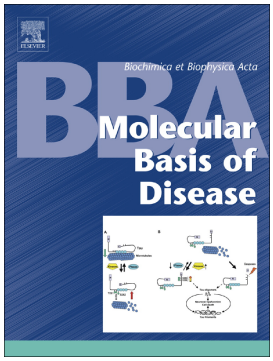
Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma

Chung, Brian; Karlsen, Tom Hemming; Folseraas, Trine

DOI:
10.1016/j.bbadis.2017.08.020

Document Version
Peer reviewed version

Citation for published version (Harvard):
Chung, B, Karlsen, TH & Folseraas, T 2017, 'Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma', Biochimica et Biophysica Acta. Molecular Basis of Disease. https://doi.org/10.1016/j.bbadis.2017.08.020



Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma

Brian K. Chung, Tom H. Karlsen, Trine Folseraas

PII: S0925-4439(17)30302-2
DOI: doi: 10.1016/j.bbadis.2017.08.020
Reference: BBADIS 64870

To appear in:

Received date: 22 June 2017
Revised date: 16 August 2017
Accepted date: 21 August 2017

Please cite this article as: Brian K. Chung, Tom H. Karlsen, Trine Folseraas , Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma, (2017), doi: 10.1016/j.bbadis.2017.08.020

Cholangiocytes in the pathogenesis of primary sclerosing cholangitis and development of cholangiocarcinoma

Brian K. Chung[1–4]*, Tom H. Karlsen[2–5], Trine Folseraas[2–5]

[1] Centre for Liver Research and NIHR Birmingham Inflammation Biomedical Research Centre, Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK.

[2] Norwegian PSC Research Center, Department of Transplantation Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway.

[3] Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.

[4] Research Institute of Internal Medicine, Division of Surgery, Inflammatory Medicine and Transplantation, Oslo University Hospital Rikshospitalet, Oslo, Norway.

[5] K.G. Jebsen Inflammation Research Centre, Institute of Clinical Medicine, Faculty of Medicine, University of Oslo, Oslo, Norway.

* Indicates corresponding author

Corresponding Author

*Brian K. Chung, PhD

Norwegian PSC Research Center

Department of Transplantation Medicine

Division Surgery, Inflammatory Diseases and Transplantation

Oslo University Hospital Rikshospitalet, Postboks 4950 Nydalen, N-0424 Oslo, Norway.

Phone: +44 747 0375 922

Fax: +47 23 07 3510

Email: b.k.chung@medisin.uio.no

Keywords

primary sclerosing cholangitis; cholangiocytes; cholangiocarcinoma; pathogenesis

Number of Figures: **5** / **Number of Tables**: **1**

Abstract

Primary sclerosing cholangitis (PSC) is an idiopathic cholangiopathy strongly associated with inflammatory bowel disease (IBD) and characterized by cholestasis, chronic immune infiltration and progressive fibrosis of the intrahepatic and extrahepatic bile ducts. PSC confers a high risk of cholangiocarcinoma (CCA) with PSC-CCA representing the leading cause of PSC-associated mortality. PSC-CCA is derived from cholangiocytes and associated progenitor cells – a heterogeneous group of dynamic epithelial cells lining the biliary tree that modulate the composition and volume of bile production by the liver. Infection, inflammation and cholestasis can trigger cholangiocyte activation leading to an increased expression of adhesion and antigen-presenting molecules as well as the release of various inflammatory and fibrogenic mediators. As a result, activated cholangiocytes engage in a myriad of cellular processes, including hepatocellular proliferation, apoptosis, angiogenesis and fibrosis. Cholangiocytes can also regulate the recruitment of immune cells, mesenchymal cells, and endothelial cells that participate in tissue repair and destruction in settings of persistent inflammation. In PSC, the role of cholangiocytes and the mechanisms governing their transformation to PSC-CCA are unclear however localization of disease suggests that cholangiocytes are a key target and potential regulator of hepatobiliary immunity, fibrogenesis and tumorigenesis. Herein, we summarize mechanisms of cholangiocyte activation in PSC and highlight new insights into disease pathways that may contribute to the development of PSC-CCA.

1. Introduction

Primary sclerosing cholangitis (PSC) is a rare hepatobiliary disease characterized by persistent biliary inflammation, concentric periductal fibrosis ("onion-skin" fibrosis) and in most cases, progression to liver cirrhosis and end-stage liver disease. PSC carries a significant risk of cholangiocarcinoma (CCA) and PSC-CCA represents the most significant cause of patient mortality in PSC [1,2]. By contrast, risk of hepatocellular carcinoma (HCC) is relatively low in PSC [3,4] and underscores the central role of cholangiocytes in the pathogenesis of disease. PSC shows a male preponderance (2 to 1) and affects all ages with a median onset of 30 – 40 years. Incidence ranges geographically from 0 – 1.3 cases per 100,000 persons per year and prevalence from 0 – 16.2 cases per 100,000 persons [5,6]. In the majority of cases, PSC associates with a distinct form of inflammatory bowel disease (IBD) that bears unique clinical [7] and genetic features to ulcerative colitis and Crohn's disease [8-10]. Up to 25% of PSC patients also develop autoimmune conditions, including autoimmune thyroid disease, type 1 diabetes, rheumatoid arthritis and celiac disease [11]. Heritability is comparable to other complex immune-mediated disorders as first-degree relatives are approximately 10-times more likely to develop PSC compared to unrelated individuals [12]. Definitive diagnosis of PSC is achieved by findings of characteristic biliary strictures using magnetic resonance- or endoscopic retrograde-cholangiography (MRCP/ERCP) following the exclusion of secondary sclerosing cholangitis etiologies (e.g. IgG4-associated cholangitis, infections, ischemia, toxins, biliary calculi or trauma) [13]. Owing to a lack of effective medical therapies [14-16], over 50% of patients progress to end-stage disease within 10-15 years of diagnosis which positions PSC as the leading indication of orthotopic liver transplantation amongst cholestatic diseases in Western countries

[17,18]. Following transplantation, the 1-year survival rate is 85%, the 5-year survival rate is 72% (United Network for Organ Sharing; www.unos.org) with disease recurring in approximately 25% of cases [19,20].

