular metabolism(67). In addition to the exchange of signals, ions and messengers between adjacent cells, functions independent of intercellular communication and related to the presence of Cx in the mitochondria have also been shown. In this case, Cx43 has important functions including modulation of mitochondrial respiration and production of reactive oxygen species(68).

Hepatic ischemia-reperfusion injury is commonly observed during partial hepatectomy and liver transplantation, and Cxs have been studied in this setting. In animal models of hepatic ischemia-reperfusion, an early decrease in Cx26 and Cx32 mRNA and protein expression was observed(69, 70). Partial prevention of this effect was obtained with actinomycin D, which prevents the degradation of Cx32 mRNA, although protein expression of Cxs remained low, suggesting that its regulation occurs by different post-transcriptional and post-translational mechanisms(71). This alteration is likely to represent an adaptive response aiming to restrict the spread of noxious signals to healthy areas. In keeping with this hypothesis, *in vitro* experiments using cell cultures, targeting Cx32 gene increased cell survival, which was associated with decreased molecular permeability of GJ(72). An alternative explanation is that a reduction of cell-to-cell communication prevents disruption of cellular metabolism(73).

Ischemic preconditioning, which attenuates and protects against ischemia-reperfusion damage, is nitric oxide (NO) dependent(74, 75). In this condition, cell-to-cell coupling seems necessary for the protective effect of preconditioning as uncoupling by chemical inhibitors significantly reduced the protection provided by hypoxic preconditioning. In addition, preconditioning led to an increase in Cx43 expression, which was associated with increased GJ permeability(76). Clearly, more research is needed to understand the pathophysiological alterations in ischemia-reperfusion injury related to Cxs, which may open potential therapeutic approaches to reverse the effects.

Role of connexins in hepatic fibrosis

Fibrosis is a consequence of pro-inflammatory cytokine release, oxidative stress, necrosis/apoptosis, and is associated with the involvement of stellate cells, which transforms from a quiescent state into a proliferative and contractile myofibroblast-like phenotype. Other neighboring non-parenchymal cells including cholangiocytes, Kupffer cells and infiltrating monocytes interact and contribute to further activation of stellate cells.

In the normal liver, fenestrated liver sinusoidal endothelial cells induce senescence of hepatic stellate cells. Capillarization of sinusoids reduces the ability of endothelial cells to suppress stellate cell activity(77, 78). Although GJs may provide a direct pathway of intercellular communication, functional communication between endothelial cells and stellate cells has yet to be consistently identified(7, 79). However, as previously discussed, Cxs may contribute to intercellular transfer of angiocrine signals after injury(80) or may be incorporated in microvesicles involved in promoting fibrogenesis(81). In this regard, Cxs, in particular Cx43, has been shown to be involved in contributing to the composition of membrane vesicles making this an important target for future research(82).

The role of GJs in liver fibrogenesis has been studied recently(83). Studies using Cx32 knock out mice showed a significant decrease in liver fibrosis compared to wild-type mice. Although the mechanism underlying this protective effect of Cx32 deletion is not clear, reduced oxidative stress was suggested as a possible explanation. In experimental models of cirrhosis induced by carbon tetrachloride, a downregulation of Cx32 was observed(84). In humans, reduced expression as well as a re-localization from the membrane to the cytoplasm was also observed(71). This evidence argues favorably for a protective role of Cx32.

In models of fibrosis such as after Schistosoma mansoni inoculation(85), and in the common bile duct ligation model(23, 86, 87), Cx43 expression was increased at the expense of a decreased expression of Cx26 and Cx32(23, 86, 88, 89). By contrast, others have observed a decreased expression(84, 87), or aberrant Cx43 positioned within the cytoplasm of cells(90), after chronic carbon tetrachloride administration. Phenobarbital, which itself decreases GJ(91), is usually co-administrated to promote fibrosis(92), possibly explaining the observed discrepancy.

Current evidence points towards a role of Cx43 in collagen matrix deposition. Administration of chronic carbon tetrachloride to Cx43 deficient mice resulted in a similar grade of fibrosis compared to wild type animals. Nevertheless, an intensification of collagen deposition and

nodule formation with retraction of liver capsule was more evident in Cx43 deficient animals(90). In apparent contradiction, another study evaluated the role of Cx43 in fibrosis, aimed at discriminating between GJ and hemichannels. In both cases when Cx43 was inhibited, mice treated chronically with thioacetamide exhibited less fibrosis. Additionally, the authors concluded that hemichannel blockade mediated reduced stellate cell activation and deposit of collagen(93). To add more complexity, pannexins are involved in the transport of ATP into the extracellular space where it is converted to adenosine, which acts on its receptors that stimulate fibrosis. In another recent study, tenofovir, acting as a pannexin hemichannel blocker had a direct antifibrotic effect(94).

