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Control of Cholangiocyte Adaptive Responses by Visceral Hormones and Neuropeptides

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Abstract Cholangiocytes, the epithelial cells lining the biliary tree, are the target cells in several liver diseases, termed cholangiopathies. Cholangiopathies are a challenge for clinicians and an enigma for scientists, as the pathogenetic mechanisms by which they develop, and the therapeutic tools for these diseases are still undefined. Several studies demonstrate that many visceral hormones, neuropeptides, and neurotransmitters modulate the adaptive changes of cholangiocytes to chronic cholestatic injury. The aim of this review is to present the recent findings that contributed to clarify the role of visceral hormones and neuropeptides in the regulation of the pathophysiology of cholestasis. These studies helped to shed light on some aspects of cholangiocyte pathophysiology, revealing novel perspectives for the clinical managements of cholangiopathies.

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Introduction

Cholangiocytes are the epithelial cells that line the biliary tree. The physiologic role of cholangiocytes is to modify the bile of canalicular origin, before it reaches duodenum, by a wide array of absorptive and secretory processes [1, 2].

There is increasing interest in gaining knowledge on cholangiocyte pathobiology and mechanisms of response to injury. Such an interest is, in a certain sense, driven by clinical issues: cholangiocytes are the target of chronic diseases, termed cholangiopathies [3, 4], that represent a daily challenge for clinicians, as definitive medical treatments are not available yet. As a consequence, the 20% of liver transplants among adults and 50% pediatric patients are because of these disorders [5]. Despite the different etiology, cholangiopathies are commonly characterized by dysregulation of the balance between cell growth and survival [4]. In the course of such diseases, there is an impaired proliferative response to duct injury and increased cell death by apoptosis, which leads to vanishing of bile ducts [3, 4] and liver failure in the end stage.

Malignant transformation of cholangiocytes gives rise to cholangiocarcinoma, a very aggressive and fatal malignancy. Although there are some risk factors (among which cholangiopathies are reported), it is common experience that the vast majority of patients affected by biliary malignancies do not show any of those conditions. These factors make cholangiocarcinoma a great challenge for clinicians as far as surveillance, management, and therapy are concerned [6].

It is generally accepted that the progression of injury in the course of cholangiopathies and promotion and progres-

sion of cholangiocarcinoma are at least in part because of the failure of the mechanisms of adaptation of cholangiocytes to the injury of the biliary tree. Many of those mechanisms still remain enigmatic [4, 6, 7].

The research on this field shed, in most recent years, some light on these aspects, by identifying those factors that contribute to the regulation of cholangiocyte ability to respond to injury.

Bile acids were the endogenous factors that have been historically studied more in this regard, and their properties in modulating cholangiocyte biology are now unanimously accepted [8–12]. More recently, several studies showed that cholangiocyte biology is also under the regulation of visceral hormones, neuropeptides, and neurotransmitters. Those data came to the point of depicting the biliary epithelium to be similar to the gastric or intestinal epithelia, for which the key role played by nerves and neuroendocrine hormones has been known for years [13]. In addition, it has been shown that the adaptive response to chronic cholestatic injury is associated to the acquisition, by cholangiocytes, of neuroendocrine phenotype, that is not specific of these cells in normal conditions [7, 14].

In this review, we discuss the evidence from studies published in recent years that have unveiled the role of visceral hormones and neuropeptides in the regulation of cholangiocyte response to liver injury and their potential clinical relevance.

Physiologic role of cholangiocytes and their adaptive responses to chronic cholestatic injury

Physiology

The physiologic role of cholangiocytes is to modify the bile of canalicular origin through various secretory and absorptive processes [1]. The major regulator of cholangiocyte functional activity is the neuroendocrine hormone secretin [15, 16]. Secretin elicits ductal secretion [1, 15, 17] by selective interaction with secretin receptors, expressed only by cholangiocytes in rat liver [18]. The interaction of secretin with its own receptors [18] leads to an increase in intracellular cyclic adenosine monophosphate (cAMP) levels [1, 17, 19–21], activation of protein kinase A (PKA) [22], opening of cystic fibrosis transmembrane regulator Cl^- channels [23] with activation of the Cl^-/HCO_3^- exchanger [1, 17, 22, 24], with the consequent secretion of bicarbonate into bile [16]. Cholangiocytes express several other transporters both at the apical and basolateral pole that allow vectorial water movement across the biliary epithelium (as reviewed by Lazaridis et al. [4]). Noteworthy is the presence of apical (apical sodium-dependent bile acid transporter [ASBT]) and basolateral (t-ASBT, multidrug resistance protein 3, organic solute transporter) transporters for bile acids [25]. This is considered the molecular substrate for the ability of cholangiocytes to absorb bile acids from bile and excrete them into the outflowing branches of the peribiliary plexus. Such a process has been termed as "chole-hepatic shunt" and makes an aliquot of bile acids secreted by hepatocytes in bile to rapidly flow back toward hepatocytes themselves [25]. Physiologically, cholangiocyte activity is quite relevant: Although in normal conditions, they represent approximately 4–5% of the liver mass, they contribute to about the 10–30% of daily bile production in humans [1, 4].