The localization of immune infiltrate and fibrosis surrounding portal areas strongly suggests that cholangiocytes are key players in PSC, either as a victim or a foe – or both. Cholangiocytes are a heterogeneous group of reactive epithelial cells that line the intrahepatic and extrahepatic bile ducts as well as the peribiliary glands and facilitate the transport of bile constituents from the liver to the duodenum [21,22]. Cholangiocytes express a variety of molecular transporters, aquaporins and ion channels that also enable cholangiocytes to modify the final composition and volume of bile entering the biliary tract [18,23-25]. In PSC, cholangiocytes lining both the intrahepatic and extrahepatic ducts can be affected and inflammation of the biliary tract is linked to cholangiocyte senescence and cholestasis [26,27]. There is also precursor cell activation, with an expansion of the peribiliary gland compartment and likely (although formally not shown) increased mucus production [22]. Impaired cholangiocyte function resulting from a combination of biliary insults (likely involving both inflammation, bile acid toxicity and microbial factors) likely contributes to PSC pathogenesis. It is currently unknown to what extent disturbances in normal cholangiocyte functions are involved in PSC causation, however as shown by sclerosing cholangitis in the context of mutations to the cholangiocyte proteins cystic fibrosis transmembrane conductance regulator (*CFTR*) [27,28], X-prolyl aminopeptidase P1 (*XPNPEP1*) and adducing 3 (*ADD3*) [29-33], the possibility exists. As it stands, the precise etiopathogenesis of PSC is unknown and what we currently believe is that a complex manifestation of multiple genetic and environmental determinants in sum results in the clinical phenotype [34-39].

Similarly, the mechanisms that precipitate the malignant transformation of cholangiocytes in PSC-CCA are poorly understood and it remains to be determined what proportion of PSC-CCA results from the interaction of genetic and environmental factors that initiate PSC versus secondary effects related to cholangiocyte activation, chronic inflammation, bile acid toxicity and cellular senescence that occur during the progression of disease.

2. Epidemiology of PSC-CCA

CCA is an adenocarcinomatous cancer arising from cholangiocytes. Overall, CCA is rare and accounts for approximately 3% of all gastrointestinal tumors worldwide [40-43]. PSC is a major risk factor for CCA with 10% of CCA cases in the Western world being associated with PSC [44,45]. In patients with PSC, the annual incidence of PSC-CCA is 0.5 – 1.5% [46-48] and the lifetime risk ranges from 6 – 12% [2,45-52]. Higher frequencies of PSC-CCA (up to 19.9 %) are reported in transplant and referral center cohorts but such studies presumably include a greater number of patients with severe PSC phenotypes and likely overestimate the true prevalence of PSC-CCA in the total patient population [46,53]. Similarly to the overall incidence of PSC [54-56], risk of PSC-CCA is higher in northern Europe and the United States than populations in southern Europe and Asia (2.8 % to 8.4%) [50,57,58]. Pediatric patients and patients with small duct PSC also have a lower risk of PSC-CCA [59-61]. Approximately one third of PSC-CCA is diagnosed within the first year of PSC diagnosis suggesting that undiagnosed PSC precedes the development of PSC-CCA in these cases with symptoms primarily related to PSC-CCA leading to the diagnosis of PSC [2,47,59,62].

Knowledge on predisposing risk factors for PSC-CCA are scarce. The duration and severity of PSC do not seem to correlate with PSC-CCA development [48,63,64] and several proposed factors, including age at diagnosis, smoking and alcohol consumption, a Mayo risk score of more than four, a history of variceal bleeding or colorectal neoplasia and the presence or duration of concurrent IBD confer only small increments to disease liability and fail to stratify PSC subgroups at high risk for PSC-CCA [48-51,65,66]. Several studies support a model of CCA development whereby inflammatory epithelial damage leads to a sequence of metaplasia to low grade dysplasia (LGD), high grade dysplasia (HGD) and carcinoma (Figure 1) [63,67-71]. In agreement with this model, cholangiocytes in explant livers from PSC-CCA patients more often show evidence of metaplasia (43% vs 19%, $P = 0.013$) and dysplasia (83% vs 36%, $P < 0.0001$) than in PSC without CCA suggesting an association between biliary dysplasia and PSC-CCA [71-73]. Supporting this notion, polysomy and homozygous loss of 9p21 indicative of bile duct dysplasia and CCA have been observed with increasing frequencies in HGD than LGD [74,75]. Thus, cholangiocyte dysplasia may serve as a predictor for PSC-CCA, however dysplasia is also relatively frequent in the absence of PSC-CCA and may regress after resolution of biliary tract infections [71,76,77]. Moreover, the mechanisms regulating the progression from inflammatory epithelial damage to neoplastic transformation are largely unknown however surveillance for cholangiocarcinoma is recommended for patients with PSC (Figure 2).

3. Genetics of PSC and PSC-CCA

To date, 23 susceptibility loci of genome-wide significance ($P \leq 5.0 \times 10^{-8}$) and several loci of suggestive significance have been identified in PSC (Table 1) [35,77-79]. The genetic architecture in PSC mimics that of prototypical antigen-driven conditions such as rheumatoid arthritis, type 1 diabetes and celiac disease with the by far strongest association residing within the human leukocyte antigen (HLA) complex. Non-HLA associations in PSC also overlap with other complex immune-mediated conditions and largely correspond to pathways of immune function, trafficking and tolerance suggesting that patients with PSC carry an underlying predisposition towards autoimmunity that precedes other biliary insults including those involving increased gut permeability and toxic accumulation of bile acids [8-10,76]. The presence of distinct T-cell clonality [80,81] and antibody production in the liver [76,82] further implicate PSC as an immune-mediated entity and supports a disease model where antigens of unknown origin presented on disease-associated HLA molecules drive the activation of pathogenic T cells and B cells [35,83]. Thus far, the antigenic sources in PSC have not been identified but given that most patients appear unresponsive to immunosuppressive therapy [84-87], a continuous exposure to a diverse variety of exogenous antigens (infectious, dietary, environmental) may underpin the development of biliary inflammation in PSC. Such a disease mechanism would be analogous to celiac disease where dietary antigens from cereal gluten proteins are necessary to trigger B- and T-cell reactivity to self-antigens and ultimately gut epithelium destruction [88-90].