Cirrhosis and its complications

Portal hypertension

In liver disease, increased intrahepatic vascular resistance contributes to the severity of portal hypertension(95). In addition to the structural component of portal hypertension due to fibrosis, a more dynamic component is also found(96). Intrahepatic vascular tone in cirrhosis is increased due to dysfunction of sinusoidal cells and decreased NO resulting in impaired vasorelaxation in response to acetylcholine(97). GJ connects endothelial cells and allows propagation of vasodilation(98, 99). Indeed, binding of acetylcholine stimulates calcium activated potassium channels in the plasma membrane, which is conducted from cell to cell through GJ.

Cx37, Cx40, Cx43 and Cx45 regulate vascular tone(100). Cx40 and Cx43 are involved in regulation of hepatic blood flow and are expressed in sinusoidal and endothelial cells of hepatic arteries and portal veins(7, 87, 101)(Figure 4). This is consistent with the observation that Cx43 expression is likely to be absent during the resting state but induced during endothelial dysfunction(102). It is noteworthy that the expression of Cx43 in stellate cells is increased in parallel with its activation and its blockade inhibits propagated contraction in response to calcium(7, 103). Experiments conducted in our laboratory showed that blocking GJ increases portal perfusion pressure and reduces vasodilatory response to acetylcholine(87). The

mechanism of this observation is not clear but the data suggests this may be modulated by Cx-mediated NO release (104).

Decreased endothelial nitric oxide synthase activity in the liver may also be due to upregulation of caveolin-1(105). Interestingly, a strong association between Cx40 and Cx43 and caveolin-1 has been identified in endothelial and epidermal cells(106). It is possible that Cx43 expression is implicated in caveolin-1 overexpression in cirrhotic livers. Shear stress is a potent inducer of NO production and its relationship with GJ has been evaluated. Shear stress promoted Cx43 expression in endothelial cells(107). Although increased expression of Cx43 seems not to be limited to the sinusoidal liver cells, it is possible that the induction of Cx43 expression seen during cirrhosis is in response to shear stress as a compensatory mechanism to favor the transfer of molecules. By contrast, changes in Cx37 expression pattern by shear stress are less clear(108). Interestingly, Kruppel-like factor 2 (KLF2), which is activated after induction of shear stress and upregulates eNOS, has been suggested to regulate Cx37 expression. Indeed, shear stress induced Cx37 expression was abrogated following KLF2 suppression suggesting that KLF2 acts as a transcription factor for Cx37. Here again, the relation between a relevant NO promoter such as KLF2 and Cxs, suggests a role for GJ in the regulation of vascular tone.

In cirrhosis, following an increase in intrahepatic resistance, a progressive cascade of events leads to splanchnic and peripheral vasodilation. Sodium retention and volume expansion increases cardiac output that in turn contributes to the development of ascites, circulatory dysfunction and renal failure(109). In opposition to the hepatic circulation, systemic NO is elevated. In addition to NO, other factors have also been hypothesized to participate in arterial vasodilation, such as the endothelium-derived hyperpolarizing factors(110) and more specifically epoxyeicosatrienoic acids(111). GJ have been described as being fundamental in conducting hyperpolarization directly from the endothelium to vascular smooth muscular cells in the arteries(111, 112). In small resistance mesenteric arteries of cirrhotic rats, inhibiting epoxyeicosatrienoic acids promoted vasoconstriction, an effect that was still observed after NO and prostaglandin inhibition, showing an independent effect(113). However, the effect of

epoxyeicosatrienoic acids was blunted following pretreatment with a GJ blocker suggesting that epoxyeicosatrienoic acids may initiate a hyperpolarizing response that is conducted to vascular smooth muscle cells by myoendothelial GJ with consequent vasorelaxation. NO is also responsible for improving Cx43 communication between endothelial and myoendothelial cells. This is because of its ability to nitrosylate proteins, thus modifying protein function(114). Indeed, NO has been shown to s-nitrosylate Cx43 channels(115).

Hepatic encephalopathy

Hepatic encephalopathy (HE) is an important neuropsychiatric complication associated with end stage liver disease and has a multifactorial pathogenesis. Work from our laboratory recently demonstrated that Cx-hemichannel functionality, and consequently lactate transport, was impaired in the cerebral cortices of bile duct ligated rats with mild HE(116). While the expression of the main astrocytic and neuronal Cxs was unaffected, the results of this study suggest that HE is associated with impairment of central nervous system hemichannel functionality, with ammonia playing a key role. The data supporting a Cx-hemichannel dysfunction provides evidence of a potential neuronal energy deficit due to impaired hemichannel-mediated lactate transport between astrocytes and neurons as a possible mechanism underlying the pathogenesis of HE.

Cholestatic disease

GJs are involved in bile secretion and regulation of bile flow(117-120), and any alteration in intercellular transmission of secondary messengers might be expected to result in cholestasis. After bile duct ligation, GJ expression was decreased(121, 122). This was associated with marked reduction in protein levels of Cx26 and Cx32(23, 86, 88, 89), which seems to be related to the associated inflammatory response(86). In addition, an increase in cholestatic bile acids such as taurolithocholate, taurolithocholate-sulfate and taurochenodeoxycholate promotes the closed state of GJ and worsens intercellular communication, making cholestasis worse(123).