Proliferative and functional response

The adaptive changes of cholangiocyte biology in response to chronic injury have been elucidated mostly by interpreting what was observed in animal models. The most employed experimental model is the bile duct ligation (BDL) rodent model. Born as an experimental set for liver fibrogenesis [26], the BDL model has been, and still is, chosen whenever one of the main features of cholangiocyte reaction to injury needs to be studied, e.g., proliferation. Normally, cholangiocytes are mitotically dormant (Go of the cell cycle) [1, 27]. The peculiar capacity of cholangiocytes to proliferate is evidenced in specific experimental conditions as well as in many different human pathological conditions [1, 7, 28]. In most of these conditions, cholangiocyte proliferation is part of the so-called "ductular reaction," a term coined by Popper [29] to identify the expanded population of epithelial cells at the interface of the biliary tree and the hepatocytes and which refers to proliferation of preexisting ductules, progenitor cell activation, and appearance of intermediate hepatocytes. Recently, the term "ductular proliferation" [30] has been suggested instead of "ductular reaction," but also, this term is not completely satisfactory, as the proliferating reactive ductules may not simply arise from proliferation of preexisting bile ductular cells, as they may also originate from activated and differentiated progenitor cells [7]. Besides the different nomenclatures, cholangiocyte proliferation, as main component of "ductular reaction," has been classified in four types; they can be recreated by different experimental models (BDL, partial hepatectomy, chronic α-naphthylisothiocyanate feeding, chronic L-proline treatment, prolonged oral administration of lithocholate, chenodeoxycholate, and taurocholate) or observed in human diseases [7, 28]. Cholangiocyte proliferation, which is coupled with enhanced functional activity, is theleologically considered a compensatory mechanism: it is an attempt to restore damaged ducts and/or to increase the intrahepatic mass of bile ducts able to host retained bile in case of obstruction.

Survival

Cholangiopathies progress because of an increasing loss of bile ducts [4] leading to vanishing of bile ducts, a phenomenon that is present virtually in all those diseases [4]. Programmed cell death by apoptosis occurs, as in many cell types, in cholangiocytes as well [31, 32]. It is currently thought that, under normal homeostasis, senescent or damaged cholangiocytes undergo apoptosis and are replaced by the proliferation of new cells, in a dynamic equilibrium [4]. Under this light, ductopenia can be considered the result of enhanced cell death that prevails over compensatory cholangiocyte proliferation [4].

The study of cholangiocyte apoptosis in humans and animal models is challenging given the transitory events involved. Nevertheless, when apoptosis is achieved, in the BDL model, by carbon-tetrachloride or 6-hydroxydopamine (6-OHDA) intoxication, vagotomy or biliary-jejunal anastomosis, a severe reduction of the previously expanded bile duct mass is observed [33–36]. Those data thus support the concept of the significance of the altered balance between cholangiocyte proliferation and death for the progression toward ductopenia.

Cell-to-cell interactions

Another major feature of cholangiocyte adaptive response to chronic injury is the interaction with other liver cells, such as inflammatory cells and hepatic stellate cells (HSC). The latter are those responsible for the deposition of extracellular matrix, thus for liver fibrosis development and progression [26]. Together with the vanishing of bile ducts, liver fibrosis is a paradigmatic feature of cholangiopathies [4]. The cross talk between cholangiocytes and HSC yet remains unclear. It is generally accepted that cholangiocyte proliferation is "the pace-maker of portal fibrosis," as in the assumption of Valeer Desmet [37]. Conceptually, it is believed that portal fibrosis follows proliferating bile ducts to support newly formed ducts and newly formed branches of the vascular peribiliary plexus (PBP) [38, 39]. The hypothesis that most of the authors support is the one that indicates proliferating cholangiocytes as the source of molecules able to activate HSC (and/or portal myofibroblasts) [26, 39, 40].

