Review article:

CONNEXINS AND PANNEXINS IN LIVER DAMAGE

Sara Crespo Yanguas§, Joost Willebrords§, Michaël Maes1, Tereza Cristina da Silva2, Isabel Veloso Alves Pereira2, Bruno Cogliati2, Maria Lucia Zaidan Dagli2#, Mathieu Vinken1#*

1 Department of In Vitro Toxicology and Dermato-Cosmetology, Faculty of Medicine and Pharmacy, Vrije Universiteit Brussel, Laarbeeklaan 103, 1090 Brussels, Belgium, mvinken@vub.ac.be
2 Department of Pathology, School of Veterinary Medicine and Animal Science, University of São Paulo, Av. Prof. Dr. Orlando Marques de Paiva 87, São Paulo SP CEP 05508-900, Brazil
* Corresponding author
§ Equally contributing first authors
# Share equal seniorship

http://dx.doi.org/10.17179/excli2016-119

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0/).

ABSTRACT

Connexins and pannexins are key players in the control of cellular communication and thus in the maintenance of tissue homeostasis. Inherent to this function these proteins are frequently involved in pathological processes. The present paper reviews the role of connexins and pannexins in liver toxicity and disease. As they act both as sensors and effectors in these deleterious events connexins and pannexins could represent a set of novel clinical diagnostic biomarkers and drug targets.

Keywords: Connexin, pannexin, acute liver injury, steatosis, hepatitis, cholestasis, fibrosis, liver cancer

Abbreviations: ATP: adenosine triphosphate, Cx: connexin, GJIC: gap junctional intercellular communication, HCC: hepatocellular carcinoma, NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis, Panx: pannexin

INTRODUCTION

Like in other organs liver homeostasis relies on the interplay between extracellular, intracellular and intercellular signaling. The latter is mediated by gap junctions which arise from the interaction of 2 hemichannels, also called connexons of adjacent cells, each connexon being composed of 6 connexin (Cx) proteins. More than 20 different connexin variants have been identified, all which are named after their molecular weight as predicted by cDNA sequencing and expressed in kilodaltons (Bai and Wang, 2014), and that are produced in a tissuespecific way. In liver, 5 different connexin species are detectable (Figure 1). Parenchymal liver cells, the hepatocytes, abundantly produce Cx32 and small quantities of Cx26. By contrast, Cx43 is the predominant connexin species present in nonparenchymal liver cells, including stellate cells, Kupffer cells, and sinusoidal endothelial cells (Fischer et al., 2005). Cx37 and Cx40 are the major connexins harbored by liver vasculature (Chaytor et al., 2001; Fischer et al., 2005; Hernández-Guerra et al., 2014;
Shiojiri et al., 2006). Nevertheless, gap junctions are mainly, if not uniquely, found between hepatocytes (Spray et al., 1994). Gap junctions provide a pathway for the intercellular flux of small and hydrophilic substances, including adenosine triphosphate (ATP), cyclic adenosine monophosphate and inositol triphosphate, as well as several ions (Alexander and Goldberg, 2003; Dbouk et al., 2009; Decrock et al., 2009). By doing so, gap junctional intercellular communication (GJIC) has been found critical for the performance of vital functions in liver, such as plasma protein synthesis (Yang et al., 2003) and xenobiotic biotransformation (Neveu et al., 1994; Shoda et al., 1999, 2000).

About 15 years ago, a novel group of connexin-like proteins was discovered, the pannexin (Panx) family, with 3 members characterized thus far. Pannexins do not form gap junctions, but rather assemble in a configuration reminiscent of connexin hemi-channels. They facilitate paracrine signaling by controlling the exchange of substances like ATP between the cytosol and the extracellular environment (Panchin et al., 2000). A number of reports published in recent years have demonstrated Panx1 expression in liver tissue, in particular produced by hepatocytes (Bruzzone et al., 2003; Csak et al., 2011; Ganz et al., 2011; Kim et al., 2015; Xiao et al., 2012) and Kupffer cells (Sáez et al., 2014). Other studies showed the presence of Panx2 protein in mouse liver (Le Vasseur et al., 2014) and rat hepatocytes (Li et al., 2008). Although their physiological roles in liver remain to be established, pannexin-mediated communication has already been associated with liver pathology. In fact this paper will review the current knowledge regarding the involvement of connexins, pannexins and their channels in liver injury, in casu occurring in the context of liver disease and toxicity.