On the other hand, the expression of Cx43 increases following bile duct ligation(86, 88) as well as after the development of cirrhosis(23, 87). The protein expression of Cx43 was further increased following LPS challenge and reduced following treatment with anti-TNF drugs(23). These data suggest that this adaptive response is contributed to by activation and infiltration of macrophages, which is involved in the synthesis and recycling of Cx43(86).

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease comprises a complex disease spectrum, including hepatic steatosis, nonalcoholic steatohepatitis (NASH), cirrhosis and eventually hepatocellular carcinoma (HCC). Intracellular signaling cascades favour the deposition of fat in hepatocytes and induce inflammation(124). Since Cxs forming GJ can modulate the transfer of molecules, their role in NASH is potentially important. Cx32 knockout rats with diet induced nonalcoholic fatty liver disease developed more pronounced oxidative stress, inflammation and liver injury compared with wild-type controls(125, 126) suggesting that GJ plays a protective role by maintaining homeostasis through cell-to-cell communication. However, blocking Cx hemichannels using specific peptides, decreased triglycerides, cholesterol, and inflammatory markers in high fat diet-fed animals compared with controls(127). This apparent paradoxical observation may be explained by the fact that hemichannels are constitutively closed and open after a pathological stimulus contrary to Cx forming GJ. During injury different deleterious molecules exchange between the extracellular and intracellular environment of cells, so blocking hemichannels may be responsible for the beneficial phenotype reported in these studies. In keeping with this study, carbenoxolone, a non-specific blocker of Cx was used as treatment in genetically modified obese rats showing decreased liver steatosis in treated animals along with a significantly decreased body fat percentage, hypertriglyceridemia, hypercholesterolemia and insulin resistance(128).

Pannexins forming channels connecting cells with the extracellular environment have also been studied in the setting of NASH(129). These channels when open participate in inflammatory processes(130). A decrease in lobular inflammation and oxidative stress was observed in

animals with pannexin deletion compared to wild type mice. However, in this study a different gene expression profile was observed in pannexin deficient animals, particularly affecting lipid metabolism and genes involved in the inflammatory and oxidative stress response suggesting that more experiments focused on specifically blocking pannexins without modifying gene expression need to be performed. Interestingly, at a cellular level, pannexins contribute to ATP release, which functions as a pro-inflammatory signal for recruitment and activation of inflammatory cells in lipoapoptosis(131). Overall, these studies suggest that improving GJ permeability, or blocking hemichannels either constituted by Cx or pannexin may represent relevant therapeutic targets.

Hepatocellular carcinoma

The ability of GJ to regulate cellular proliferation(132, 133) supports the idea that these channels could be involved in cancer pathophysiology. In addition, there is evidence based on experimental studies suggesting a possible role of GJ in liver carcinogenesis. Targeted disruption of the Cx32 gene was associated with an increase in hepatic tumors possibly because of a reduction in the propagation of apoptotic signals to adjacent cells(134-136). In keeping with this and further supporting the idea that Cxs may show tumor suppressive properties, both Cx26 and Cx32 expression are decreased in HCC while a mislocalisation (and dysfunction) of the Cxs from the cell membrane to the cytoplasm has also been observed(137-141). In HCC tissues, a reduction in the expression of Cx32 was associated with more aggressive tumor with increased tumor size, vascular invasion, and poorer survival. Thus, experiments exploring the potential benefit of GJ opening drugs should be explored. Concerning this observation, doxorubicin resistant HCC cell lines showed reduced expression of Cx32 and overexpression of Cx32 resulted in increased sensitivity of these cells to doxorubicin supporting the hypothesis that Cx32 could be an important target for counteracting drug resistance of HCC(142, 143). More recently, sorafenib, an oral multikinase inhibitor approved for advanced HCC was more efficacious after increasing GJ intercellular communication with all-trans retinoic acid. This effect was abolished after co-incubation with GJ inhibitor 18-alpha glycirrhetinic acid and oleamide(142, 143).

In an apparent contradiction, the observation that Cx43 expression is increased in HCC cells suggests that Cx43 may possibly have oncogenic properties instead of suppressing tumorigenesis(144-147). In fact, the magnitude of expression of Cx43 and its localization correlated with the malignant potential(148), migration, invasion capacity and metastatic ability of HCC(149). However, an alternative explanation could be that the increased expression of Cx43 is a compensatory response to mislocalisation of Cx43 as has been postulated to occur in breast cancer(150). Additional, studies are needed to elucidate the exact role of Cx43 in hepatocarcinogenesis.

CONCLUSIONS

In conclusion, there is accumulating evidence that GJ have important functions related to cell-to-cell communication and contribute to tissue homeostasis among other functions. These functions have relevant consequences on liver tolerance to acute injury as well as chronic insult, such as that observed in cirrhosis. It is clear that Cxs are expressed in multiple cell types and have distinct or even opposite roles depending on the liver cell type studied and type of constituted channel. Reduction or upregulation in the expression of different Cx subtypes are observed in many liver disease conditions, which may be the basis to new therapeutic strategies by specifically limiting or improving the traffic of messengers. However, more research is needed to elucidate the exact molecular mechanisms involved in order to exploit this pathway for treatment of liver diseases.