However, it is known that, in cholangiopathies (like in primary biliary cirrhosis [PBC], for example), liver fibrosis progresses in late stages, when apoptosis and ductopenia prevail over cell proliferation [4, 38].

In light of what is said above, however, it becomes unreliable to ascribe the development of liver fibrosis in the course of ductopenic syndromes "only" to cholangiocyte proliferation, especially in late stages. In this regard, there are evidences that support the hypothesis that inflammation, typical of these disorders, plays a major role in sustaining the fibrogenic process [26, 40, 41].

At this time, it is also possible to hypothesize that cell death by apoptosis may significantly contribute to the development of the fibrogenic process in the course of ductopenic syndromes. In particular, it has been demonstrated that apoptosis activates liver fibrogenesis, by different means, e.g., by the local release of soluble, profibrogenic factors and by the HSC activation that results by their ability to engulf apoptotic bodies [40, 42, 43].

Neuropeptides and neurotransmitters affecting cholangiocyte adaptive changes to cholestatic injury

The liver is innervated by both sympathetic and parasympathetic nerves, whose fibers are located around the hepatic artery, portal vein, intrahepatic and extrahepatic bile ducts [44, 45]. Sympathetic nerves originate from the celiac ganglion, whereas the parasympathetic from the vagus nerve [44, 45]. Besides catecholamines and acetylcholine (ACh), autonomic fibers that innervate the liver can also release other neurotransmitters, like neuropeptide Y [46, 47], calcitonin gene-related peptide (CGRP), somatostatin, vasoactive intestinal polypeptide (VIP), enkephalin, and bombesin [48–51]. Nervous terminations mostly follow the vascular structures of the portal tract and have been identified around bile ducts and vessels. Many of the above mentioned neurotransmitters have been shown to modulate intrahepatic hemodynamic [52–54].

Cholinergic neurotransmitters

A number of studies showed that cholinergic nerves regulate bile secretion [55, 56]. In bile-fistula dogs with interrupted enterohepatic circulation, distal stimulation of the vagus nerve increases bile bicarbonate secretion, whereas vagotomy decreases basal bile flow and bicarbonate output [55, 56].

In early studies [57], it seemed that cholangiocytes might express both the M1 and the M3 ACh receptor subtypes and that the cholinergic agonist, carbachol, stimulates bile flow of the isolated perfused rat liver. These observations were not confirmed by successive studies. In 1996, Nathanson et al. [58] demonstrated that, in accordance with the concept that cholangiocytes, but not hepatocytes, express ACh receptors, ACh did not affect the functions of hepatocytes, but elicited Ca^{2+} increase and oscillation in isolated bile duct units (IBDUs), because of both an influx of extracellular Ca^{2+} and the mobilization of intracellular Ca^{2+} stores. Subsequently, it was shown that cholangiocytes express, at the basolateral domain, M3 (but not M1 and M2) ACh receptors [24]. In that study, it was shown

that ACh has no effect on the basal activity of the Cl^-/HCO_3^- exchanger, but it significantly potentiates the stimulatory effect of secretin on this exchanger [24]. It can be postulated that the role of ACh on cholangiocyte functional activity aims to enrich bile of bicarbonate during the digestive phase, when, indeed, the parasympathetic system activity is high. By selectively interacting with M3 receptor subtypes, ACh elicits the secretin-induced adenylyl cyclase activity, which leads to activation of the Cl^-/HCO_3^- exchanger with bicarbonate secretion into bile [24]. Recent studies indicate that ACh plays a significant permissive role in sustaining cholangiocyte reaction to cholestasis also as far as survival is concerned (Fig. 1). Interruption of the cholinergic innervation by vagotomy induces a marked decrease in total bile duct mass caused by both impaired cholangiocyte proliferative capacity and intracellular cAMP levels and enhanced cell death by apoptosis [33, 59]. These studies also show that maintenance of intracellular cAMP levels (by chronic administration of forskolin) prevents the effects of vagotomy on cholangiocyte apoptosis, proliferation, and secretion [33].

