Knockout of histidine decarboxylase decreases bile duct ligation-induced biliary hyperplasia via downregulation of the histidine decarboxylase/VEGF axis through PKA-ERK1/2 signaling

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Hodges K, Ueno Y, Nguyen Q, Greene JF, Francis H. Knockout of histidine decarboxylase decreases bile duct ligation-induced biliary proliferation and protein expression of PKA/ERK1/2 compared with BDL WT mice. Histamine decreases bile duct ligation-induced biliary proliferation via downregulation of the histidine decarboxylase/VEGF axis through PKA-ERK1/2 signaling. Am J Physiol Gastrointest Liver Physiol 307: G813–G823, 2014. First published August 28, 2014; doi:10.1152/ajpgi.00188.2014.—Histidine is converted to histamine by histidine decarboxylase (HDC). We have shown that cholangiocytes (1) express HDC, 2 secrete histamine, and 3 proliferate after histamine treatment via ERK1/2 signaling. In bile duct-ligated (BDL) rodents, there is enhanced biliary hyperplasia, HDC expression, and histamine secretion. This study aimed to demonstrate that knockdown of HDC inhibits biliary proliferation via downregulation of PKA/ERK1/2 signaling. HDC−/− mice and matching wild-type (WT) mice were subjected to sham or BDL. After 1 wk, serum, liver blocks, and cholangiocytes were collected. Immunohistochemistry was performed for 1) hematoxylin and eosin, 2) intrahepatic bile duct mass (IBDM) by cytokeratin-19, and 3) HDC biliary expression. We measured serum and cholangiocyte histamine levels by enzyme immunoassay. In total liver or cholangiocytes, we studied: 1) HDC and VEGF/HIF-1α expression and 2) PCNA and PKA/ERK1/2 protein expression. In vitro, cholangiocytes were stably transfected with shRNA-HDC plasmids (or control). After transfection we evaluated pPKA, pERK1/2, and cholangiocyte proliferation by immunoblots and MTT assay. In BDL HDC−/− mice, there was decreased IBDM, PCNA, VEGF, and HDC expression compared with BDL WT mice. Histamine levels were decreased in BDL HDC−/−. BDL HDC−/− livers were void of necrosis and inflammation compared with BDL WT. PKA/ERK1/2 protein expression (increased in WT BDL) was lower in BDL HDC−/− cholangiocytes. In vitro, knockdown of HDC decreased proliferation and protein expression of PKA/ERK1/2 compared with control. In conclusion, loss of HDC decreases BDL-induced biliary mass and VEGF/HIF-1α expression via PKA/ERK1/2 signaling. Our data suggest that HDC is a key regulator of biliary proliferation.

cholangiocyte proliferation; histamine; HDC knockout; BDL

HISTAMINE, a vital biogenic amine, plays several key roles in mammalian tissues from neurotransmission in the central nervous system to embryogenesis (19, 34, 44, 48). We have observed that histamine also regulates biliary repair and regeneration in the liver (19). Histamine is formed from the one-step decarboxylation reaction of the amino acid histidine by L-histidine decarboxylase (HDC) and, following release, is either stored or degraded by histamine-N-methyltransferase and monoamine oxidase B (15, 21, 32). Histamine exerts its effects through interaction with one of four G protein-coupled receptors, H1–H4 histamine receptor (HR). These receptors are both stimulatory (H1 and H2 HR) and inhibitory (H3 and H4 HR) depending on the location and tissue (48).

Cholangiocytes, the epithelial cells that line bile ducts in the liver, are the target cells of cholangiopathies like primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC), as well as cholangiocarcinoma (19). Cholangiocytes do not typically undergo mitosis, except when proliferation occurs in cholangiopathies, and in animal models of cholestasis (16, 36). The bile duct ligation (BDL) model has been extensively used to mimic human cholestasis in the rodent (14, 18, 37). After BDL, large cholangiocytes (that line large bile ducts) proliferate via the cAMP/PKA/ERK1/2-signaling pathway. In fact, we have shown that stimulation with histamine increases large cholangiocyte proliferation via this pathway, whereas small cholangiocytes (that line small bile ducts) proliferate via a calcium-dependent pathway (16, 21). It has also been previously shown that, in BDL rats treated with an H3HR agonist, cholangiocyte proliferation decreases by the cAMP-dependent PKA/ERK1/2/ELK-1 pathway (16).

