Connexin and pannexin signaling in gastrointestinal and liver disease

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Gap junctions, which mediate intercellular communication, are key players in digestive homeostasis. They are also frequently involved in gastrointestinal and liver pathology. This equally holds true for connexin (Cx) hemichannels, the structural precursors of gap junctions, and pannexin (Panx) channels, Cx-like proteins assembled in a hemichannel configuration. Both Cx hemichannels and Panx channels facilitate extracellular communication and drive a number of deteriorative processes, such as cell death and inflammation. Cxs, Panxs, and their channels underlie a wide spectrum of gastrointestinal and liver diseases, including gastritis and peptic ulcer disease, inflammatory intestinal conditions, acute liver failure, cholestasis, hepatitis and steatosis, liver fibrosis and cirrhosis, infectious gastrointestinal pathologies, and gastrointestinal and liver cancer. This could open promising perspectives for the characterization of new targets and biomarkers for therapeutic and diagnostic clinical purposes in the area of gastroenterology and hepatology. (Translational Research 2015;166:332–343)

Abbreviations: ATP = adenosine triphosphate; CagA = cytotoxin-associated gene A; Cx = connexin; GJIC = gap junctional intercellular communication; HCC = hepatocellular carcinoma; mRNA = messenger RNA; Panx = pannexin; VacA = vacuolating toxin A

INTRODUCTION

Gap junctional intercellular communication (GJIC) relies on the exchange of small and hydrophilic substances between adjacent cells, including adenosine triphosphate (ATP), cyclic adenosine monophosphate, and inositol triphosphate as well as ions.[1-3] Hence, GJIC is considered to be a key mechanism in the maintenance of tissue functioning. In the gastrointestinal system, gap junctions indeed drive processes such as gastroduodenal[4] and gut motility,[5,6] gastric acid secretion,[7] gastric cytoprotection,[8,9] and intestinal innate immune defense.[10] Similarly, GJIC underlies critical hepatic functions, including xenobiotic biotransformation[11-13] and plasma protein synthesis.[14]

Gap junctions are composed of 2 hemichannels of neighboring cells, which in turn are composed of 6 connexin (Cx) proteins. Today, more than 20 different Cx species have been identified, which all are named after their molecular weight.[15] Cxs share a structure consisting of 4 membrane-spanning domains, 2 extracellular loops, a cytoplasmic loop, a cytosolic *N*-terminal area, and a *C*-terminal region.[2,3] Cxs are expressed in a tissue-specific way. Thus, gastric tissue produces Cx26, Cx32, and Cx43.[4,8,9,16-20] At least 10 different Cx variants have been characterized in the intestinal system, namely Cx26, Cx31, Cx32, Cx36, Cx37, Cx40, Cx43, Cx45, and Cx57 in the small

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intestine,[6,18,20-23] and Cx26, Cx31, Cx31.1, Cx32, Cx36, Cx40, Cx43, and Cx45 in the colon.[20,24-32] As much as 5 Cx family members are detectable in liver, among which Cx26 and Cx32 are predominantly expressed by hepatocytes, whereas nonparenchymal liver cells harbor Cx37, Cx40, and Cx43[19,33-35] (Fig 1).

In the last decade, it has been well documented that Cx hemichannels, in addition to acting as structural precursors of gap junctions, also provide a pathway for communication, albeit between the cytosol and extracellular environment.[36,37] The messengers that permeate Cx hemichannels and pannexin (Panx) channels show great overlap with those involved in GJIC. Nevertheless, although some physiological roles have been attributed to Cx hemichannels, in particular in the intestine,[28] they primarily become active during disease. Furthermore, a novel class of Cx-like proteins was discovered in 2000, the Panxs, which gather in a configuration reminiscent of Cx hemichannels and also facilitate extracellular communication.[38] Only 3 Panxs have yet been identified. Of those, Panx1 and Panx2 are expressed in gastric tissue,[39,40] the intestine,[40,41] and the liver[40,42-47] (Fig 1). Recently, it has become clear that Panx channels, like Cx hemichannels, are also essentially involved in pathologic processes.[41,48-52] In this article, the role of Cxs, Panxs, and their channels in gastrointestinal (Table I) and liver (Table II) diseases is discussed.