Adrenergic and dopaminergic neurotransmitters

Rather than cholinergic innervation per se, it is the visceral innervation as a whole that seems to be required to allow the adaptive reaction of cholangiocytes to cholestasis. Intact sympathetic nervous fibers are required for hepatocyte and cholangiocyte proliferation after partial hepatectomy [60]. In fact, recent evidences indicate that cholangiocytes from BDL rats express alpha-1, alpha-2, beta-1, and beta-2 adrenergic receptors. If activation of alpha-1 adrenergic receptor increases secretin-stimulated ductal secretion [61], activation of alpha-2 adrenergic receptor has an inhibitory effect on ductal secretion in the course of cholestasis [62]. Similar to what is shown in the gut, adrenergic innervation may play a role in counterbalancing the stimulatory effects of cholinergic nerves [33] on ductal bile secretion in chronic cholestatic liver diseases. In addition, it is now known that adrenergic innervation is required for growth and survival of cholangiocytes in reaction to cholestasis as well (Fig. 1). Administration of a single intraportal injection of 6-OHDA, which induces degeneration of dopaminergic terminal fibers [63, 64], blunts the cholangiocyte functional and proliferative responses to cholestasis, and induces cell death by apoptosis [34]. Chronic administration of clenbuterol (a beta-2 adrenergic agonist) [65] and dobutamine (a beta-1 adrenergic agonist) [66] prevents the decrease in cAMP levels and secretion induced by 6-OHDA, maintains cholangiocyte proliferation, and decreases cholangiocyte apoptosis because of 6-OHDA [34]. Furthermore, it has been shown that cholangiocytes express the D2 (but not the D1 and D3) dopaminergic receptors and that the D2 dopaminergic agonist, quinelorane, inhibits secretin-induced ductal secretion in BDL rats through activation of the Ca^{2+}-dependent protein kinase C (PKC) gamma (but not PKC alpha, beta I, and II) and inhibition of secretin-stimulated cAMP levels and PKA activity [67] (Fig. 1).

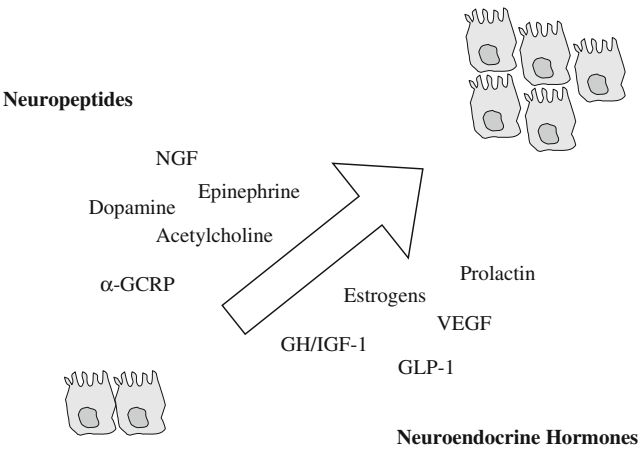

Fig. 1 Neuropeptides (*top left*) and neuroendocrine hormones (*bottom right*) enhancing cholangiocyte proliferative response to cholestatic injury

NGF and sensory innervation

The relationship between the biliary epithelium and nerves appears to be closer than what was thought at the beginning. Cholangiocytes express the receptor for nerve growth factor (NGF), the activation of which strongly promotes biliary cell growth [68] (Fig. 1). Moreover, it has been found that cholangiocytes themselves can produce and secrete NGF and that, when this neurotrophin is immunoneutralized, the growth of the biliary tree in the BDL rat is strongly diminished [68].

There is evidence that also the sensory innervation may play a significant role in the regulation of cholangiocyte adaptive response to cholestasis. The sensory neuropeptide α-CGRP increases cholangiocyte proliferation in vitro [71]. Most interestingly, a marked reduction of bile duct mass is observed both 3 and 7 days after BDL in CGRP receptor knockout mice [71] (Fig. 1).

Altogether, these data suggest that the innervation plays a substantial role in the regulation of cholangiocyte biology, thus enlightening the need of studying whether the liver denervation after transplantation might affect the functions of the grafted biliary tree. Of major interest would also be to investigate whether other endogenous factors, instead of nerves, can support cholangiocyte functions in the denervated organ. In this view, some evidences suggest that bile acids could be important. It has been found that administration of taurocholic [59] or ursodeoxycholic acid [69] counteracts the loss of bile ducts induced by cholinergic denervation in the BDL rat. Similarly, taurocholic acid administration also prevents the loss of bile ducts induced by adrenergic denervation [70].