Cholangiocytes express HDC and HRs and secrete histamine (21, 40). After BDL, there is increased 1) HDC expression and histamine secretion (21, 40) and 2) VEGF expression and secretion (22, 23). We have recently demonstrated that short-term inhibition of HDC via the compound α-methyl-ηm-histidine decreases biliary proliferation and the expression of VEGF both in vivo and in vitro (40). The goal of this study was to evaluate the long-term effects of the depletion of HDC on biliary proliferation and VEGF expression with use of genetically modified HDC−/− mice.

METHODS

Materials

All reagents and kits were obtained from Sigma-Aldrich (St. Louis, MO) unless otherwise indicated. Reagents for the histamine enzyme immunoassay (EIA) were purchased from Cayman Chemical (Ann Arbor, MI). The commercially available kits for measuring VEGF-C levels were purchased from Abcam (Cambridge, MA). The antibodies used for immunohistochemistry and immunoblots were obtained from Santa Cruz Biotechnology (Santa Cruz, CA) unless specified otherwise. The commercially available kits for immunohistochemistry were purchased from Vector Laboratories (Burlingame, CA). All

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primers, reagents, and plasmids for shRNA transfection were purchased from SABiosciences (Fredrick, MD).

**In Vivo Models**

Homozygous HDC<sup>−/−</sup> mice were obtained from Dr. J. R. Goldenring, Vanderbilt University (42). HDC<sup>−/−</sup> mice and the corresponding wild-type (WT) mice were subjected to BDL (25) for up to 7 days or sham surgery. Animals were housed at Scott & White Hospital Animal Facility and given free access to drinking water and chow. All animals were kept in a temperature-controlled environment with a 12:12 light-dark cycle and all protocols were strictly adhered to as approved by the local Institutional Animal Care and Use Committee (IACUC). From these animals, we obtained serum, liver blocks (frozen and paraffin embedded), and cholangiocytes. Virtually pure

Fig. 1. Evaluation of liver morphology by hematoxylin and eosin staining. In sham wild-type (WT) and sham histidine decarboxylase (HDC)<sup>−/−</sup> mice, there was no evidence of necrosis, lobular damage, or inflammation. Bile duct ligation (BDL)-induced increase in necrosis, lobular damage, and inflammation was diminished in BDL HDC<sup>−/−</sup> mouse liver. Black arrows indicate fibrotic tissue. Original magnification ×20.

Fig. 2. Masson’s trichrome evaluation in liver sections. A: immunostaining for Masson’s trichrome demonstrates an increase in collagen content (stained blue) in BDL WT compared with sham WT or sham HDC<sup>−/−</sup> mice. In BDL HDC<sup>−/−</sup> mice, there was a decrease in collagen content compared with BDL WT. Original magnification ×20. By real-time PCR in total liver, we found that in BDL WT there was a significant increase in α-smooth muscle actin (α-SMA; B) and collagen I (C) compared with both sham WT and sham HDC<sup>−/−</sup>. In BDL HDC<sup>−/−</sup>, the expression of α-SMA and collagen I was significantly downregulated compared with BDL WT. Data are means ± SE of 6 experiments. *P < 0.05 compared with sham WT.
cholangiocytes were isolated as described by us (25) using a monoclonal antibody (a rat IgG2a, a gift from Dr. R. Faris, Brown University, Providence, RI) against an unidentified antigen expressed by all mouse cholangiocytes (29). Cell viability was evaluated by usingTrypan blue stain. Freshly isolated hepatocytes were obtained bystandard collagenase perfusion (29).

Characterization and Morphology of HDC−/− Mice

We performed hematoxylin and eosin (H&E) staining and Masson’s trichrome staining in paraffin-embedded liver sections (4 μm thick) from the selected groups of animals to evaluate lobular damage, necrosis, inflammation, and overall liver architecture. Liver sections were stained by a commercially available kit purchased from Sigma Aldrich (catalog no. HT15). We also evaluated the architecture of other major organs (heart, lung, kidney, stomach, and spleen) by H&E staining. Light microscopy and immunohistochemical observations were performed in a blinded manner by two independent observers. Real-time PCR was performed for α-smooth muscle actin (α-SMA) and collagen I in total liver from all groups using mouse primers (SABiosciences). A ΔΔCt (delta delta threshold cycle) analysis was performed for real-time PCR; 1 μg of total cellular RNA was used for all PCR reactions. In total liver RNA, cytokeratin-19 (CK-19, a cholangiocyte-specific marker) (22) was utilized to determine the ratio of expression per cholangiocytes; all reactions were compared with GAPDH (22) to ensure proper loading.