Figure 1: Expression of connexins and pannexins in liver cells
Table 1: Expression of connexins and pannexins in liver cells

| Liver cell                  | Cx/Panx expression                  | Reference                                                                 |
|-----------------------------|-------------------------------------|---------------------------------------------------------------------------|
| Hepatocyte                  | Cx32, Cx26, Panx1, Panx2            | Bruzzone et al., 2003; Csak et al., 2011; Fischer et al., 2005; Fowler et al., 2013; Ganz et al., 2011; Kim et al., 2015; Kumar and Gilula, 1986; Kuraoka et al., 1993; Le Vasseur et al., 2014; Li et al., 2008; Nicholson et al., 1987; Paul, 1986; Xiao et al., 2012; Zhang and Nicholson, 1989 |
| Kupffer cell                | Cx43, Cx26, Panx1                   | Eugenin et al., 2007; Fischer et al., 2005; Sáez et al., 2014              |
| Stellate cell               | Cx43, Cx26                          | Fischer et al., 2005; Hernández-Guerra et al., 2014                       |
| Sinusoidal endothelial cell | Cx43, Cx32, Cx26                    | Fischer et al., 2005; Hernández-Guerra et al., 2014                       |
| Cholangiocyte               | Cx43, Cx32                          | Bode et al., 2002                                                         |
| Hepatic artery endothelial cell | Cx43                                | Chaytor et al., 2001; Hernández-Guerra et al., 2014; Shiojiri et al., 2006 |

ACUTE LIVER INJURY

Acute liver failure is a clinical syndrome from a variety of causes resulting from rapid loss in hepatocyte function, typically associated with coagulopathy and encephalopathy in a patient without preexisting liver disease. Upon administration of prototypical liver toxicants including thioacetamide, acetaminophen, D-galactosamine or carbon tetrachloride, to Cx32-lacking rodents, decreased aminotransferase serum levels and less liver damage is observed in comparison with wild-type littermates (Asamoto et al., 2004; Naiki-Ito et al., 2010; Patel et al., 2012). Along the same line, hepatocytes originating from Cx32-deficient mice show reduced cell death when treated with acetaminophen in vitro (Saito et al., 2014). This points to a role for Cx32-based signaling either in spreading noxious messengers or in the removal of dead cells in order to restore the homeostatic balance. In contrast to this is a recent report, describing protective effects of Cx32 in acetaminophen-triggered liver toxicity, possibly linked to the trafficking of glutathione between hepatocytes via gap junctions (Igarashi et al., 2014). This can be reconciled with the well-known decay of Cx32 production and concomitant reduced channel activity upon exposure of hepatocytes to liver toxicants both in vitro and in vivo (Vinken et al., 2009; Maes et al., 2016). Hepatocellular gap junctions persist in the early phases of centrilobular necrotic cell death induced by thioacetamide in rat, yet they fade away during the subsequent restorative proliferative response. In a later stage, gap junctions initially emerge in perinecrotic areas and ultimately in all zones (Kojima et al., 1994). Of note, in liver of rodents overdosed with acetaminophen, Cx43 is upregulated and de novo expressed in hepatocytes (Naiki-Ito et al., 2010, Maes et al., 2016). In rat liver, this Cx43 expression is colocalized with caspase 3, suggesting a role for Cx43 in cell death. However, a recent study showed that Cx43-deficient mice display increased liver cell death, inflammation and oxidative stress in comparison to wild-type littermates after acetaminophen overdose (Maes et al., 2016). Furthermore, high Cx43 immunoreactivity is observed around inflamed and necrotic areas in a rat model of acute-on-chronic liver failure (Balasubramaniyan et al., 2013).