Visceral hormones affecting cholangiocyte adaptive changes to cholestatic injury

Neuroendocrine hormones are secreted by neuroendocrine cells that are diffused in the whole gastrointestinal tract, in particular in the stomach, in the small bowel [72], as well as in the pancreas [73]. If these cells are considered the main source of the neuroendocrine hormones further on in this review, we will describe the results of some studies suggesting that cholangiocytes themselves can synthesize some of these peptides in the course of cholestasis.

Secretin

The close link between cholangiocytes and neuroendocrine hormones has its basis on the early studies that showed how the hormone secretin is the most potent regulator of cholangiocyte functional activity [15, 16]. In 1988, Alpini et al. [16] demonstrated that, in a rat model of cholestasis (induced by BDL), the infusion of secretin was associated with a marked increase of the bile flow and bicarbonate biliary excretion. After that "landmark" manuscript, a wide series of following investigations defined the intracellular mechanisms by which secretin induce such a potent functional stimulus. This challenge represented, for several years, the main aim of this field of research. It is now known that secretin, as mentioned above, is the main regulator of cholangiocyte functional activity in normal state and cholestasis [1, 15–17].

Somatostatin

Other neuroendocrine hormones were then discovered to affect cholangiocyte functional activity. One of the first molecules studied was somatostatin. It was demonstrated that cholangiocytes express the $SSTR_2$ receptor; the interaction of somatostatin with this receptor markedly diminished the effect of secretin on the biliary excretion of water and bicarbonate by cholangiocytes in cholestatic conditions [74]. Such an effect of somatostatin was because of the fact that the activation of the $SSTR_2$ receptor prevented the increase of the adenylyl cyclase elicited by secretin [74]. In later studies conduced in IBDUs isolated from wild-type and $SSTR_2$-knockout mice, it was also found that somatostatin not only reduces cholangiocyte choleresis, but it also stimulates ductal bile absorption [75].

Insulin and ET-1

Cholangiocytes express at the apical pole the receptor for insulin [76]. Upon its activation, a marked reduction of the secretin-induced choleresis was observed, both if the hormone was administered in vivo to BDL rats and if microinjected into the lumen of IBDUs isolated from animals with cholestasis. It was observed that the activation of the insulin receptor determined a cascade of intracellular events that resulted in the inhibition of secretin-stimulated cAMP and PKA activity. Such a chain of events seemed to have its core event in the enhancement of the intracellular Ca^{2+} levels and the consequent activation of the Ca^{2+}-dependent PKCα [76]. An effect similar to the one of insulin was described for endothelin-1 (ET-1), which by interacting with the specific receptors expressed in the biliary epithelium (ET_A and ET_B), blunts the secretin-induced choleresis of the BDL rat and also reduces the expression of the secretin receptor on cholangiocytes [77].

VIP and bombersin

In contrast to somatostatin and insulin, VIP and bombesin were found to be able to enhance cholangiocyte choleresis. Both the hormones induced a potent fluid and bicarbonate excretion in IBDUs, but not in hepatocytes, isolated from normal and cholestatic rats. Interestingly, neither VIP nor bombesin had any significant effect on modulating intracellular cAMP levels [78–80]. Detailed pH studies indicated that the underlying intracellular mechanism is, at least for bombesin, its ability to stimulate the activity of Cl^-/HCO_3^- exchanger in association with a counterbalancing secondary activation of electrogenic Na^+/HCO_3^- symport [80].

Gastrin and thyroid hormone

In more recent studies, increasing evidences regarding the ability of neuroendocrine hormones to affect also cholangiocyte growth and survival, and not only their functional activity, were reported.

If the infusion of gastrin to BDL rats is associated with the reduction of the choleretic response to secretin by cholangiocytes [21], its chronic administration through an intraperitoneal mini-pump resulted not only in reduced functional activity but also in a marked decrease of the bile duct mass [81] (Fig. 2). Cholangiocytes indeed express the CCK-B/gastrin receptors [81], which upon activation, elicit intracellular Ca^{2+} release, increase of IP_3 levels, membrane translocation (e.g., activation) of the Ca^{2+}-dependent PKCα [81]. In turn, the gastrin-activated PKCα, as abovementioned, is able to interfere with the secretin signaling and modulate the adenylyl-cyclase activity, thus reducing the intracellular cAMP levels and PKA activity. These observations, together with others that demonstrated that bile acids also activate this intracellular pathway to modulate cholangiocyte growth [8], contributed to elucidate as to which intracellular signaling cascades sustain the proliferative response of cholangiocytes to cholestasis and by which mechanisms it is possible to modulate them.