Evaluation of HDC and HR Expression and Histamine Secretion in Vivo

From the selected groups of mice, we measured HDC expression by 1) immunohistochemistry in paraffin-embedded liver sections (4 μm thick) and 2) real-time PCR in total RNA (1 μg) (15) from total liver and isolated cholangiocytes. In total liver we measured the expression of the four HRs by real-time PCR. For immunohistochemistry, the anti-HDC antibody was diluted 1:50; negative controls were performed in parallel with nonimmune serum as the primary antibody. Light microscopy (Leica Microsystems, Wetzlar, Germany) was performed to obtain and quantify images. Quantitative real-time PCR (15) was performed in total RNA extracted from total liver, isolated cholangiocytes, and hepatocytes from all animal groups to determine HDC and HR gene expression as described above. Standard end point RT-PCR (1) was performed to determine HDC expression in purified hepatocytes (40).

Histamine levels were measured by commercially available EIA kits (Cayman Chemical) (15) in serum as well as isolated cholangiocyte supernatants (after incubation for 6 h at 37°C) as previously described by us (21, 40).

Evaluation of Biliary Mass and Proliferation

Intrahepatic bile duct mass (IBDM) and cholangiocyte proliferation were determined in all animal groups by semiquantitative immunohistochemistry for CK-19 and PCNA (21, 38). In isolated cholangiocytes, we performed immunoblotting (using 20 μg of protein) for PCNA to determine cholangiocyte proliferation (21).

Determination of VEGF/HIF-1α Expression and Secretion

Because we have previously shown that pharmacological inhibition ofHDC decreases VEGF levels, we measured VEGF-A/C expression byreal-time PCR and immunoblotting in total liver and isolated cholangio-cyte (see above) from selected groups of mice. To determine VEGF
secretion, commercially available EIA kits were used to measure VEGF-A and VEGF-C levels from serum and cholangiocyte supernatants (collected after incubation for 6 h at 37°C) (15, 23). Hypoxia-inducible factor (HIF-1α) has been shown to regulate VEGF (53); therefore we measured the expression of this transcription factor in cholangiocytes from WT, BDL WT, and BDL HDC−/− mice by immunoblots.

**Determination of the PKA/ERK-Dependent Signaling Pathway**

Since histamine increases biliary proliferation via activation of PKA/ERK1/2 phosphorylation (21), we evaluated the role of this pathway in our study. Phosphorylation of protein kinase A (PKA) and the MAPK kinase, ERK1/2 was evaluated by immunoblotting (40) in isolated cholangiocytes from the selected groups of animals. Data were expressed as ratio of protein expression of total PKA (tPKA) and total ERK1/2 (tERK1/2), respectively. Immunoblotting was performed with 20 μg of protein; we used β-actin as the housekeeping gene (21). Band intensity was determined Odyssey from LI-COR Biosciences (Lincoln, NE), model no. 9120.

**In Vitro Models**

**Cell culture.** The in vitro studies were performed in our SV-40 transformed large mouse cholangiocyte cultures (54) that were stably transfected with an shRNA plasmid directed to HDC (LMCC-HDC) along with an empty vector control (LMCC-neg) per our previous studies (40). Knockdown of HDC was verified by real-time PCR and immunoblotting (40).

**Evaluation of the PKA/ERK1/2 signaling pathway.** Immunoblotting was performed to determine the phosphorylation of PKA and ERK1/2 in transfected cells (lacking HDC or negatively transfected) as described (40). Proliferation was measured by PCNA immunoblots and MTT assays in stably transfected cells (LMCC-neg or LMCC-HDC) as previously described (40).

**Statistical Analysis**

Data are expressed as means ± SE. Differences between groups were analyzed by the Student’s unpaired t-test when two groups were
analyzed, and by ANOVA when more than two groups were analyzed, followed by an appropriate post hoc test. The null hypothesis was rejected at $P < 0.05$.