CX AND PANX CHANNELS IN GASTROINTESTINAL AND HEPATIC PATHOLOGY

Involvement of Cx signaling in gastric disease. ***Gastritis and peptic ulcer disease.*** Loss of GJIC has been associated with gastric ulcer formation. Electron microscopic studies of human gastric ulcers indeed showed a marked reduction in gap junction numbers. In areas of intestinal metaplasia, gap junctions have been occasionally seen between absorptive cells of the villi, but not in the lateral membranes of goblet cells.[53] On the border of human gastric ulcers, Cx32 spots in the surface mucous cells are significantly fewer than in the surface mucous cells of the body and the antrum distant from the ulcer area. Most foveolar cells adjacent to gastric erosions display decreased or even absent Cx32 staining.[54] Gastric expression of Cx32 is also reduced in experimentally induced atrophic gastritis in rat.[55,56]

Infectious gastric disease. *Helicobacter pylori* colonizes the human stomach and confers an increased risk for the development of peptic ulceration, gastric adenocarcinoma, and lymphoma. Among the various virulence factors secreted by *H. pylori* is cytotoxin-associated gene A (CagA), which is associated with gastric cancer.[57] Indeed, CagA-positive *H. pylori*, especially the East Asian type, and CagA-negative strains abolish GJIC in cultured human gastric epithelial cells, which is accompanied by the inhibition of cell proliferation.[58,59] On administration of water extracts of CagA-positive *H. pylori* to rats, in which gastric ulcers were induced by acetic acid, healing and reappearance of Cx32 protein expression in gastric mucosa are significantly delayed.[60] CagA-positive *H. pylori* also downregulates Cx43 production in cultured human gastric carcinoma cells.[61] Likewise, in precancerous gastric lesions of patients with *H. pylori* infection, especially with the CagA-positive variant, Cx32 and Cx43 levels are reduced compared with noninfected patients.[62-64] This is paralleled by hypermethylation of their corresponding gene promoters.[65] Eradication of *H. pylori* usually results in the restoration of Cx expression in human gastric cells both *in vitro*[61] and *in vivo*.[62] Another toxin produced by *H. pylori* is vacuolating toxin A (VacA), which can cause multiple alterations in gastric epithelial cells, including cell death. In fact, it has been reported that Cx43 is a host cell constituent that contributes to VacA-induced cell death. Furthermore, variation among cell types in the susceptibility to VacA-induced cell death is attributable, at least in part, to cell type–specific differences in Cx43 production.[66]

Miscellaneous gastric disease. Gastroparesis or delayed gastric emptying is a condition frequently seen in people with *diabetes mellitus*. Streptozotocin-induced *diabetes mellitus* in rats causes functional impairment of neuromuscular transmission, reduces the maximum activity of the electrogenic pump, increases the sensitivity of muscarinic receptors, negatively affects the sensitivity of adrenoceptors, and decreases the myogenic activity in gastric smooth muscles.[67] This has been linked to a reduced amount of gap junctions in interstitial cells of Cajal in the antrum.[68] The number of gap junctions of muscle cells and interstitial cells of Cajal is also decreased in infantile hypertrophic pyloric stenosis, a functional gastric outlet obstruction as a result of hypertrophy and hyperplasia of the muscular layers of the pylorus.[69] In addition, Cx43 protein production declines in spontaneous neonatal gastric perforation.[70]

Gastric cancer. Cx26 becomes located in the cytoplasm in human gastric carcinoma and is associated with a biologically less aggressive phenotype and pathologic early stage of gastric carcinoma. For this reason, Cx26 has been proposed to act as a gastric tumor suppressor.[16] In human and murine gastric tumors, Cx32 protein is strongly downregulated and is located in the cytosol[71,72] or may even be absent.[17,54,56] Overexpression of Cx32 in cultured human gastric cancer cells inhibits cell proliferation by upregulating the cell cycle inhibitors $p21^{Cip1}$ and $p27^{Kip1}$. This