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Table 1. Experimental studies describing the role of connexins in acute liver injury.

Studied Cx	Animal model	Type and dose of toxic	Effects	Reference
Cx32	Sprague-Dawley male transgenic rat	Single intraperitoneal injection of D-galactosamine (300 mg/kg body wt) and carbon tetrachloride (0.5 mL/kg body wt)	Less evident necrosis and ballooning findings in Cx32 deficient rats	Asamoto et al. (45)
Cx32 and Cx43	Sprague-Dawley male transgenic rat	Single intraperitoneal injection of acetaminophen (250, 500, 1000 mg/kg body wt)	Less inflammation after insult in rats lacking Cx32, and induction of Cx43 expression	Naiki-Ito et al. (46)
Cx32	Sprague-Dawley male rats	Single intraperitoneal injection of carbon tetrachloride (1.0 mL/kg body wt) and Dimethylnitrosamine (6.3 -25 mg/kg body wt)	Decrease in hepatic Cx32 expression after injury and inverse correlation with the increase in plasmic alanine-aminotransferase activity	Miyashita T et al. (43)
Cx32	Ceramide synthase (CerS2) null mice (altered gap junction function)	Intraperitoneal injection of acetaminophen (300 mg/kg body wt), D-galactosamine (800 mg/kg body wt), carbon tetrachloride (2 ml/kg body wt) and thioacetamide (200 mg/kg body wt)	Less acetaminophen-induced hepatotoxicity after ablation of Cx32	Park WJ et al. (47)
Cx26 and Cx32	C57BL/6 Knock-out mice and wildtype mice treated with 2-aminoethoxydiphenyl-borate (Cx blocker)	Intraperitoneal injection of thioacetamide (200, 500 or 1000 mg/kg body wt) and acetaminophen (500 or 750 mg/kg body wt)	Mice deficient in Cx32 and wildtype mice (cotreated with 2APB) showed reduced inflammation and oxidative stress	Patel SJ et al. (50)
Cx32	C57BL/6 mice treated with 2-aminoethoxydiphenyl-borate	Intraperitoneal injection of acetaminophen (400 mg/kg body wt)	Protection by attenuation of c-jun-N-terminal kinase but not related to a specific role for Cx32	Du K et al. (151)
Cx32	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (300 mg/kg body wt)	No influence of Cx32 deletion	Maes M et al. (152)
Cx32	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (100, 200, or 300 mg/kg body wt)	More susceptible to liver damage 24 hours after the insult in Cx32 deficient mice	Igarashi I et al. (53)
Cx43	C57BL/6 Knock-out mice	Intraperitoneal injection of acetaminophen (300 mg/kg body wt)	Cx43-deficient animals tended to show increased liver cell death, inflammation and oxidative stress in comparison with wild type counterparts	Maes M et al. (44)

Cx, connexin; wt, weight

Table 2. Experimental studies describing the role of connexins in inflammation induced by lipopolysaccharide (LPS).

Studied Cx	Animal model	Type and dose of toxic	Effects	Reference
Cx32	C57BL/6 Knock-out mice	Intravenous injection of LPS	Hypoglycemia was slightly prolonged and cholestasis was much worse in Cx32-deficient mice	Correa PR et al. (54)
Cx26, Cx32 and Cx43	Sprague-Dawley male rats	Intravenous injection of LPS 2 mg/kg body wt	Cx26 and Cx32 were reduced after LPS whereas Cx43 increased associated with prominent inflammation	Gonzalez HE et al. (86)
Cx32	Sprague-Dawley male rats	Intravenous injection of LPS 1 mg/kg body wt	A decrease in the level of Cx32 mRNA in rat liver occurred at the posttranscriptional level	Gingalewski C et al. (56)
Cx26 and Cx32	Sprague-Dawley male rats	Intravenous injection of LPS 1 mg/kg body wt	Decreased communication was observed associated to Cx mislocalization and decreased Cx32 mRNA	De MA et al. (153)
Cx43	Cell culture	LPS in culture medium	Cx43 is tyrosine phosphorylated showing intercellular resistance following exposure to LPS	Lidington D et al. (58)
Cx26, Cx32 and Cx43	Sprague-Dawley male rats induced to bile-duct ligation	Intraperitoneal injection of LPS 1 mg/kg body wt	Cx26/32 expression inversely correlates with Cx43 expression after LPS. However, inhibiting Cx43 produced hepatocellular necrosis	Balasubramaniyan V et al. (23)
Cx26, Cx32 and Cx43	Cell culture from Wistar male rats	LPS 1 µg/mL in culture medium	LPS up-regulate Cx43 protein and messenger RNA expression, and enhance intercellular communication in hepatic stellate cells	Fischer R et al. (7)
Cx43	Sprague-Dawley male rats	Intraperitoneal injection of LPS 6 mg/kg body wt	Kuppfer cells exposed to LPS showed Cx43 at cell-cell contacts associated with higher dye coupling	Eugenin EA et al. (61)

LPS, lipopolysaccharide; Cx, connexin; wt, weight

Figure 1. Representation of hepatocytes and gap junction functions, structure, trafficking messengers and strategies to evaluate gap junction functions. a) Gap junctions participate in different functions. b) Individual connexins assemble intracellularly into hexamers, called connexons (hemichannels), which dock with other connexons in adjacent cells, assembling an axial channel spanning two plasma membranes and a narrow extracellular gap. c) Different molecules pass through the gap junctions. d) With different approaches the function of different connexins (Cx) has been evaluated.