LIVER STEATOSIS

Non-alcoholic fatty liver disease (NAFLD) is currently the most common chronic liver disease worldwide. NAFLD is defined by the presence of liver fat accumulation exceeding 5% of hepatocytes in the absence of significant alcohol intake. As
such, NAFLD encompasses a broad histopathological spectrum, ranging from steatosis to non-alcoholic steatohepatitis (NASH) and even liver cancer (Loomba and Sanyal, 2013; Willebrords et al., 2015). NASH relies, at least in part, on the activation of inflammasomes, being multiprotein complexes involved in innate immunity and caspase 1 processing, which in turn leads to cleavage and extracellular release of interleukin 1 beta and interleukin 18 (Wree et al., 2014). Panx1 channels have been repeatedly found to facilitate inflammasome activation (Pelegrin and Surprenant, 2006, 2007). As a matter of fact, these pores support ATP release during lipotoxic apoptosis induced by saturated free fatty acids in cultured hepatocytes, which is a hallmark of NASH. Panx1 channels are therefore thought to play a critical role in inflammation associated with lipotoxic liver injury (Xiao et al., 2012). Panx1 channels have been repeatedly found to facilitate inflammasome activation (Pelegrin and Surprenant, 2006, 2007). As a matter of fact, these pores support ATP release during lipotoxic apoptosis induced by saturated free fatty acids in cultured hepatocytes, which is a hallmark of NASH. Panx1 channels are therefore thought to play a critical role in inflammation associated with lipotoxic liver injury (Xiao et al., 2012). As a matter of fact, these pores support ATP release during lipotoxic apoptosis induced by saturated free fatty acids in cultured hepatocytes, which is a hallmark of NASH. Panx1 channels are therefore thought to play a critical role in inflammation associated with lipotoxic liver injury (Xiao et al., 2012).

HEPATITIS

Hepatitis refers to a general inflammatory response of the liver to a number of factors, such as drugs or viruses (Vinken et al., 2013). Hepatitis patients present reduced amounts of Cx32 in the liver (Nakashima et al., 2004; Yamaoka et al., 2000), a feature that can be reproduced in rodents when treated with lipopolysaccharide (Correa et al., 2004; Gonzalez et al., 2002; Temme et al., 2000). Deterioration of Cx32 expression hereby results from mRNA degradation (Theodorakis and De Maio, 1999). Downregulation of Cx32 production by proinflammatory cytokines in cultures of primary hepatocytes is controlled by nuclear factor kappa beta signaling and mitogen-activated protein kinase, and is accompanied by abrogation of GJIC (Yamamoto et al., 2004). Hepatic Cx26, however is positively affected by proinflammatory stimuli both in vitro and in vivo (Temme et al., 2000, 1998). Likewise, Cx43 expression and GJIC become enhanced in cultures of primary stellate cells and Kupffer cells in inflammatory conditions (Eugenin et al., 2007; Fischer et al., 2005). Cx43 hereby moves from the cytosol to the membrane surface in order to assemble into functional gap junctions. Upregulated Cx43 production also occurs during liver inflammation in vivo (Eugenin et al., 2007; Gonzalez et al., 2002). This is thought to reflect the activation of Kupffer cells, which assists in the removal of debris and apoptosis of damaged hepatocytes following inflammation (Eugenin et al., 2007).

CHOLESTASIS

Acute or chronic impairment of bile flow from the liver to the duodenum is referred to as cholestasis. Upon cholestasis, hepatocytes adopt a brownish-green stippled appearance within the cytoplasm, which reflects bile accumulation. Canalicular bile plugs between hepatocytes or within bile ducts may also be observed, representing bile that has been excreted and that is obstructed in the duct. Because of increased pressure, such bile duct plugs may cause rupture and hence spilling of bile into surrounding tissue. This can induce hepatic necrosis and inflammation (Vinken et al., 2013). Cholestasis can be experimentally induced by bile duct ligation. This is associated with decreased gap junction quantities and low Cx32 amounts in the liver (Balasubramaniyan et al., 2013; Fallon et al., 1995; Gonzalez et al., 2002; Kojima et al., 2003), which is mediated by the p38 mitogen-activated protein kinase (Kojima et al., 2003). Cx26 levels also drop, while Cx43 production increases following bile duct ligation (Balasubramaniyan et al., 2013; Fallon et al., 1995).