**RESULTS**

Morphological and Pathological Characteristics of HDC$^{-/-}$ Mice

We measured the overall morphology of the liver in all groups of animals. There was no significant difference between the sham WT and the sham HDC$^{-/-}$ liver with regard to inflammation, necrosis, and lobular damage as shown by H&E staining (Fig. 1). In the BDL WT mice, there was a marked increase in lobular damage, inflammation, and necrosis (marked by black arrows); however, these parameters were decreased in BDL HDC$^{-/-}$ mice, suggesting that depletion of HDC improves these morphological changes; representative images are shown in Fig. 1. By immunohistochemistry for Masson’s trichrome, we found that the amount of fibrotic tissue (stained blue) was increased in BDL WT compared with sham WT mice (Fig. 2). In BDL HDC$^{-/-}$ mice, the amount of fibrotic tissue was reduced compared with BDL WT mice (Fig. 2). The expression of α-SMA and collagen I was decreased in sham HDC$^{-/-}$ mice compared with sham WT; however, this was markedly increased in BDL WT mice. In BDL HDC$^{-/-}$ mice, the expression of α-SMA and collagen I was significantly downregulated compared with BDL WT (Fig. 2). In other organs from HDC$^{-/-}$ and BDL HDC$^{-/-}$ mice, there was no significant evidence of morphological alterations compared with WT counterparts (data not shown). Together these data indicate that genetic loss of HDC in WT mice does not significantly alter liver morphology and structure. In the BDL HDC$^{-/-}$ mice, there was a decrease in BDL-induced damage, α-SMA, and collagen I, suggesting that the HDC/histamine axis may be a contributor to this process.

Biliary Expression of HDC and Secretion of Histamine Is Decreased in HDC$^{-/-}$ Mice

HDC expression was measured by immunohistochemistry and real-time PCR in liver sections, and in total liver and...
isolated cholangiocytes, respectively. As shown in Fig. 3, in sham WT mice, there was minimal expression of HDC; however, in BDL WT mice there was a marked increase in HDC expression in liver sections (Fig. 3A), total liver lysates (Fig. 3B), and isolated cholangiocytes (Fig. 3C). As expected, HDC expression was virtually undetected in liver sections, total liver lysates, and isolated cholangiocytes from sham HDC−/− mice (Fig. 3, A–C). In BDL HDC−/− mice, there was minimal expression of HDC, which is likely due to the dietary intake of histidine (Fig. 5A). In sham WT mice, there was a small detectable amount of histamine release that is likely due to the dietary intake of histidine (Fig. 5). In cholangiocyte supernatant from BDL WT mice, there was a significant increase in histamine release compared with both sham WT mice, there was a decrease in intrahepatic ductal mass compared with sham WT (Fig. 6). In BDL WT mice there was a significant increase in ductal mass compared with sham WT and sham HDC−/− mice, and in BDL HDC−/− mice there was a significant decrease in intrahepatic ductal mass compared with BDL WT mice (Fig. 6).

By immunohistochemistry for CK-19 (a specific marker of bile ducts) (33), we found that in sham HDC−/− mice there was similar bile duct mass (marked by red arrows) compared with sham WT mice (Fig. 6). In BDL WT mice there was a significant increase in ductal mass compared with sham WT and sham HDC−/− mice, and in BDL HDC−/− mice there was a significant decrease in intrahepatic ductal mass compared with BDL WT mice (Fig. 6). Like previous studies (25), PCNA expression increased in isolated cholangiocytes from BDL WT mice compared with sham WT or sham HDC−/− mice (Fig. 7). In cholangiocytes from BDL HDC−/− mice there was a striking decrease in PCNA gene expression compared with BDL WT mice (Fig. 7) demonstrating that HDC modulates biliary proliferation.