Figure 2. Communication between liver cells through gap junctions. Diagram showing how different liver cells express connexins (Cx).

Figure 3. Connexin types implicated in different organ diseases. Connexins (Cx) are express in cells of almost every organ where dysfunction provokes different diseases.

Figure 4. Role of connexins in portal hypertension in cirrhosis. Different cells in the liver participate in fibrosis and vascular tone, contributing to increased intrahepatic resistance. Connexins (Cx) participate in arterial vasodilation by conducting hyperpolarization directly from endothelium to vascular smooth muscular cell in the arteries.

Figures

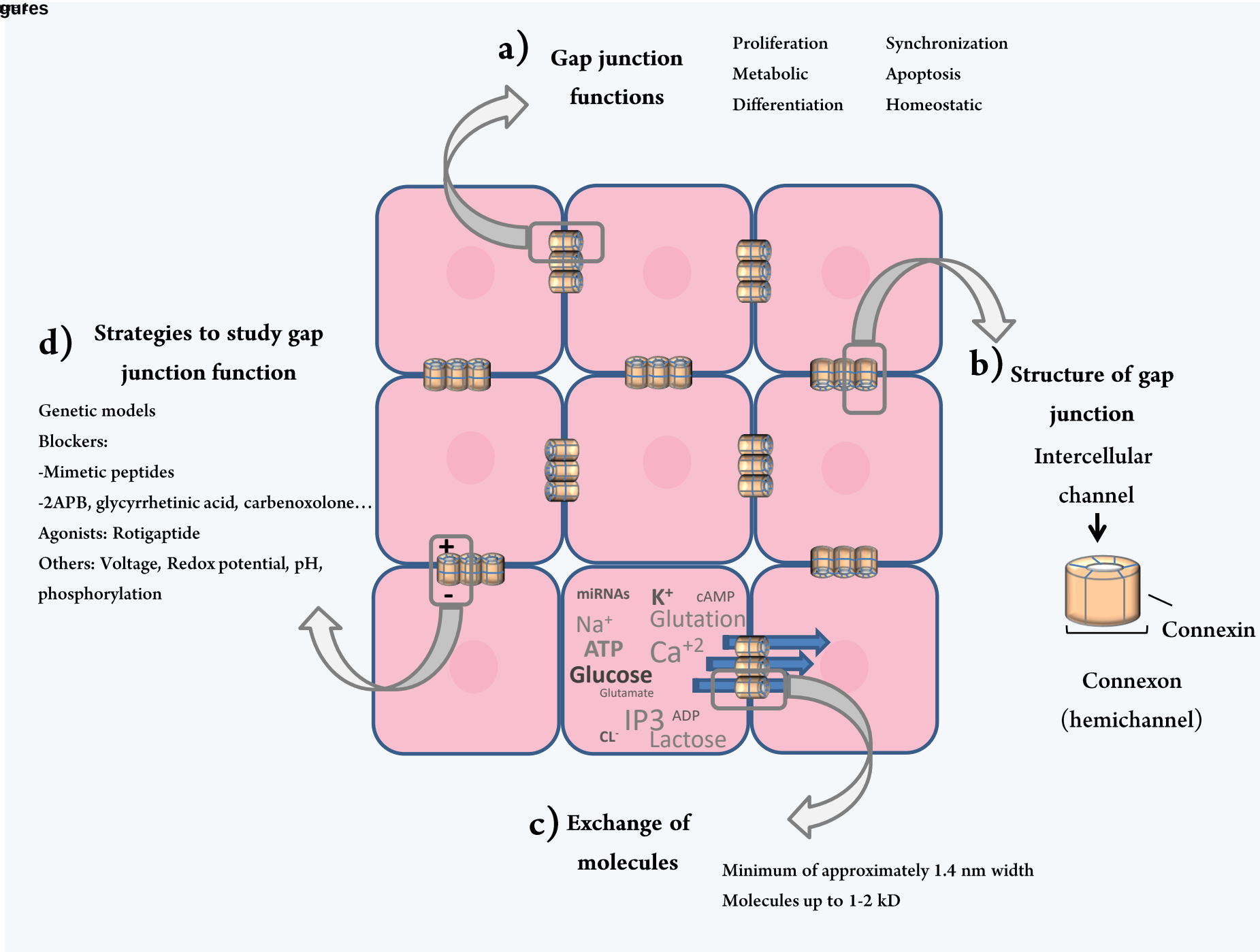

a) Gap junction functions
Proliferation
Synchronization
Metabolic
Apoptosis
Differentiation
Homeostatic
d) Strategies to study gap junction function
Genetic models
Blockers:
-Mimetic peptides
-2APB, glycyrrhetinic acid, carbenoxolone…
Agonists: Rotigaptide
Others: Voltage, Redox potential, pH, phosphorylation
b) Structure of gap junction
Intercellular channel
Connexin
Connexon (hemichannel)
miRNAs
K+
cAMP
Na+
Glutation
ATP
Ca+2
Glucose
Glutamate
IP3
ADP
CL-
Lactose
c) Exchange of molecules
Minimum of approximately 1.4 nm width
Molecules up to 1-2 kD