Moreover, as cAMP/PKA resulted to be the key molecules implicated in cholangiocyte proliferation, this helped to explain the association between increase of duct mass and enhancement of biliary choleresis [8, 16].

Similarly to gastrin, somatostatin inhibits the proliferation of large cholangiocytes in BDL rats by selectively interacting with $SSTR_2$ receptors and decreasing intracellular cAMP levels [20]. The same sequence of intracellular events follows histamine receptor activation; the consequence of which is reduced cell proliferation in response to cholestasis [82]. Inhibition of cell growth is also observed after exposure to high concentrations of thyroid hormone, even if this occurs via a direct cross talk between the Ca^{2+} and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways [83] (Fig. 2).

Estrogens

A major contribution to this field of research has been given by the studies that clarified the effects of estrogens on the biliary epithelium that expresses both the estrogen receptors (ERs) α and β, with their expression being upregulated after BDL [84] (Fig. 1). When cholangiocytes were stimulated in vitro with 17-β-estradiol, their proliferation was markedly increased, as a consequence of the ER-dependent activation of Src/Shc/ERK1/2 intracellular pathway [85]. As a demonstration of the physiological and pathophysiological relevance of estrogens as far as cholangiocyte proliferative response to cholestasis, when BDL male rats were treated in vivo with anti-estrogens like tamoxifen or Ici 182,780 [86] or when BDL female rats were subjected to ovariectomy [84], the growth of the biliary tree was blunted, and the biliary epithelium underwent programmed cell death by apoptosis [84, 86]. This evidence suggested that estrogens are required for a correct response of the biliary tree to injury. These studies produced in the very last few years by Alvaro et al. [38] represent a significant change of perspective in the knowledge of the role played by the neuroendocrine system on the biliary cell biology. Indeed, PBC, the most common of the cholangiopathies [4], is much more frequent in women than in men and has its clinical outcome typically after menopause, when the endogenous estrogen levels suddenly drop [38]. Therefore, these observations seem to provide the biological confirmation of the role of estrogens in the progression of PBC, a role that was, before, only hypothesized on the basis of epidemiological data. To further support this concept, it has been also demonstrated that the ER expression in cholangiocytes is markedly reduced in late stages of PBC [38]. Altogether, these studies made clear that gaining knowledge on the role of neuroendocrine hormones on cholangiocyte biology might also be an effective strategy to further understand the pathophysiology of the cholangiopathies and thus eventually to design novel therapeutic strategies.

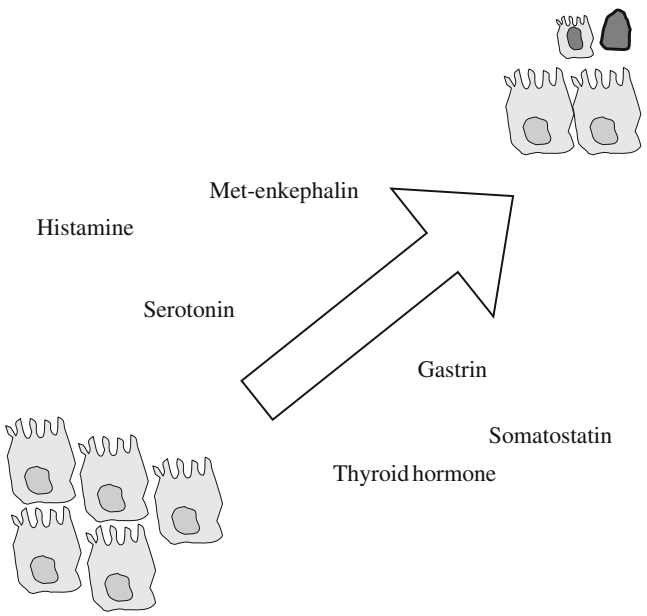

Fig. 2 Neuropeptides and neuroendocrine hormones inhibiting cholangiocyte proliferative response and/or inducing cell death by apoptosis

Prolactin

Besides estrogens, other sexual hormones have been considered to be involved in the regulation of cholangiocyte biology. Normal cholangiocytes express both isoforms (long and short) of prolactin receptors, whose expression increases after BDL. The administration of prolactin to normal female rats increased cholangiocyte proliferation. In purified normal female cholangiocytes, prolactin stimulated cholangiocyte proliferation, which is associated with increased intracellular Ca^{2+} levels and $PKC_{\beta I}$ phosphorylation. Administration of an anti-prolactin antibody to BDL female rats decreased cholangiocyte proliferative response to cholestasis [87] (Fig. 1).