HDC Regulates VEGF/HIF-1α Expression and Secretion

HDC has been shown to regulate VEGF expression (15, 40) and similar to our previous findings, we found that after BDL, biliary VEGF-A/C expression significantly increased compared with sham WT mice (Fig. 8, A and B). Consistent with previous studies showing that inhibition of HDC reduces VEGF gene and protein expression (40), in BDL HDC−/− mice there was a marked decrease in VEGF levels compared with BDL WT (Fig. 8, A and B). We measured VEGF-C levels in cholangiocyte supernatants and we found, similar to our previous studies (22), that in BDL WT supernatant there was an increased release of VEGF-C compared with sham WT cholangiocyte supernatant (data not shown). In cholangiocyte

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Fig. 8. Determination of VEGF expression and secretion and HIF-1α expression. In total liver, VEGF-A gene expression (A) and VEGF-C protein expression (B) increased in BDL WT compared with sham WT mice. In the BDL HDC−/− mice, gene expression of VEGF-A and protein expression of VEGF-C was significantly decreased compared with BDL WT. Similar results were found for VEGF-C secretion evaluated by enzyme immunoassay (EIA) (C). In cholangiocyte supernatant from BDL HDC−/− cholangiocyte supernatant, VEGF-C release was diminished compared with BDL WT mice. D: by immunoblots, HIF-1α expression was significantly increased in BDL WT compared with sham WT mice, whereas in BDL HDC−/− mice, there was a decrease in HIF-1α expression. Data are means ± SE of 6 experiments for real-time PCR, 6 experiments for immunoblots, and 4 experiments run in triplicates for EIA. *P < 0.05 compared with sham WT mice; #P < 0.05 compared with sham WT mice.
supernatant from BDL HDC−/− mice, VEGF-C secretion was significantly decreased compared with BDL WT mice, supporting the concept that the HDC/histamine axis regulates VEGF (Fig. 8C). Since it has been demonstrated that HIF-1α regulates VEGF expression (53), we examined this protein by immunoblots and found that in BDL WT there is a significant upregulation of HIF-1α expression compared with WT alone (Fig. 8D). In BDL HDC−/− mice, the expression of HIF-1α was significantly lower compared with BDL WT, which suggests that HIF-1α is a potential mediator by which histamine regulates VEGF expression and secretion (Fig. 8D).

**HDC Regulates Biliary Proliferation via PKA/ERK Signaling**

Since large cholangiocytes are the main biliary type to proliferate after BDL via a PKA/ERK1/2-dependent signaling pathway (3, 16), we evaluated this signaling pathway in our HDC−/− model after BDL. By immunoblots, we found that in BDL WT cholangiocytes there was a significant increase in the phosphorylation of both PKA (Fig. 9A) and ERK1/2 (Fig. 9B). PKA and ERK1/2 phosphorylation decreased in BDL HDC−/− cholangiocytes suggesting that HDC regulates biliary proliferation via PKA/ERK1/2 signaling (Fig. 9, A and B).

**In Vitro Knockdown of HDC Decreases Biliary Proliferation via pPKA/pERK1/2 Signaling**

In our final experiments we validated our in vivo data by using cultured cholangiocytes stably transfected with an shRNA for HDC or empty vector [knockdown (~90%) was validated by real-time PCR, data not shown]. By immunoblots, we found that loss of HDC (LMCC-HDC) decreases the phosphorylation of PKA (Fig. 10A) and ERK1/2 (Fig. 10B) and biliary proliferation (Fig. 10C) compared with LMCC-neg.

**DISCUSSION**

In this study, we demonstrated that the genetic knockdown of HDC has significant inhibitory effects on biliary proliferation, fibrosis, and VEGF expression via PKA/ERK1/2 signaling. In HDC−/− mice subjected to BDL, there was decreased intrahepatic bile duct mass, proliferation, fibrosis, and VEGF expression compared with BDL WT mice by downregulation of the PKA/ERK1/2 signaling pathway. Overall, the liver morphology was not significantly altered in HDC−/− mice compared with WT mice. Histamine secretion in both serum and cholangiocyte supernatant was markedly decreased in sham HDC−/− and BDL HDC−/− mice compared with WT mice (Fig. 11, working model). Our study suggests that autocrine/paracrine downregulation of the HDC/histamine axis by cholangiocytes as well as other cells such as mast cells induces a change of the PKA/ERK1/2 signaling pathway with downregulation of VEGF, thus decreasing biliary hyperplasia. Besides mast cells, which are the primary cellular source of histamine release (8, 9), it has been demonstrated that other cells may express HDC and secrete histamine, namely Kupffer cells (28, 52), which have previously been reported to contain the machinery to synthesize and secrete histamine. In data not shown here, we verified that Kupffer cells (isolated from sham and BDL rats) contain the machinery to synthesize and degrade histamine; however, these cells secrete a very limited amount of histamine compared with mast cells or cholangiocytes. These data contrast with previous findings but this may be due to the fact that, in previous studies, liver injury was induced by toxins (LPS and CCl4), and not through a model of biliary damage like BDL. Furthermore, we found that both quiescent and culture-activated myofibroblasts express small amounts of HDC compared with both cultured mast cells and cholangiocytes, suggesting that these cells are not the primary target of histamine-mediated biliary proliferation; however, they may contribute to PBC or PSC through cross talk with cholangiocytes via the HRs. It would be interesting to study and understand the paracrine contributions of mast cells and potentially Kupffer cells or myofibroblasts in biliary proliferation and fibrosis.