Figure 2

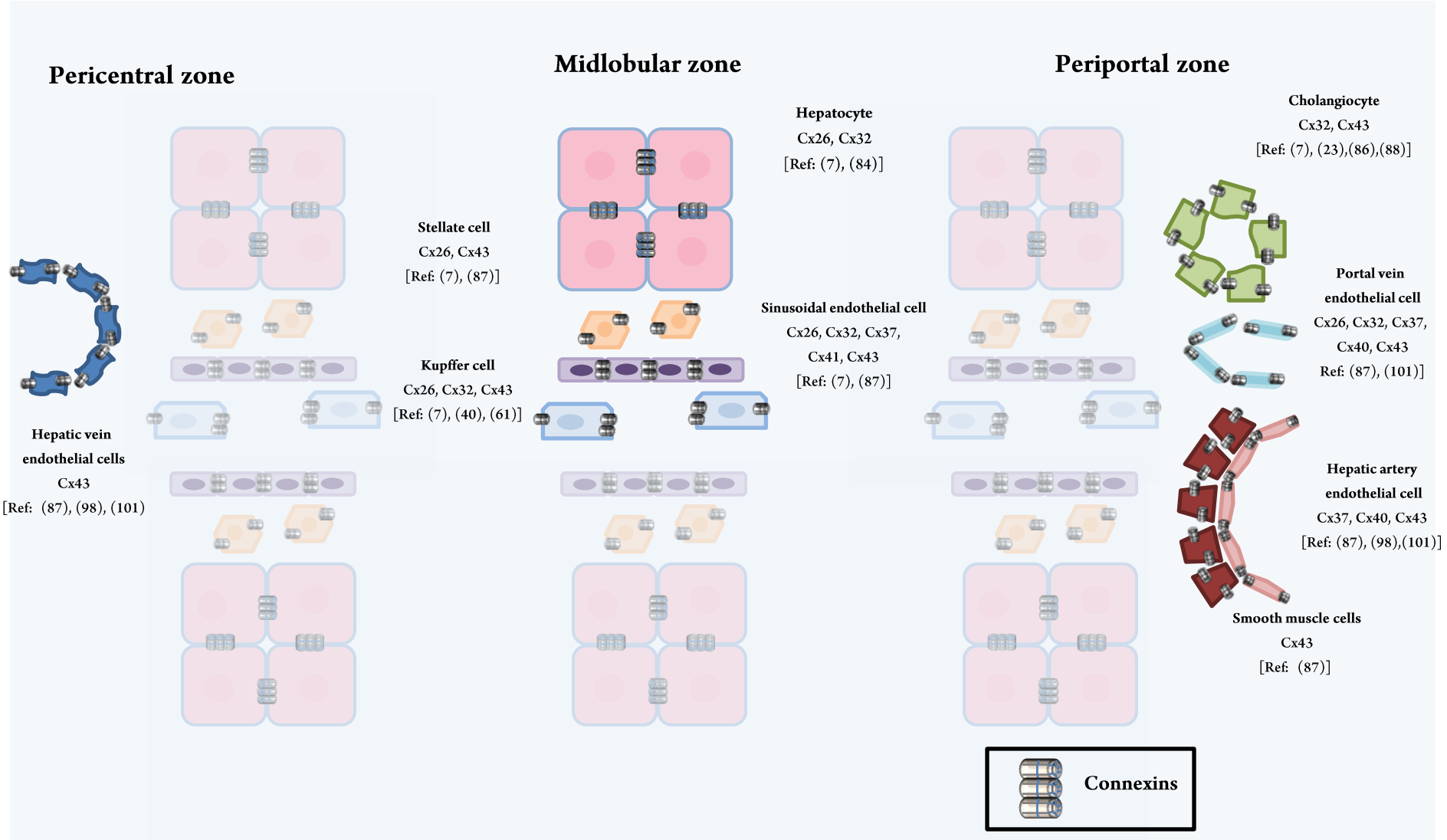