LIVER FIBROSIS

Fibrosis is a wound-healing response to various types of injury, whereby quiescent stellate cells transform into proliferative, fibrogenic and contractile myofibroblast-like cells. This is associated with a cascade of biochemical events, such as proinflammatory
cytokine release and extracellular matrix deposition, all which result in drastic phenotypic changes, including scarring. The final stage of fibrosis is called cirrhosis and is considered irreversible (Crespo Yanguas et al., 2016; Friedman, 2008, 2010; Lee et al., 2015). Cx32 steady-state protein levels are reduced in cirrhosis patients, a process that goes hand in hand with its relocalization in the cytoplasm of hepatocytes (Nakashima et al., 2004; Yamaoka et al., 2000, 1995). Furthermore, upregulated Cx43 production has been observed in human cirrhotic liver tissue (Hernández-Guerra et al., 2014). These findings are identical to those in rodents following chronic administration of thioacetamide or carbon tetrachloride (Nakata et al., 1996). In Cx43-lacking mice, strongly reduced cell death and hepatocellular injury is observed after treatment with carbon tetrachloride (Cogliati et al., 2011). The latter induces translocation of both Cx26 and Cx43 from the plasma membrane to the cytoplasm and nuclei of sinusoidal endothelial cells, a scenario that is equally seen for Cx32 in hepatocytes. Perinuclear residing of Cx26 and Cx43 also occurs in cultures of spontaneously activated primary stellate cells (Fischer et al., 2005). This could underlie the establishment of heterologous communication between stellate cells and hepatocytes under these conditions (Rojkind et al., 1995), whilst homologous GJIC in cultured hepatocytes is suppressed by carbon tetrachloride (Saez et al., 1987). Collectively, these observations suggest distinct roles for connexins in each liver cell type in the process of fibrogenesis (Oloris et al., 2007).

**LIVER CANCER**

Chronic liver disease may burgeon into the onset of liver cancer, mainly hepatocellular carcinoma (HCC). GJIC is strongly reduced in HCC cells (Mesnil et al., 2005; Yang et al., 2003; Yano et al., 2001). This is paralleled by cytoplasmic Cx32 localization, which is believed to promote motility and metastatic potential (Li et al., 2007). Decrease of Cx26 production in HCC has been related to epigenetic modifications, in particular DNA methylation (Shimizu et al., 2007; Tsujiuchi et al., 2007). Concomitantly, Cx43 gradually appears in the cytoplasm and at the plasma membrane of HCC cells (Krutovskikh et al., 1994; Oyamada et al., 1990; Wang et al., 2013b). In fact, the extent of cytoplasmic Cx43 localization corresponds with the malignant potential of the liver tumor (Kawasaki et al., 2007). In addition, Cx43 expression in HCC is linked to migration, invasion and metastatic ability (Ogawa et al., 2012). Silencing of Cx43 production in liver cancer cells inhibits proliferation and favors the differentiated phenotype, whereas the opposite has been observed in HCC cells that artificially overexpress Cx43. Not surprisingly, Cx32 amounts and gap junction activity inversely correlate with Cx43 presence in HCC cells. Cx43 is therefore considered a hepatic oncogene (Zhang et al., 2007). By contrast, Cx32 acts as a liver tumor suppressor, a notion that is supported by the observation that Cx32 knockout rodents display increased susceptibility to chemically induced hepatocarcinogenesis (Dagli et al., 2004; Igarashi et al., 2013).

**CONCLUSIONS**

Because of its unique localization and position in the organism, the liver is a major target for systemic toxicity and disease (Vinken et al., 2013). Connexins are goalkeepers in hepatic homeostasis and hence are routinely involved in liver pathology. They act both as sensors and effectors in this process. Regarding the former, a general observation in liver disease is that Cx32 production gradually decreases at the expense of Cx43 (Krutovskikh et al., 1994; Oyamada et al., 1990; Wang et al., 2013b). This renders Cx43 a potential biomarker that can be used for diagnostic purposes. In addition, connexins can also represent drugable targets due to their active role in liver pathogenesis. Research in this direction is nowadays challenged with the complex multifaceted communication capacities of connexins. Indeed, in the last decade, it has become clear that
connexin hemichannels not only are the structural building blocks of gap junctions, but also are equally signaling entities on their own. They specifically establish a circuit for trafficking of messengers, such as ATP, between the cytosol and the extracellular space, similar to pannexin-based communication. Unlike their full channel counterparts however, hemichannels have a low open probability (Chandrasekhar and Bera, 2012; D’hondt et al., 2014; Decrock et al., 2009). In fact, although heavily debated and still highly criticized, it seems that hemichannels specifically open during pathological circumstances, which is another difference with gap junctions. In this respect, hemichannels consisting of Cx32 and to a lesser extent of Cx43, but not their corresponding gap junctions have been found to drive hepatocyte cell death (Vinken et al., 2010, 2012). Therefore, inhibition of hemichannels could introduce a novel strategy for the clinical management of liver disease. This also holds true for pannexin channels that underlie inflammatory processes, including in liver disease (Csak et al., 2011; Diezmos et al., 2013; Ganz et al., 2011; Gulbransen et al., 2012; Xiao et al., 2012). Focus should thereby be put on the development of pharmacological inhibitors of hemichannels and pannexin channels. Most of the currently available inhibitors of these channels are not able to distinguish between connexin and pannexin signaling on the one hand and between hemichannel communication and GJIC on the other hand (Bodendiek and Raman, 2010). An exception includes the group of so-called mimetic peptides, which reproduce specific amino acid sequences in the connexin protein structure. Some of these mimetic peptides have the ability to inhibit hemichannels without affecting gap junctions (Abudara et al., 2014; Iyyathurai et al., 2013) and have been found to protect against cell death in vivo (Wang et al., 2013a). Similarly, specific pannexin mimetic peptides are able to counteract inflammation and cell death (Orellana et al., 2011; Pelegrin et al., 2008). Such compounds should be further explored in future, as they may open new avenues for the clinical treatment of liver disease.