As non-HLA risk regions primarily point to the relevance of immune pathways [35,91,92], development of PSC-CCA could most likely serve a secondary event related to the disease

process and not result from a primary genetic effect. This does not exclude the possibility that the current identified risk loci have undefined roles in cholangiocyte biology and play relevant roles in carcinogenic processes [35,93]. Delineating such pathophysiological implications in PSC will require further translational studies. For example, the *MST1* gene at chromosome 3p21, which represents one of the strongest susceptibility loci outside the HLA-region in PSC (Table 1), encodes a serine-threonine kinase involved in cell proliferation, apoptosis and tumour suppression in the liver [62,65,94,95]. *MST1* variants have been implicated in the risk of sporadic extrahepatic CCA [96,97], moreover MST1 has been shown to suppress hepatocellular carcinoma development through the suppression of the *YAP1* oncogene [65,96]. Thus, the role of MST1 in the context of cancer development and PSC-CCA merits further investigation. The *BCL2L11* risk locus at 2q13 serves as another example of a putative combined PSC and PSC-CCA risk locus. *BCL2L11* encodes the BCL-2-Like protein-11 (BCL2-L-11) and marked BCL2-L-11 overexpression has been shown in CCA tissue from rats [98,99]. BCL2-L-11 is critical for apoptosis of B-and T cells, granulocytes and macrophages [96,100] and the early induction of BCL2-L-11 may eliminate cytotoxic T cells prematurely resulting in chronic inflammation and tumour progression [101-103]. *CTLA4* at 2q33 represents another plausible PSC candidate risk gene of interest in the context of PSC-CCA. Surface expression of CTLA-4 is present on a variety of human malignant solid tumours [104,105] and CTLA-4 may induce immune dysregulation as antibodies blocking CTLA4 have been utilized as checkpoint inhibitors in cancer immunotherapy [106,107]. Interestingly, the antitumor effects from CTLA4 inhibition may rely on the composition of the gut microbiome as tumours in germ-free mice do not to respond to CTLA4 blockade [108]. Other PSC candidate risk genes with putative roles in carcinogenesis based on insight from other diseases or animal models include *FOXP1*, *NFKB1*,

CD226 and *STRN4* (Figure 3), and future investigation is required to elucidate the impact of specific genetic variants within these and other established risk genes identified in the general PSC population in the PSC-CCA subpopulation.

Lastly, a limited number of studies exploring the contribution of non-PSC risk loci known to confer an increased risk for PSC-CCA susceptibility have been performed. Two polymorphisms of the natural killer cell receptor G2D (*NKG2D*) have been implicated in PSC-CCA in a Scandinavian PSC cohort (rs1105378, OR = 2.08, P = 0.011 and rs2617167, OR = 2.32, P = 0.0020) [109]. NKG2D is an activating receptor expressed on the surface of natural killer (NK) cells, invariant natural killer T (NKT) cells, CD8+ T cells and subsets of CD4+ T cells [110]. In healthy tissue, NKG2D ligands are generally absent on the cell surface, but can be expressed at sites of inflammation and on cells that have undergone hyper-proliferation and transformation. Although the precise role of the *NKG2D* variants associated with PSC-CCA needs to be confirmed, these variants may impair NKG2D surveillance and recognition of the NKG2D ligand; major histocompatibility complex class I chain-related molecule A (MICA). The *MICA* 5.1 allele is associated with PSC and carriership of this particular variant is associated with PSC-CCA resistance (OR = 0.43, 95%, P = 0.032) [109,111]. It is possible that MICA 5.1 interactions with low-risk *NKG2D* alleles provide protection against PSC-CCA [109]. Suggestive associations to genetic variants within the DNA repair genes MutY homology (*MUTYH*) and Nei-like DNA glycosylase 1 (*NEIL1*) have been implicated with PSC-CCA development [112].

Genetically-driven subsets of PSC are likely to exist, including phenotypes associated with a higher risk of PSC-CCA development, and studies focusing on this yet undefined high-risk PSC-

CCA subset may reveal oncogenic mutations hitherto not identified at robust significance levels in the overall PSC population. Exome and/or whole genome sequencing studies not yet performed in PSC may also reveal rare sequence variants with strong phenotypic effects contributing to risk of PSC-CCA [113].

4. Cholangiocyte heterogeneity in PSC and PSC-CCA

Cholangiocytes are heterogeneous and differences in their morphology, function and ontogeny may relate to PSC phenotypes and development of PSC-CCA (Figure 4). Intrahepatic ducts are mostly comprised of small cholangiocytes and demonstrate a flattened or cuboidal morphology with microvilli extending towards the biliary lumen [114,115]. By contrast, large cholangiocytes found mainly in the extrahepatic bile ducts appear columnar and project a primary cilium on the apical surface which is capable of sensing chemical and mechanical stimuli of the biliary lumen [116]. Morphological distinctions amongst small and large cholangiocytes also reflect differences in embryological origin as small cholangiocytes are thought to originate from a shared hepatoblast precursor whereas large cholangiocytes share a common pancreaticoduodenal progenitor [114,117]. Although perihilar CCA is topographically the most common subtype of PSC-CCA (~ 65%), primary tumors may less frequently arise from cholangiocytes lining the large intrahepatic (15%) and extrahepatic (20%) bile ducts [118].

The role of small versus large cholangiocytes in the development of PSC-CCA is unclear however PSC-CCA is predominantly thought to originate from large cholangiocytes lining

intrahepatic and extrahepatic bile ducts [1,2,119] and data from animal models of chronic liver injury show that large cholangiocytes undergo greater levels of proliferation [120] and apoptosis [121] compared to small cholangiocytes. Additionally, damage to bile ducts can also trigger a reparative process known as ductular reaction which features the proliferation and expansion of bile ducts and induction of liver progenitors that can differentiate into both hepatocytes and cholangiocytes [122]. Taken together, these results suggest that differential responses to biliary stimuli and injury may explain the higher incidence of PSC-CCA amongst patients with large duct PSC compared to those with small duct disease. Ongoing efforts towards defining the specific roles of small and large cholangiocyte in various biological pathways may uncover important mechanisms underlying disease progression and development of PSC-CCA.