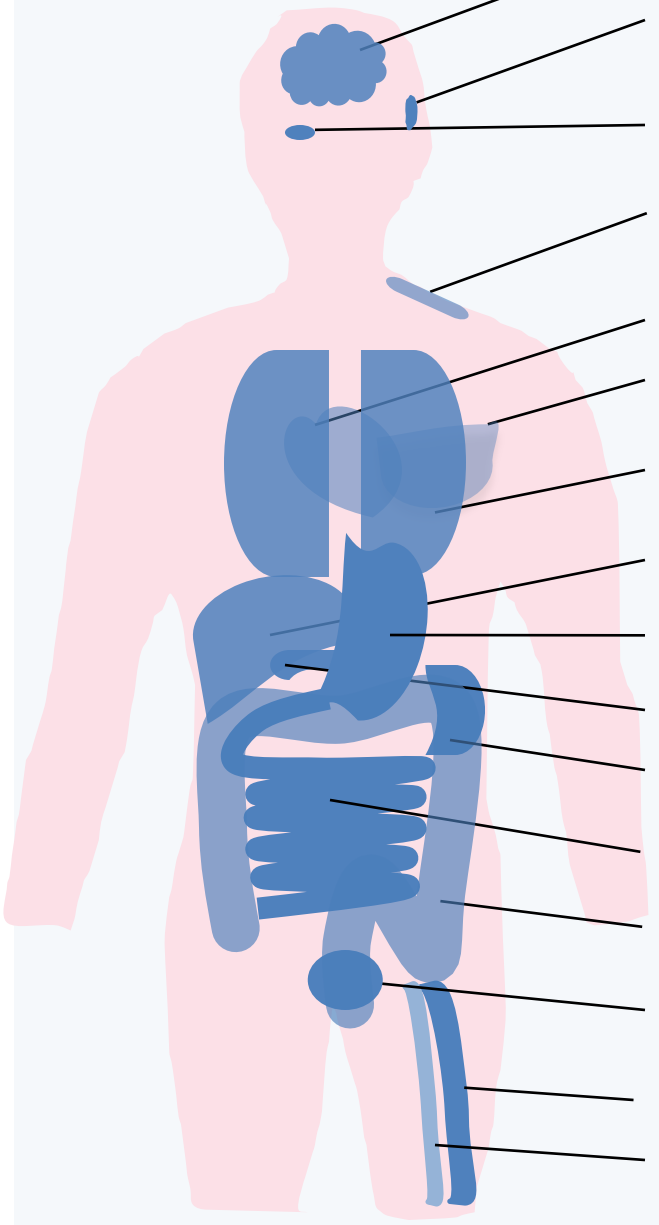
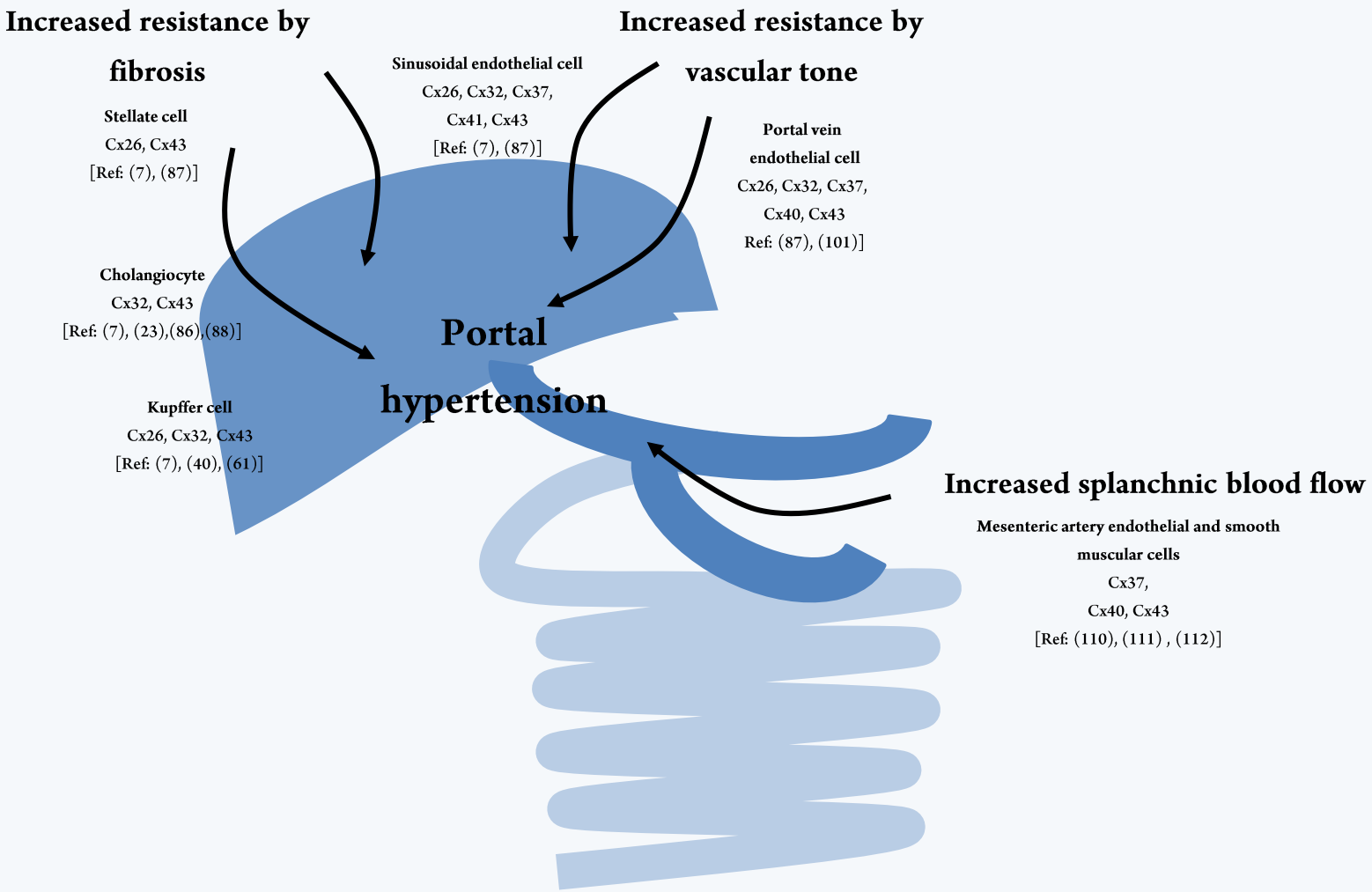