Acknowledgements

This work was financially supported by the grants of Agency for Innovation by Science and Technology in Flanders-Belgium (IWIT) the University Hospital of the Vrije Universiteit Brussel-Belgium (“Willy Gepts Fonds” UZ-VUB) the Fund for Scientific Research Flanders-Belgium (FWO grants G009514N and G010214N) the European Research Council (ERC Starting Grant 335476) the University of São Paulo-Brazil and the Foundation for Research Support of the State of São Paulo-Brazil (FAPESP SPEC grant 2013/50420-6).

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

Abudara V, Bechberger J, Freitas-Andrade M, De Bock M, Wang N, Bultynck G, et al. The connexin43 mimetic peptide Gap19 inhibits hemichannels without altering gap junctional communication in astrocytes. Front Cell Neurosci. 2014;8:306.

Alexander DB, Goldberg GS. Transfer of biologically important molecules between cells through gap junction channels. Curr Med Chem. 2003;10:2045-58.

Asamoto M, Hokaiwado N, Murasaki T, Shirai T. Connexin 32 dominant-negative mutant transgenic rats are resistant to hepatic damage by chemicals. Hepatology. 2004;40:205-10.

Bai D, Wang AH. Extracellular domains play different roles in gap junction formation and docking compatibility. Biochem J. 2014;458:1-10.

Balasubramaniyan V, Dhar DK, Warner AE, Vivien Li WY, Amiri AF, Bright B, et al. Importance of Connexin-43 based gap junction in cirrhosis and acute-on-chronic liver failure. J Hepatol. 2013;58:1194-200.

Bode HP, Wang L, Cassio D, Leite MF, St-Pierre MV, Hirata K, et al. Expression and regulation of gap junctions in rat cholangiocytes. Hepatology. 2002;36:631-40.
Bodendiek SB, Raman G. Connexin modulators and their potential targets under the magnifying glass. Curr Med Chem. 2010;17:4191-230.

Bruzzone R, Hormuzdi SG, Barbe MT, Herb A, Monyer H. Pannexins a family of gap junction proteins expressed in brain. Proc Natl Acad Sci USA. 2003;100:13644-9.

Chandrasekhar A, Bera AK. Hemichannels: permeants and their effect on development physiology and death. Cell Biochem Funct. 2012;30:89-100.

Chaytor AT, Martin PE, Edwards DH, Griffith TM. Gap junctional communication underpins EDHF-type relaxations evoked by ACh in the rat hepatic artery. Am J Physiol. 2001;280:H2441-50.

Cogliati B, Da Silva TC, Aloia TP, Chaible LM, Real-Lima MA, Sanches DS, et al. Morphological and molecular pathology of CCL4-induced hepatic fibrosis in connexin43-deficient mice. Microsc Res Tech. 2011;74:421-9.

Correa PR, Guerra MT, Leite MF, Spray DC, Nathanson MH. Endotoxin unmasks the role of gap junctions in the liver Biochem Biophys Res Commun. 2004;322:718-26.

Crespo Yanguas S, Cogliati B, Willebrords J, Maes M, Colle I, van den Bossche B, et al. Experimental models of liver fibrosis. Arch Toxicol. 2016; in press.

Csak T, Ganz M, Pespisa J, Kodyš K, Dolganiuć A, Szabo G. Fatty acid and endotoxin activate inflammasomes in mouse hepatocytes that release danger signals to stimulate immune cells. Hepatology. 2011;54:133-44.