Serotonin

The neuroendocrine hormone serotonin has been hypothesized to be involved in the genesis of certain clinical features of PBC, like fatigue and pruritus [88, 89]. Interestingly, it has been recently shown that cholangiocytes express the serotonin 1A and 1B receptors [90]. When they are activated by selective agonists, the proliferation of BDL cholangiocytes is dramatically reduced (Fig. 2), both in vivo and in vitro [90], in association with the loss of the response to secretin of the typical markers of cholangiocyte functional activity [90], like bile flow, bicarbonate excretion, and intracellular cAMP synthesis [15]. Such an effect seems to be mediated by the cross talk between the $Ca^{2+}$and

the cAMP/PKA signalings. If the serotonin 1A and 1B receptors are activated, there is an increase of Ca^{2+} and IP_3 levels and PKCα activity, with the consequent reduction of intracellular cAMP levels and PKA activity. Most interestingly, it was observed that hyperplastic cholangiocytes isolated from BDL rats are able to synthesize and secrete serotonin [90]. If, in vitro or in vivo, cholangiocyte-secreted serotonin is neutralized, cholangiocyte proliferation in response to cholestasis further increases [90].

Endogenous opioid peptides

Pruritus of chronic cholestatic disorders is thought to be elicited, at least in part, to the central action of endogenous opioid peptides [88, 91, 92]. In the course of cholestasis opioidergic neurotransmission and opioid peptide plasma and hepatic levels are markedly increased [92–96]. Interestingly, it has been also demonstrated that, in the course of experimental cholestasis, the mRNA for preproenkephalin, which codes for Met- and Leu-enkephalins and Met-enkephalin-containing peptides, is expressed in the liver. In addition, Met-enkephalin immunoreactivity is detected in the liver, particularly in cholangiocytes, of patients with PBC [93] and of rats with cholestasis [97]. The reason why liver cells, cholangiocytes in particular, should synthesize endogenous opioid peptides has been enigmatic for long. Recently, we got to some evidences that, at least in part, may answer that question. Cholangiocytes express the three classic opioid receptors (δ, μ, and κ OR); in the course of chronic cholestasis, the functional one is the δOR [98]. The autocrine–paracrine interaction of ligands with that receptor results in a marked inhibition of cholangiocyte proliferation, both in vivo and in vitro. δOR activation, indeed, elicits the IP_3 (D-myo-inositol 1,4,5-triphosphate)-dependent Ca^{2+} signaling (CamKIIα, PKCα) in cholangiocytes, which antagonizes pro-proliferative pathways like cAMP/PKA, PI3K, and ERK1/2 [98]. When BDL rats are treated with a general opioid receptor antagonist such as naloxone, the proliferative and functional response of cholangiocytes to cholestasis is further increased [98] (Fig. 2).

Altogether, these data suggest that serotonin and endogenous opioid peptides form an autocrine loop of peptides secreted by cholangiocytes to limit their adaptive outgrowth and functional activity in reaction to cholestasis.

GH/IGF-1 axis and VEGF

Recent evidences demonstrated that a "stimulatory" autocrine/paracrine loop may exist. It has been found that cholangiocytes are the target of the growth hormone (GH)/insulin-like growth factor-1 (IGF-1) axis (Fig. 1) [99]: GH induces IGF-1 expression and releases in isolated cholangiocytes, with the consequent stimulation of cell growth by IGF-1 [99]. Proliferating cholangiocytes from BDL rats express and secrete vascular endothelial growth factor (VEGF)-A and VEGF-C [100]. By interacting with specific receptors (VEGFR-2 and VEGFR-3), they stimulate cholangiocyte proliferation, both directly and playing synergistic effects with estrogens and IGF-1 on modulation of cholangiocyte proliferation with convergence on common signaling pathways, including the ERK system [7, 100]. However, when VEGF is blocked by specific neutralizing antibodies, no proliferation of PBP occurs in spite of BDL, suggesting that VEGF secreted by cholangiocytes drives the proliferative adaptive response of PBP to cholestasis [101] (Fig. 1).