Pericentral zone
Midlobular zone
Periportal zone
Hepatocyte
Cx26, Cx32
[Ref: (7), (84)]
Cholangiocyte
Cx32, Cx43
[Ref: (7), (23),(86),(88)]
Stellate cell
Cx26, Cx43
[Ref: (7), (87)]
Portal vein
endothelial cell
Cx26, Cx32, Cx37,
Cx40, Cx43
Ref: (87), (101)]
Sinusoidal endothelial cell
Cx26, Cx32, Cx37,
Cx41, Cx43
[Ref: (7), (87)]
Kupffer cell
Cx26, Cx32, Cx43
[Ref: (7), (40), (61)]
Hepatic vein
endothelial cells
Cx43
[Ref: (87), (98), (101)
Hepatic artery
endothelial cell
Cx37, Cx40, Cx43
[Ref: (87), (98),(101)]
Smooth muscle cells
Cx43
[Ref: (87)]
Connexins

Figure 3



Central nervous system Cx30, Cx32, Cx43, Cx47 [Ref:163,183]	Lost of memory, leukodystrophy
Cochlea Cx26, Cx30, Cx31, Cx43 [Ref:156, 159, 162,164,174,175,176]	Deafness, oculodentodigital dysplasia, keratoderma-hypotrhichosis-leukonychia totalis syndrome
Eye Cx46, Cx50 [Ref:172,173,182,185]	Cataract
Skin Cx26, Cx30, Cx31, Cx43 [Ref:154,155,160,161]	Palmoplantar keratoderma, psoriasis, erythrokeratodermia variabilis et progressiva
Heart Cx37, Cx40 [Ref:168,169,177,62]	Atrial fibrillation, infarct
Breast Cx26, Cx43 [Ref:158,181]	Breast cancer
Lung Cx37, Cx40, Cx43 [Ref:167,171,178]	Asthma, pulmonary arterial hypertension, idiopathic pulmonary fibrosis
Liver Cx26, Cx32, Cx40, Cx43	DILI, portal hypertension, hepatocellular carcinoma, cholestasis, NASH, ischemia-reperfusion, acute liver failure, cirrhosis
Stomach Cx30, Cx32, Cx43 [Ref:186]	Gastric cancer, gastric erosions
Pancreas Cx36 [Ref:166]	Diabetes
Kidney Cx37, Cx40, Cs43, Cx45 [Ref:170]	Hypertension
Duodenum Cx37 [Ref:187]	Celiac disease
Colon Cx26, Cx32, Cx43 [Ref:157,179,180]	Dysentery, diverticulosis, colon cancer, Crohn´s disease, Hirschsprung´s disease
Prostate Cx43 [Ref:37]	Prostate cancer metastasis
Vessels Cx47 [Ref:184]	Lymphedema
Peripheral nervous system Cx32 [Ref: 165]	Charcot-Marie-Tooth disease

Figure 4


Increased resistance by fibrosis
Stellate cell
Cx26, Cx43
[Ref: (7), (87)]
Cholangiocyte
Cx32, Cx43
[Ref: (7), (23),(86),(88)]
Kupffer cell
Cx26, Cx32, Cx43
[Ref: (7), (40), (61)]
Sinusoidal endothelial cell
Cx26, Cx32, Cx37,
Cx41, Cx43
[Ref: (7), (87)]
Increased resistance by vascular tone
Portal vein endothelial cell
Cx26, Cx32, Cx37,
Cx40, Cx43
Ref: (87), (101)]
Portal hypertension
Increased splanchnic blood flow
Mesenteric artery endothelial and smooth muscular cells
Cx37,
Cx40, Cx43
[Ref: (110), (111) , (112)]

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