Dagli ML, Yamasaki H, Krutovskikh V, Omori Y. Delayed liver regeneration and increased susceptibility to chemical hepatocarcinogenesis in transgenic mice expressing a dominant-negative mutant of connexin32 only in the liver. Carcinogenesis. 2004;25:483-92.

Dbouk HA, Mroue RM, El-Sabban ME, Talhoub RS. Connexins: a myriad of functions extending beyond assembly of gap junction channels. Cell Commun Signal. 2009;7:4.

Decrock E, Vinken M, De Vuyst E, Krysko DV, D’Herde K, Vanhaecke T, et al. Connexin-related signaling in cell death: to live or let die? Cell Death Diff. 2009;16:524-36.

D’hondt C, Iyathurai J, Himpens B, Leybaert L, Bultynck G. Cx43-hemichannel function and regulation in physiology and pathophysiology: insights from the bovine corneal endothelial cell system and beyond Front Physiol. 2014;5:348.

Diezmos EF, Sandow SL, Markus I, Shevy Perera D, Lubowski DZ, King DW, et al. Expression and localization of pannexin-1 hemichannels in human colon in health and disease. Neurogastroenterol Motil. 2013;25:e395-405.

Eugenin EA, Gonzalez HE, Sanchez HA, Branes MC, Saez JC. Inflammatory conditions induce gap junctional communication between rat Kupffer cells both in vivo and in vitro. Cell Immunol. 2007;247:103-10.

Fallon MB, Nathanson MH, Mennone A, Saez JC, Burgstahler AD, Anderson JM. Altered expression and function of hepatocyte gap junctions after common bile duct ligation in the rat. Am J Physiol. 1995;268:C1186-94.

Fischer R, Reinehr R, Lu TP, Schonick A, Warkfulat U, Dienes HP, et al. Intercellular communication via gap junctions in activated rat hepatic stellate cells. Gastroenterology. 2005;128:433-48.

Fowler SL, Akins M, Zhou H, Figey D, Bennett SA. The liver connexin32 interactome is a novel plasma membrane-mitochondrial signaling nexus J Proteome Res. 2013;12:2597-610.

Friedman SL. Mechanisms of hepatic fibrogenesis. Gastroenterology. 2008;134:1655-69.

Friedman SL. Evolving challenges in hepatic fibrosis. Nat Rev Gastroenterol Hepatol. 2010;7:425-36.

Ganz M, Csak T, Nath B, Szabo G. Lipopolysaccharide induces and activates the Nalp3 inflammasome in the liver. World J Gastroenterol. 2011;17:4772-8.

Gonzalez HE, Eugenin EA, Garces G, Solis N, Pizarro M, Accatino L, et al. Regulation of hepatic connexins in cholestasis: possible involvement of Kupffer cells and inflammatory mediators. Am J Physiol. 2002;282:G991-G1001.

Gulbransen BD, Bashashati M, Hirata SA, Gui X, Roberts JA, MacDonald JA, et al. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. Nat Med. 2012;18:600-4.

Hernández-Guerra M, González-Méndez Y, de Ganzo ZA, Salido E, García-Pagán JC, Abrante B, et al. Role of gap junctions modulating hepatic vascular tone in cirrhosis. Liver Int. 2014;34:859-68.

Igarashi I, Makino T, Suzuki Y, Kai K, Teranishi M, Takasaki W, et al. Background lesions during a 24-month observation period in connexin 32-deficient mice. J Vet Med Sci. 2013;75:207-10.
Igarashi I, Maejima T, Kai K, Arakawa S, Teranishi M, Sanbuisho A. Role of connexin 32 in acetaminophen toxicity in a knockout mice model. Exp Toxicol Pathol. 2014;66:103-10.

Iyyathurai J, D’hondt C, Wang N, De Bock M, Himpens B, Retamal MA, et al. Peptides and peptide-derived molecules targeting the intracellular domains of Cx43: gap junctions versus hemichannels. Neuropharmacology. 2013;75:491-505.

Kawasaki Y, Kubomoto A, Yamasaki H. Control of intracellular localization and function of Cx43 by SEMA3F. J Membr Biol. 2007;217:53-61.

Kim HY, Kim SJ, Lee SM. Activation of NLRP3 and AIM2 inflammasomes in Kupffer cells in hepatic ischemia/reperfusion. Febs J. 2015;282:259-70.