Glucagon-like peptide-1

An additional, recently discovered member of the stimulatory autocrine loop for cholangiocyte reaction to cholestasis is glucagon-like peptide-1 (GLP-1; Fig. 1). GLP-1 is secreted by enteroendocrine L cells and modulates the biology of a number of cells, by interacting with a specific G-protein coupled receptor (GLP-1R) [102]. GLP-1 is known to modulate glucose homeostasis; specifically, it has been demonstrated that GLP-1 prevents pancreatic β-cell death by apoptosis and sustains their proliferation [102]. We observed that GLP-1 strongly stimulates cholangiocyte growth, by interacting with a specific receptor (GLP-1R) present on cell surface [103]. Moreover, when cholangiocytes proliferate, they synthesize and release GLP-1 that then acts in the microenvironment. GLP-1 becomes thus required for cell adaptive response to cholestasis: if a GLP-1R antagonist is administered to BDL rats, a marked reduction of bile duct mass and choleresis is observed [103]. These data gain further interest if it considered that, among its many biological properties, GLP-1 was found able to induce pancreatic ductal cells to acquire a neuroendocrine phenotype [104, 105]; pancreatic ductal cells share features in common with cholangiocytes, in terms of embryologic origin, morphology, functional activity, and response to injury [106, 107]. As mentioned above, cholangiocytes themselves acquire a neuroendocrine phenotype in the course of cholestasis: what it is driven by, however, is still unclear. These recent findings indicate GLP-1 as a likely candidate.

Pathobiological implications

The studies of most recent years indicate that the biliary tree is not only the target of hormones and neuropeptides. Its adaptive changes in reaction to injury is characterized by the ability of cholangiocytes to synthesize and secrete a wide array of peptides, that affect their own and other liver

cell biological adaptation to injury [7]. Rather than being just a target, proliferating cholangiocytes are to be considered a sort of "neuroendocrine compartment" of the liver, as proposed by Alvaro et al [7]. Those "active" properties of cholangiocytes also give a mechanistic explanation to the neuroendocrine transdifferentiation of these cells, already observed years ago [14].

The regulation of cholangiocyte adaptive response to cholestasis by visceral neuropeptides and hormones has several pathobiological implications, both in terms of progression of diseases and cholangiocarcinoma development. As mentioned above, the ability of these peptides to maintain cell survival could be a major determinant for disease progression and activation of the fibrogenetic process. Most interestingly, however, is the fact that several of those factors that participate in the autocrine loop of regulation of cholangiocyte biology are also able to activate HSC, thus to elicit the "wound-healing" process. It is known, indeed, that the cholangiocyte secreted platelet-derived growth factor, transforming growth factor-β1, ET-1, VEGF, serotonin, met-enkephalin, IGF-1, and NGF are able to elicit HSC fibrogenetic activity [40].

The knowledge gained on the regulation of cholangiocyte biology has further implications: many of the neuropeptides or hormones that affect cholangiocyte adaptive response to cholestasis have been found also able to modulate cholangiocarcinoma growth [108]. This suggests the possibility that cholangiocarcinoma development may be associated with the failure of the complex interplay of peptides that govern the proper response of cholangiocyte to long-standing injury.

Clinical significance and perspectives

Does the knowledge on the neuropeptides/hormonal regulation of adaptive response of cholangiocytes have potential clinical implications? Probably it does. Compounds able to stimulate/antagonize the action of several factors involved in the regulation of cholangiocyte biology, such as VEGF, serotonin, opioid peptides, estrogens, and somatostatin, are already available in clinical practice. It would be worth to consider, thus, the possibility of employing them in clinical sets to verify if they do play a significant role in modulating the progression of cholangiopathies. Probably, a particular attention should be paid to those molecules specifically designed to act on the balance between cell proliferation and death. In this regard, the future may be not too far: GLP-1 prevents pancreatic β-cell death by apoptosis and sustains their proliferation [102]. As a consequence, the GLP-1R selective agonist exendin-4 is now employed as a novel anti-diabetic therapy in humans [109].

If not for therapeutic purposes, the knowledge on the neuron/endocrine regulation of cholangiocyte adaptive reaction to injury may reveal an immediate significance at least in diagnosis/surveillance of cholangiopathies and cholangiocarcinoma. We have recently demonstrated that biliary IGF-1 levels are an accurate marker in the determination of biliary obstruction, being increased only in cholangiocarcinoma but not in other causes of biliary obstruction [110]. Dosage of biliary IGF-1, thus, is a reliable candidate to be entered in clinical practice for diagnosis of cholangiocarcinoma or surveillance of conditions of risk, like some of the cholangiopathies.

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