HDC−/− mice have been used extensively to study gastric secretion, allergy-mediated diseases, and neurotransmission (43, 44). Our studies are the first to characterize HDC−/− mice regarding the pathophysiology of liver and biliary epithelium, in particular. Characteristically, HDC−/− mice are viable and fertile; however, their mast cell number is reduced and the granule contents (i.e., histamine) are altered compared with the WT counterpart (24, 47). Other studies have shown that the gastric mucosal morphology is altered in HDC−/− mice compared with WT since these mice experience hypergastrinemia (41). Morphologically, we did not find any significant differences in the HDC−/− mice that might influence biliary/liver function; however, it was noted that as HDC−/− mice aged (15+ wk) their liver and body weight ratios increased and fatty deposits were found in liver histology (data not shown). As expected,
there was an alteration of liver morphology in BDL WT mice as demonstrated by an increase in collagen staining, inflammation, and α-SMA and collagen I expression. However, these parameters were downregulated in the BDL HDC mice, suggesting that lack of histamine may ameliorate BDL-induced liver damage and fibrosis. Interestingly, the fibrotic markers α-SMA and collagen I were significantly downregulated in sham HDC mice compared with sham WT, which is likely due to the altered number and morphology of the mast cells that are found in HDC mice (44). Mast cells have been shown to infiltrate the liver and contribute to fibrosis (5, 51), and, therefore, altered mast cell number and distorted morphology may explain why there is a decrease in fibrosis in HDC mice compared with sham WT.

Histamine has been shown to be a trophic, growth-promoting agent during both hyperplastic and neoplastic growth (15, 16). Like our previous studies (40), we found that HDC expression is upregulated following BDL (40) and is expressed primarily in cholangiocytes, thus supporting the concept of an autocrine regulation of biliary homeostasis that is mediated by the HDC/histamine axis. In support of our findings, it has been shown that HDC and histamine levels are significantly increased and contribute to tumor growth (12). HDC levels were increased within the oxyntic mucosa in models of hypergastrinemia (31). Furthermore, HDC expression has been found in Leydig tumor cells and stimulation with histamine increases their expression levels (45). Parallel to other studies (43), we found little to no expression of HDC in organs and tissues from our HDC mice. Although there is the deletion of a segment of the HDC gene, HDC mice can still produce small amounts of histamine, which we suspect is taken from the dietary intake of histidine.

The HRs which are G protein-coupled receptors, are known to induce both stimulatory (H1 and H2) and inhibitory (H3 and H4) effects on cellular proliferation (6, 7, 10, 50). In fact, our previous studies have confirmed this differential action in cholangiocytes (16, 17, 20, 39). We found in our present study that H1–H4 all increase in the BDL WT mice; however, knockout of HDC decreases the expression of all four HRs. In data not presented here we found that Kupffer cells from both sham and BDL rats express HRs as well as myofibroblasts.
expression levels of the four HRs varied between sham and BDL Kupffer cells and quiescent vs. culture-activated myofibroblasts, which is similar to our findings in cholangiocytes. In a recent study it was found that H3HR was significantly downregulated in the hippocampus of HDC−/− mice, whereas H1HR and H2HR were relatively unchanged between WT and HDC−/− mice (11), demonstrating that specific receptors have particular targets and differential effects. The loss of all four receptors in our model of BDL HDC−/− suggests that there is a coordinated regulation of the receptors to allow for the balance between the stimulatory and inhibitory actions. Further studies are warranted to understand fully which receptor might play a key role in our model.