Kojima T, Sawada N, Zhong Y, Oyamada M, Mori M. Sequential changes in intracellular junctions between hepatocytes during the course of acute liver injury and restoration after thioacetamide treatment. Virchows Arch. 1994;425:407-12.

Li X, Cao J, Jin Q, Xie C, He Q, Cao R, et al. A proteomic study reveals the diversified distribution of plasma membrane-associated proteins in rat hepatocytes. J Cell Biochem. 2008;104:965-84.

Loomba R, Sanyal AJ. The global NAFLD epidemic. Nat Rev Gastroenterol Hepatol. 2013;10:686-90.

Li X, Cao J, Jin Q, Xie C, He Q, Cao R, et al. A proteomic study reveals the diversified distribution of plasma membrane-associated proteins in rat hepatocytes. J Cell Biochem. 2008;104:965-84.

Mesnil M, Crespin S, Avanzo JL, Zaidan-Dagli ML. Defective gap junctional intercellular communication in the carcinogenic process. Biochim Biophys Acta. 2005;1719:125-45.

Naiki-Ito A, Asamoto M, Naiki T, Ogawa K, Takahashi S, Sato S, et al. Gap junction dysfunction reduces acetaminophen hepatotoxicity with impact on apoptotic signaling and connexin 43 protein induction in rat. Toxicol Pathol. 2010;38:280-6.

Nakashima Y, Ono T, Yamanoi A, El-Assal ON, Kohno H, Nagasue N. Expression of gap junction protein connexin32 in chronic hepatitis liver cirrhosis and hepatocellular carcinoma. J Gastroenterol. 2004;39:763-8.

Nakata Y, Iwai M, Kimura S, Shimazu T. Prolonged decrease in hepatic connexin32 in chronic liver injury induced by carbon tetrachloride in rats. J Hepatol. 1996;25:529-37.

Neveu MJ, Babcock KL, Hertzberg EL, Paul DL, Nicholson BJ, Pitot HC. Colocalized alterations in connexin32 and cytochrome P450IIB1/2 by phenobarbital and related liver tumor promoters. Cancer Res. 1994;54:3145-52.

Nicholson B, Dermietzel R, Teplow D, Traub O, Willecke K, Revel JP. Two homologous protein components of hepatic gap junctions. Nature. 1987;329:732-4.

Nicholson B, Dermietzel R, Teplow D, Traub O, Willecke K, Revel JP. Two homologous protein components of hepatic gap junctions. Nature. 1987;329:732-4.

Ogawa K, Pitchakarn P, Suzuki S, Chewonarin T, Tang M, Takahashi S, et al. Silencing of connexin 43 suppresses invasion migration and lung metastasis of rat hepatocellular carcinoma cells. Cancer Sci. 2012;103:860-7.

Oloris SC, Mesnil M, Reis VN, Sakai M, Matsuzaki P, Fonseca Ede S, et al. Hepatic granulomas induced by Schistosoma mansoni in mice deficient for connexin 43 present lower cell proliferation and higher collagen content. Life Sci. 2007;80:1228-35.
Orellana JA, Shoji KF, Abudara V, Ezan P, Amigou E, Saez PJ, et al. Amyloid beta-induced death in neurons involves glial and neuronal hemichannels. J Neurosci. 2011;31:4962-77.

Oyamada M, Krutovskikh VA, Mesnil M, Partensky C, Berger F, Yamasaki H. aberrant expression of gap junction gene in primary human hepatocellular carcinomas: increased expression of cardiac-type gap junction gene connexin 43. Mol Carcinog. 1990;3:273-8.

Panchin Y, Kelmanson I, Matz M, Lukyanov K, Usman N, Lukyanov S. A ubiquitous family of putative gap junction molecules. Curr Biol. 2000;10:R473-4.

Patel SJ, Milwid JM, King KR, Bohr S, Iracheta-Velle A, Li M, et al. Gap junction inhibition prevents drug-induced liver toxicity and fulminant hepatic failure. Nat Biotechnol. 2012;30:179-83.

Paul DL. Molecular cloning of cDNA for rat liver gap junction protein. J Cell Biol. 1986;103:123-34.

Pelegrin P, Surprenant A. Pannexin-1 mediates large pore formation and interleukin-1beta release by the ATP-gated P2X7 receptor. EMBO J. 2006;25:5071-82.