5. Dysbiosis and the gut-liver axis

Understanding the initiating and propagating factors responsible for cholangiocyte activation in PSC is of considerable interest given that cholangiocytes have the dynamic capacity to detect a multitude of stimuli and broadly influence various immune cells that may resolve biliary injury but also contribute to cholangiopathy in an inflammatory context [123]. Similar to, and in continuity with the intestinal epithelium, cholangiocytes are often considered a first-line of defense with relevant functional capacities (e.g. expression of pathogen pattern recognition receptors [PRRs]) that including the transmembrane family of Toll-like receptors (TLRs) and intracellular proteins containing the nucleotide binding oligomerization domain (NOD) [124,125] enables cholangiocytes to recognize a variety of pathogenic molecules and

endogenous components released from host cells following infection and cellular injury [126]. TLR and NOD ligation initiates the translocation of the pleiotropic transcription factor NF-κB and signaling through the mitogen-activated protein kinase (MAPK) cascade, both of which lead the production of inflammatory chemokines/cytokines, upregulation of surface adhesion molecules as well as the activation of pathways regulating cell growth, apoptosis and tumorigenesis [127]. Dysregulation of the NF-κB1 pathway may contribute to biliary inflammation and development of PSC-CCA as NF-κB subunit 1 (*NF-κB1*) has been identified as a PSC risk loci (Table 1) [9]. However, no direct assessments of NF-κB or MAPK signaling have been conducted in PSC and further assessment of these pathways, preferably in primary cholangiocytes from patients and healthy subjects, is necessary to substantiate any such involvement.

Several lines of emerging evidence argue that microbes in the gut and/or biliary tract participate in the activation of cholangiocytes and development of PSC-CCA. Gut commensals are known to affect bile acid metabolism [128,129] and dysbiosis identified in fecal and mucosal patient samples highlights that altered or reduced microbial diversity is a risk factor in PSC [34,36-39]. Supporting this concept, an absence of commensal microbiota in the multidrug resistance 2 knockout ($mdr2^{-/-}$) murine model of PSC exacerbates hepatobiliary disease and further suggests a role for microbiota in the pathogenesis of PSC [130]. A scenario in PSC may exist whereby gut dysbiosis leads to an altered metabolism of primary bile acids and dysfunctional reabsorption of secondary bile acids across the colonic epithelium [131]. Dysbiosis in chronic liver disease has also been shown to alter systemic levels of secondary bile acids and the accumulation of secondary bile acids in the enterohepatic circulation is suspected to contribute to liver cirrhosis

development [132] and is also noted in patients with colon cancer [133]. Hence, a positive feedback mechanism initiated by PRR signaling in cholangiocytes and enhanced by the intrinsic toxicity of biliary constituents may lead to dysbiosis and an even greater accumulation of toxic bile acids within the enterohepatic circulation.

6. Cholangiocyte immunobiology in the progression of PSC

Cholangiocytes play an integral role in the regulation of liver immunity and upregulate the expression of adhesion molecules and proinflammatory signals that orchestrate the recruitment and activation of innate and adaptive immune cells [104]. Based on the localization of lymphocytes and expression of adhesion molecules (Figure 5), cholangiocytes appear to interact with CD4+ and CD8+ T cells, but no convincing evidence of antigen-dependent activation has thus far been demonstrated in PSC. Resting cholangiocytes are known to constitutively express high levels of HLA class I [18,23] and can activate NKT cells, a subset of innate-like T cells which recognize lipid antigens presented on the HLA class I-like molecule CD1d [19]. Upon activation, cholangiocytes are known to express detectable levels of HLA class II molecules [134,135] and the adhesion molecules intracellular cell adhesion molecule-1 (ICAM-1) and lymphocyte function-associated antigen-3 (LFA-3) which enable the direct interaction of T cells and cholangiocytes via LFA-1 and CD2 [18,23,25]. However, it remains debatable if cholangiocytes possess the appropriate repertoire of co-stimulatory molecules necessary to activate conventional T cells in an antigen-dependent manner as cholangiocytes do not express the B7 family members B7.1 (CD80) and B7.2 (CD86). These important co-stimulatory

molecules engage CD28 and CTLA-4 expressed on T cells and are widely regarded as the prototypical signature of professional antigen-presenting cells [26]. Whilst future data is needed to clarify if cholangiocytes present endogenous or exogenous peptide antigens to conventional T cells, the proximity of lymphocytes around portal areas in PSC may in fact support a model where lymphocytes activated from the gut or draining lymph nodes traffic to the liver [136] and target reactive cholangiocytes due to their expression of proinflammatory cytokines [27].

In addition to conventional T lymphocytes, NK cells, NKT cells and macrophages are likely to contribute to the inflammatory setting in PSC given these cell types interact with hepatic stellate cells (HSCs) and regulate the remodeling of the local biliary microenvironment during liver fibrosis and wound healing (Figure 5) [29,31-33]. Liver macrophages in particular are likely to play an integral role in PSC as alterations to the gut microflora leading to an increase of gut permeability and bacterial translocation into the enterohepatic circulation [34,36-39] presumably results in the activation of macrophages and secretion of pro-inflammatory and fibrogenic cytokines [40,42]. Recent data also suggests that mucosal-associated invariant T (MAIT) cells, an innate-like T cell subset, may participate in PSC as cholangiocytes and liver B cells exposed to bacterial components upregulate the antigen receptor for MAIT cells known as MR1 and induce MAIT cell activation in an MR1-dependent, cytokine-independent fashion [44].