Numerous endocrine factors (serotonin, secretin, and VEGF) are known to regulate biliary proliferation by both autocrine and paracrine signaling (22, 26, 38). After BDL, there is a marked proliferation of large cholangiocytes (which line large bile ducts) (2, 4) that is regulated by the cAMP/PKA/ERK-dependent signaling pathway (16). Our study demonstrates that, after BDL in WT mice, there is enhanced intrahepatic biliary mass and proliferation that is reduced in BDL HDC−/− mice through downregulation of a PKA/ERK1/2-dependent mechanism; these findings were further confirmed in vitro by use of HDC shRNAs. Because this model induces global depletion of HDC and an alteration in mast cell content (42), our results suggest that both autocrine (by cholangiocytes) and paracrine (mast cells) regulation of biliary proliferation may be at play. Histamine has been demonstrated to induce cellular proliferation via activation of cAMP-dependent signaling in a number of epithelial including cholangiocytes. In fact, in splenocytes, treatment with histamine increased ERK1/2 phosphorylation and splenocyte proliferation (13). Also, inhibition of PKA signaling has been shown to reduce histamine-induced neurotrophin-3 production in astrocytes (30), supporting the concept that HDC/histamine induces its effects via PKA/ERK signaling. Our previous study also found that stimulation with histamine increases large cholangiocyte proliferation via the cAMP/PKA/ERK1/2-dependent pathway (21). Although histamine has been shown to stimulate the proliferation of small cholangiocytes by activation of IP3/Ca2+/Ca2+ signaling (17, 21), this signaling pathway may not be important in our model since (1) BDL induces the proliferation of only cAMP-dependent large cholangiocytes and (2) in BDL HDC−/− mice there is no proliferation of small ducts (see Fig. 6) that is regulated by IP3/Ca2+ signaling (17, 21).

The loss of HDC does not completely ablate the BDL-induced hyperplasia since there are other factors (secretin and VEGF) that stimulate cholangiocyte proliferation, factors that are increased after BDL (25, 26). However, our results demonstrate that HDC/histamine is a key trophic, autocrine regulator of biliary proliferation/loss. Supporting our findings, in other systems histamine has been shown to also be required for cellular proliferation. For example, during neural stem cell proliferation, histamine is necessary to increase cellular differentiation (49). Histamine has also been shown to induce airway smooth muscle cell hyperplasia (46). Furthermore, inhibition of HDC decreases melanoma cell proliferation (27) and Leydig cell proliferation via ERK1/2 signaling (45).

As stated above, VEGF levels increase during BDL-induced biliary proliferation. Our present study and previous work demonstrate that HDC may be a regulator of VEGF expression (15, 40). VEGF is required to sustain the increased demand for nutrition following BDL and the majority of VEGF is derived from the peribiliary vascular plexus (PBP); however, cholangiocytes also produce and secrete VEGF in response to BDL prior to the proliferation of the PBP, thus supporting the concept of an autocrine regulation of biliary mass by VEGF (23). In support of our findings that genetic loss of HDC decreases VEGF expression, a study by Ghosh et al. (24) found that VEGF expression and secretion were significantly downregulated in granulation tissue from HDC−/− mouse compared with HDC+/− mice and treatment with histamine increased VEGF levels, angiogenesis, and wound healing in HDC−/− mice, thus supporting the concept that HDC/histamine are key players in proliferation. In our previous studies we have demonstrated treatment with an HDC inhibitor, α-methyl decreased VEGF expression and angiogenesis in cholestatic and cholangiocarcinoma models (15, 40), and our present studies further support the concept that HDC is a key regulator of VEGF. VEGF can be regulated by numerous factors including the transcription factor HIF-1α. In both normal cartilage and chondrosarcoma, the expression of VEGF was HIF-1α dependent (35). Our study demonstrates that HIF-1α is decreased in BDL HDC−/− mice, suggesting that histamine regulation of VEGF expression (also decreased in BDL HDC−/− mice) is mediated by HIF-1α.

In summary, we have demonstrated that long-term depletion of HDC in genetically altered HDC−/− mice has a significant impact on biliary proliferation and VEGF expression (Fig. 11). Our findings support our previous work using a chemical inhibitor to HDC, which induced a short-term response and reduction in biliary mass, proliferation, and VEGF expression (40). Targeting HDC and histamine is proving to be a valuable resource in the treatment of numerous liver pathologies including PBC and PSC.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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