Pelegrin P, Surprenant A. Pannexin-1 couples to majotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. J Biol Chem. 2007;282:2386-94.

Pelegrin P, Surprenant A. Pannexin-1 couples to majotoxin- and nigericin-induced interleukin-1beta release through a dye uptake-independent pathway. J Biol Chem. 2007;282:2386-94.

Temme A, Traub O, Willecke K. Downregulation of connexin32 protein and gap-junctional intercellular communication by cytokine-mediated acute-phase response in immortalized mouse hepatocytes. Cell Tissue Res. 1998;294:345-50.

Theodorakis NG, De Maio A. Cx32 mRNA in rat liver: effects of inflammation on poly(A) tail distribution and mRNA degradation. Am J Physiol. 1999;276:R158-64.
Vinken M, Decrock E, De Vuyst E, De Bock M, Vandenbroucke RE, De Geest BG, et al. Connexin32 hemichannels contribute to the apoptotic-to-necrotic transition during Fas-mediated hepatocyte cell death. Cell Mol Life Sci. 2010;67:907-18.

Vinken M, Decrock E, Vanhaecke T, Leybaert L, Rogiers V. Connexin43 signaling contributes to spontaneous apoptosis in cultures of primary hepatocytes. Toxicol Sci. 2012;125:175-86.

Vinken M, Maes M, Vanhaecke T, Rogiers V. Drug-induced liver injury: mechanisms types and biomarkers. Curr Med Chem. 2013;20:3011-21.

Wang N, De Vuyst E, Ponsaerts R, Boengler K, Palacios-Prado N, Wauman J, et al. Selective inhibition of Cx43 hemichannels by Gap19 and its impact on myocardial ischemia/reperfusion injury. Basic Res Cardiol. 2013a;108:309.

Wang ZS, Wu LQ, Yi X, Geng C, Li YJ, Yao RY. Connexin-43 can delay early recurrence and metastasis in patients with hepatitis B-related hepatocellular carcinoma and low serum alpha-fetoprotein after radical hepatectomy. BMC Cancer. 2013b;13:306.

Willebrords J, Pereira IV, Maes M, Crespo Yanguas S, Colle I, Van Den Bossche B, et al. Strategies, models and biomarkers in experimental non-alcoholic fatty liver disease research. Prog Lipid Res. 2015;59:106-25.

Wree A, McGeough MD, Peña CA, Schlattijan M, Li H, Inzaugarat ME, et al. NLRP3 inflammasome activation is required for fibrosis development in NAFLD. J Mol Med (Berl). 2014;92:1069-82.

Xiao F, Waldrop SL, Khimji AK, Kilic G. Pannexin1 contributes to pathophysiological ATP release in lipoapoptosis induced by saturated free fatty acids in liver cells. Am J Physiol. 2012;303:C1034-44.

Yamamoto T, Kojima T, Murata M, Takano K, Go M, Chiba H, et al. IL-1beta regulates expression of Cx32 occludin and claudin-2 of rat hepatocytes via distinct signal transduction pathways. Exp Cell Res. 2004;299:427-41.

Yamaoka K, Nouchi T, Tazawa J, Hiranuma S, Marumo F, Sato C. Expression of gap junction protein connexin 32 and E-cadherin in human hepatocellular carcinoma. J Hepatol. 1995;22:536-9.

Yamaoka K, Nouchi T, Kohashi T, Marumo F, Sato C. Expression of gap junction protein connexin 32 in chronic liver diseases. Liver. 2000;20:104-7.

Yang J, Ichikawa A, Tsuchiya T. A novel function of connexin 32: marked enhancement of liver function in a hepatoma cell line. Biochem Biophys Res Commun. 2003;307:80-5.

Yano T, Hernandez-Blazquez FJ, Omori Y, Yamasaki H. Reduction of malignant phenotype of HEPG2 cell is associated with the expression of connexin 26 but not connexin 32. Carcinogenesis. 2001;22:1593-600.

Zhang D, Kaneda M, Nakahama K, Arii S, Morita I. Connexin 43 expression promotes malignancy of HuH7 hepatocellular carcinoma cells via the inhibition of cell-cell communication. Cancer Lett. 2007;252:208-15.

Zhang JT, Nicholson BJ. Sequence and tissue distribution of a second protein of hepatic gap junctions Cx26 as deduced from its cDNA. J Cell Biol. 1989;109:3391-401.