The pathognomonic lesion in PSC is characterized by an accumulation of fibroblasts and matrix deposition around the biliary epithelium. Cholangiocarcinoma in PSC also shows excessive "scirrhous" features, suggesting that interactions between the mesenchymal and cholangiocyte compartment are relevant in both PSC development and the associated neoplastic cholangiocyte

transformation. Local secretion of pro-inflammatory cytokines, such as inducible nitric oxide synthase (iNOS), promotes nitric oxide (NO) production and IL-6 from cholangiocytes and surrounding cells, leading to cholangiocyte proliferation and oxidative DNA damage [46]. iNOS expression is significantly upregulated in both PSC and PSC-CCA cholangiocytes as a result of the secretion of pro-inflammatory cytokines such as TNF-α in the microenvironment [46]. In oxidative DNA-damaged cholangiocytes, 8-oxodeoxyguanine represents the most abundant oxidative DNA lesion [46]. NO counteracts effective DNA repair by inhibiting 8-oxodeoxyguanine base excision allowing for the excessive accumulation of oxidative 8-oxodeoxyguanine and further promotion of DNA damage and putatively malignant cholangiocyte transformation [54].

Production of IL-6 via gp130, a co-receptor for IL-6, leads to activation of IL-6/STAT3 signaling in cholangiocytes which in turn promotes the proliferation of malignant cholangiocytes by p38 and p42/44 MAPK [57]. In addition IL-6 may trigger activation of yes-associated protein (YAP) and Notch, transcriptional regulators that control cell growth and regeneration independently of the gp130 effector IL-6/STAT3 pathway [59]. In mucosal injury, intestinal gp130/IL-6 signaling stimulates epithelial cell proliferation and causes aberrant differentiation, and it can be speculated if sustained YAP-expression may lead to excessive and oncogenic cholangiocyte proliferation in the setting of biliary epithelial injury in PSC [59,62]. In a murine model biliary transduction of constitutively active YAP combined with bile duct ligation and IL-33 administration lead to CCA formation [63]. Interestingly, the PSC risk gene *MST1* (Table 1), has been implicated in regulating YAP activation [65]. The biliary mitogen IL-33 has also been implicated in cholangiocyte carcinogenesis by promoting downstream activation of IL-6 and

inflammation and fibrosis of the biliary tract [63,67]. After neoplastic transformation, cholangiocytes secrete a range of mediators such as platelet-deriver growth factor-D (PDGF-D) which induce a stromal reaction promoting further tumor growth and invasiveness. PDGF-D recruits cancer-associated fibroblasts to the stroma, which release vascular endothelial growth factor-C (VEGF-C) that stimulates lymphangiogenesis and may promote lymphatic spread of tumors [72,73].

7. Cholestasis and bile acid toxicity in malignant cholangiocyte development in PSC

Some lines of evidence suggest that the cholestasis that occurs in the setting of PSC is critical for CCA development. In cholestasis, bile acids accumulate and exert abnormal effects on cholangiocytes. In particular, accumulation of the bile acid component deoxycholic acid activates epidermal growth factor receptor (EGFR), an upstream activator of Ras in cholangiocytes [74]. EGFR has been implicated in the growth of neoplastic cholangiocytes and several studies has shown increased expression and activating mutations of EGFR in sporadic CCA [76,77]. Sustained activation of EGFR via a TGF-α-dependent mechanism in CCA induces expression of cyclooxygenase-2 (COX-2) and p38 and p42/44 MAPK and activation of this pathway may contribute to CCA [77,78]. Lipopolysaccharide (LPS) which is frequently elevated in chronic liver disease has been shown to transactivate EGFR through release of TGF-α [76]. Furthermore, it has been demonstrated that phosphorylated EGFR is elevated in PSC and that LPS-induced Ras activation correlates with levels of EGFR phosphorylation, observations that putatively link EGFR to malignant cholangiocyte transformation in PSC [80].

Presence of deoxycholic acid in addition prolongs the activity of myeloid-cell leukemia-1 (Mcl-1), an anti-apoptotic member of the B-cell leukemia-2 family [137]. Mcl-1 promotes cellular proliferation and participates in tumour necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated resistance in CCA putatively via the IL-6/protein kinase B (Akt) signaling pathway [83]. In the setting of biliary tract inflammation, oxygenated derivatives of cholesterol (oxysterols) are abundant in bile acids and induces sustained COX-2 expression and putatively malignant cholangiocyte transformation by promoting cell proliferation, angiogenesis and inhibition of apoptosis [84,86,87]. Oxysterol-accumulation in bile may also lead to induction of the cancer-related hedgehog signaling pathway [88,90]. Alluding to the previously described relevance of oxidative stress and DNA damage in biliary tract carcinogenesis, the bile acid glycochenodeoxycholate has been shown to induce oxidative stress and DNA damage in cholangiocytes using experimental models [91].

8. MicroRNAs in PSC and PSC-CCA

MicroRNAs (miRNAs) are evolutionarily-conserved endogenous non-coding RNAs that target complementary binding sites in the 3'-untranslated region (UTR) of coding RNAs to regulate post-transcriptional gene expression [93]. Emerging data from many autoimmune and chronic inflammatory disorders [94,95] implicate the expression of specific miRNAs in the initiation and progression of disease. Little is known about miRNAs in PSC however a recent study reported decreased miR-200C in PSC compared to healthy subjects, increased miR-483-5p and miR-194

in PSC-CCA, and elevated miR-222 and miR-483-5p in PSC-CCA compared to patients with PSC alone [96]. These findings suggest that specific miRNAs in PSC and PSC-CCA may serve as useful biomarkers for the stratification of PSC phenotypes. In addition to diagnostic applications, transcriptomic miRNA profiling may also provide insights into the pathogenesis and oncogenesis of PSC. For example, decreased miR-200C expression in PSC [96] may contribute to the increased risk of PSC-CCA since the inactivation of miR-200c is known to trigger epithelial-mesenchymal transition in CCA [98]. By contrast, elevated miR-194 and miR-483-5p in PSC-CCA [96] could represent the activation of tumour suppressor pathways given that both miRNAs have been shown to strongly repress epithelial-mesenchymal transition and colonization of metastases in the liver [101,103]. Future transcriptomic approaches utilizing bile or liver mononuclear cells from large, well-defined PSC cohorts may identify miRNAs in PSC and PSC-CCA that point towards novel disease pathways that are targetable by new and existing therapeutics strategies.

9. Chronic biliary inflammation leads to cholangiocyte apoptosis, senescence and senescence-associated secretory phenotype (SASP) in PSC

Whilst the responsiveness of cholangiocytes to exogenous and endogenous stimuli is well documented [104], the direct relationship between cholangiocyte activation and the etiopathogenesis of PSC remains unclear. Persistent cholangiocyte activation in cholangiopathies such as PSC is known to increase apoptosis [106] which eliminates cells that have accumulated DNA damage and is important mechanism for inhibiting cancer development [138,139].

Apoptosis can be suppressed by mutations that dysregulate the tumour suppressor p53 and overexpression of p53 is reported in cholangiocytes from patients with PSC-CCA [69]. p53 mutations contributes to CCA development by dysregulating DNA repair and cell cycle arrest and by suppressing the normal apoptotic response [140]. Death signal mediated by binding of (TRAIL to TRAIL-R2/death receptor 5 (DR5) has also been implicated in cholestatic liver injury. Human cholangiocytes constitutively express DR5, and TRAIL expression and apoptosis has been shown to be significantly elevated in cholangiocytes in PSC [141,142]. TRAIL has been reported to promote the migration and invasiveness of CCA via a nuclear-factor κB (NF-κB)-dependent pathway in human cholangiocarcinoma cell lines [141]. Furthermore, treatment with sub-lethal concentrations of TRAIL has been shown to be mutagenic putatively by inducing misrepair of DNA damaged cholangiocytes and chromosomal instability [143].

Chronic cholangiocyte activation in PSC can also initiate a process known as cellular senescence whereby cholangiocytes enter a state of permanent cell cycle G_1 arrest to limit the proliferation of damaged cells at high risk of malignant transformation [101]. Cholangiocytes from PSC patients feature a higher expression of senescence markers, including β-galactosidase (SA-β-GAL), $p16^{INK4a}$ and N-Ras compared to cholangiocytes from patients with primary biliary cholangitis (PBC) and hepatitis C [27] indicating that cholangiocyte senescence may be a key process in the progression of PSC. Prolonged stimulation can also induce the transition of senescent cells to a highly pro-inflammatory and potentially pathogenic state known as senescence-associated secretory phenotype (SASP) [101]. SASP in PSC is associated with the robust secretion of cytokine/chemokines (IL-6, IL-8, CCL2, PAI-1), various growth factors and matrix metalloproteases (MMPs) that trigger endothelial cell invasion [144] and promote

tumorigenesis in models of epithelial cancer [145,146]. The production of these molecules in combination with the presence of endogenous (cholestane-3,5,6-oxysterol) and exogenous (LPS) bile constituents can also induce bystander senescence and amplify SASP in the biliary tract [27]. Thus, pharmacologic inhibition of cholangiocyte senescence may represent a novel therapeutic approach in PSC as inhibition of N-Ras activation by farnesylthiosalicylic acid reduced the expression of SA-β-GAL on PSC cholangiocytes activated by LPS [27]. The long-term consequences of cholangiocyte senescence in the pathophysiology of PSC remains to be explored, but SASP induction could represent an integral link between intestinal dysbiosis, bile acid toxicity, recruitment of immune cells, liver fibrosis and development of PSC-CCA in patients with PSC [27]

10. Conclusion

Over the last two decades, a better understanding of PSC and PSC-CCA has greatly increased the appreciation of cholangiocytes in liver physiology and disease processes. Many of these advances have been based on genetic findings in Mendelian cholestasis syndromes and the development and application of novel culture and experimental animal model systems [147,148]. The collective data available suggests that cholangiocytes should be considered both a primary target and active contributor in PSC and that chronic cholangiocyte activation can lead to the inhibition of apoptosis and induction of cellular senescence, processes that likely promote biliary damage and oncogenesis in PSC. Immunogenetic susceptibilities, a number of potentially relevant effector cell types and epigenetic phenomena continue to emerge as key players in PSC,

but the precise mechanisms underlying the activation of cholangiocytes, their role in fibrogenesis and their malignant transformation in PSC remains to be determined. Future studies dissecting known factors at an interaction with environmental factors, particularly the gut and biliary microbiota, in parallel with detailed mechanistic assessments of genetic and epigenetic determinants are likely to outline useful insights and provide the framework to prioritize targetable molecules and pathways for the prevention, diagnosis and treatment of PSC and PSC-CCA.

Acknowledgements

Project funding was provided by the Norwegian PSC Research Center and South-Eastern Norway Regional Healthy Authority (Grant No. 2015024). BKC is supported by the European Union Seventh Framework Programme (FP7-PEOPLE-2013-COFUND) under grant agreement #609020 (Scientia Fellows) and by the National Institute for Health Research (NIHR) Birmingham Biomedical Research Centre. This article presents independent research and the views expressed are those of the authors and not necessarily those of the National Health Service, the NIHR or the Department of Health.

References

[1] T.J. Weismüller, P.J. Trivedi, A. Bergquist, M. Imam, H. Lenzen, C.Y. Ponsioen, et al., Patient Age, Sex, and Inflammatory Bowel Disease Phenotype, Gastroenterology. (2017) 1–18. doi:10.1053/j.gastro.2017.02.038.

[2] K. Boonstra, R.K. Weersma, K.J. van Erpecum, E.A. Rauws, B.W.M. Spanier, A.C. Poen, et al., Population-based epidemiology, malignancy risk, and outcome of primary sclerosing cholangitis, Hepatology. 58 (2013) 2045–2055. doi:10.1002/hep.26565.

[3] D.M. Harnois, G.J. Gores, J. Ludwig, J.L. Steers, N.F. LaRusso, R.H. Wiesner, Are patients with cirrhotic stage primary sclerosing cholangitis at risk for the development of hepatocellular cancer? J. Hepatol. 27 (1997) 512–516.

[4] R. Zenouzi, T.J. Weismüller, P. Hubener, K. Schulze, M. Bubenheim, N. Pannicke, et al., Low Risk of Hepatocellular Carcinoma in Patients With Primary Sclerosing Cholangitis With Cirrhosis, Clinical Gastroenterology and Hepatology. 12 (2014) 1733–1738. doi:10.1016/j.cgh.2014.02.008.

[5] N.A. Molodecky, H. Kareemi, R. Parab, H.W. Barkema, H. Quan, R.P. Myers, et al., Incidence of primary sclerosing cholangitis: a systematic review and meta-analysis, Hepatology. 53 (2011) 1590–1599. doi:10.1002/hep.24247.

[6] K. Boonstra, U. Beuers, C.Y. Ponsioen, Epidemiology of primary sclerosing cholangitis and primary biliary cirrhosis: a systematic review, J. Hepatol. 56 (2012) 1181–1188. doi:10.1016/j.jhep.2011.10.025.

[7] E.V. Loftus, PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis, Gut. 54 (2005) 91–96. doi:10.1136/gut.2004.046615.

[8] J.Z. Liu, J.R. Hov, T. Folseraas, E. Ellinghaus, S.M. Rushbrook, N.T. Doncheva, et al.,

Dense genotyping of immune-related disease regions identifies nine new risk loci for primary sclerosing cholangitis, Nat. Genet. 45 (2013) 670–675. doi:10.1038/ng.2616.

[9] D. Ellinghaus, L. Jostins, S.L. Spain, A. Cortes, J. Bethune, B. Han, et al., Analysis of five chronic inflammatory diseases identifies 27 new associations and highlights disease-specific patterns at shared loci, Nat. Genet. 48 (2016) 510–518. doi:10.1038/ng.3528.

[10] S.-G. Ji, B.D. Juran, S. Mucha, T. Folseraas, L. Jostins, E. Melum, et al., Genome-wide association study of primary sclerosing cholangitis identifies new risk loci and quantifies the genetic relationship with inflammatory bowel disease, Nat. Genet. advance online publication SP - EP - (2016) 1–8. doi:10.1038/ng.3745.

[11] G.F. Mells, A. Kaser, T.H. Karlsen, Novel insights into autoimmune liver diseases provided by genome-wide association studies, Journal of Autoimmunity. 46 (2013) 41–54. doi:10.1016/j.jaut.2013.07.004.

[12] A. Bergquist, S.M. Montgomery, S. Bahmanyar, R. Olsson, A. Danielsson, S. Lindgren, et al., Increased risk of primary sclerosing cholangitis and ulcerative colitis in first-degree relatives of patients with primary sclerosing cholangitis, Clin Gastroenterol Hepatol. 6 (2008) 939–943. doi:10.1016/j.cgh.2008.03.016.

[13] European Society of Gastrointestinal Endoscopy, European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu, European Association for the Study of the Liver, Role of endoscopy in primary sclerosing cholangitis: European Society of Gastrointestinal Endoscopy (ESGE) and European Association for the Study of the Liver (EASL) Clinical Guideline, J. Hepatol. 66 (2017) 1265–1281. doi:10.1016/j.jhep.2017.02.013.

[14] European Association for the Study of the Liver, EASL Clinical Practice Guidelines: Management of cholestatic liver diseases, J. Hepatol. 51 (2009) 237–267. doi:10.1016/j.jhep.2009.04.009.

[15] R. Chapman, J. Fevery, A. Kalloo, D.M. Nagorney, K.M. Boberg, B. Shneider, et al., Diagnosis and management of primary sclerosing cholangitis, Hepatology. 51 (2010) 660–678. doi:10.1002/hep.23294.

[16] K.D. Lindor, K.V. Kowdley, M.E. Harrison, American College of Gastroenterology, ACG Clinical Guideline: Primary Sclerosing Cholangitis, Am. J. Gastroenterol. 110 (2015) 646–59– quiz 660. doi:10.1038/ajg.2015.112.

[17] R. Adam, V. Karam, V. Delvart, J. O'Grady, D. Mirza, J. Klempnauer, et al., Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR), J. Hepatol. 57 (2012) 675–688. doi:10.1016/j.jhep.2012.04.015.

[18] M.K. Auth, R.A. Keitzer, M. Scholz, R.A. Blaheta, E.C. Hottenrott, G. Herrmann, et al., Establishment and immunological characterization of cultured human gallbladder epithelial cells, Hepatology. 18 (1993) 546–555.

[19] E. Schrumpf, C. Tan, T.H. Karlsen, J. Sponheim, N.K. Björkström, O. Sundnes, et al., The biliary epithelium presents antigens to and activates natural killer T cells, Hepatology. 62 (2015) 1249–1259. doi:10.1002/hep.27840.

[20] B. Fosby, T.H. Karlsen, E. Melum, Recurrence and rejection in liver transplantation for primary sclerosing cholangitis, World J. Gastroenterol. 18 (2012) 1–15. doi:10.3748/wjg.v18.i1.1.

[21] M.H. Nathanson, J.L. Boyer, Mechanisms and regulation of bile secretion, Hepatology.

14 (1991) 551–566.

[22] G. Carpino, V. Cardinale, A. Renzi, J.R. Hov, P.B. Berloco, M. Rossi, et al., Activation of biliary tree stem cells within peribiliary glands in primary sclerosing cholangitis, J. Hepatol. 63 (2015) 1220–1228. doi:10.1016/j.jhep.2015.06.018.

[23] R.C. Ayres, J.M. Neuberger, J. Shaw, R. Joplin, D.H. Adams, Intercellular adhesion molecule-1 and MHC antigens on human intrahepatic bile duct cells: effect of pro-inflammatory cytokines, Gut. 34 (2006) 1245–1249.

[24] K.N. Lazaridis, N.F. LaRusso, The Cholangiopathies, Mayo Clin. Proc. 90 (2015) 791–800. doi:10.1016/j.mayocp.2015.03.017.

[25] S.M. Cruickshank, J. Southgate, P.J. Selby, L.K. Trejdosiewicz, Expression and cytokine regulation of immune recognition elements by normal human biliary epithelial and established liver cell lines in vitro, J. Hepatol. 29 (1998) 550–558.

[26] A.H. Sharpe, Mechanisms of costimulation, Immunol. Rev. 229 (2009) 5–11. doi:10.1111/j.1600-065X.2009.00784.x.

[27] J.H. Tabibian, S.P. O'Hara, P.L. Splinter, C.E. Trussoni, N.F. LaRusso, Cholangiocyte senescence by way of N-ras activation is a characteristic of primary sclerosing cholangitis, Hepatology. 59 (2014) 2263–2275. doi:10.1002/hep.26993.

[28] S. O'Brien, M. Keogan, M. Casey, G. Duffy, D. McErlean, M.X. Fitzgerald, et al., Biliary complications of cystic fibrosis, Gut. 33 (1992) 387–391.

[29] R. Safadi, M. Ohta, C.E. Alvarez, M.I. Fiel, M. Bansal, W.Z. Mehal, et al., Immune stimulation of hepatic fibrogenesis by CD8 cells and attenuation by transgenic interleukin-10 from hepatocytes, Gastroenterology. 127 (2004) 870–882.

[30] M.-M. Garcia-Barceló, M.-Y. Yeung, X.-P. Miao, C.S.-M. Tang, G. Cheng, G. Chen,

et al., Genome-wide association study identifies a susceptibility locus for biliary atresia on 10q24.2, Hum. Mol. Genet. 19 (2010) 2917–2925. doi:10.1093/hmg/ddq196.

[31] O. Park, W.-I. Jeong, L. Wang, H. Wang, Z.-X. Lian, M.E. Gershwin, et al., Diverse roles of invariant natural killer T cells in liver injury and fibrosis induced by carbon tetrachloride, Hepatology. 49 (2009) 1683–1694. doi:10.1002/hep.22813.

[32] S. Radaeva, R. Sun, B. Jaruga, V.T. Nguyen, Z. Tian, B. Gao, Natural killer cells ameliorate liver fibrosis by killing activated stellate cells in NKG2D-dependent and tumor necrosis factor-related apoptosis-inducing ligand-dependent manners, Gastroenterology. 130 (2006) 435–452. doi:10.1053/j.gastro.2005.10.055.

[33] A. Melhem, N. Muhanna, A. Bishara, C.E. Alvarez, Y. Ilan, T. Bishara, et al., Anti-fibrotic activity of NK cells in experimental liver injury through killing of activated HSC, J. Hepatol. 45 (2006) 60–71. doi:10.1016/j.jhep.2005.12.025.

[34] M. Kummen, K. Holm, J.A. Anmarkrud, S. Nygård, M. Vesterhus, M.L. Høivik, et al., The gut microbial profile in patients with primary sclerosing cholangitis is distinct from patients with ulcerative colitis without biliary disease and healthy controls, Gut. (2016). doi:10.1136/gutjnl-2015-310500.

[35] X. Jiang, T.H. Karlsen, Genetics of primary sclerosing cholangitis and pathophysiological implications, Nature Publishing Group. (2017) 1–17. doi:10.1038/nrgastro.2016.154.

[36] N.G. Rossen, S. Fuentes, K. Boonstra, G.R. D'Haens, H.G. Heilig, E.G. Zoetendal, et al., The mucosa-associated microbiota of PSC patients is characterized by low diversity and low abundance of uncultured Clostridiales II, J Crohns Colitis. 9 (2015) 342–348. doi:10.1093/ecco-jcc/jju023.

[37] D. Kevans, A.D. Tyler, K. Holm, K.K. Jorgensen, M.H. Vatn, T.H. Karlsen, et al., Characterization of Intestinal Microbiota in Ulcerative Colitis Patients with and without Primary Sclerosing Cholangitis, J Crohns Colitis. 10 (2016) 330–337. doi:10.1093/ecco-jcc/jjv204.

[38] M.N. Quraishi, M. Sergeant, G. Kay, T. Iqbal, J. Chan, C. Constantinidou, et al., The gut-adherent microbiota of PSC-IBD is distinct to that of IBD, Gut. (2016). doi:10.1136/gutjnl-2016-311915.

[39] J. Sabino, S. Vieira-Silva, K. Machiels, M. Joossens, G. Falony, V. Ballet, et al., Primary sclerosing cholangitis is characterised by intestinal dysbiosis independent from IBD, Gut. 65 (2016) 1681–1689. doi:10.1136/gutjnl-2015-311004.

[40] E. Liaskou, H.W. Zimmermann, K.-K. Li, Y.H. Oo, S. Suresh, Z. Stamataki, et al., Monocyte subsets in human liver disease show distinct phenotypic and functional characteristics, Hepatology. 57 (2013) 385–398. doi:10.1002/hep.26016.

[41] K.N. Lazaridis, G.J. Gores, Cholangiocarcinoma, Gastroenterology. 128 (2005) 1655–1667.

[42] M.L. Balmer, E. Slack, A. de Gottardi, M.A.E. Lawson, S. Hapfelmeier, L. Miele, et al., The liver may act as a firewall mediating mutualism between the host and its gut commensal microbiota, Sci Transl Med. 6 (2014) 237ra66. doi:10.1126/scitranslmed.3008618.

[43] S.A. Khan, M.B. Toledano, S.D. Taylor-Robinson, Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma, HPB (Oxford). 10 (2008) 77–82. doi:10.1080/13651820801992641.

[44] H.C. Jeffery, B. van Wilgenburg, A. Kurioka, K. Parekh, K. Stirling, S. Roberts, et al.,

Biliary epithelium and liver B cells exposed to bacteria activate intrahepatic MAIT cells through MR1, J. Hepatol. 64 (2016) 1118–1127. doi:10.1016/j.jhep.2015.12.017.

[45] N.F. LaRusso, B.L. Shneider, D. Black, G.J. Gores, S.P. James, E. Doo, et al., Primary sclerosing cholangitis: summary of a workshop, in: Hepatology, 2006: pp. 746–764. doi:10.1002/hep.21337.

[46] M. Jaiswal, N.F. LaRusso, L.J. Burgart, G.J. Gores, Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism, Cancer Res. 60 (2000) 184–190.

[47] A. Bergquist, A. Ekbom, R. Olsson, D. Kornfeldt, L. Lööf, A. Danielsson, et al., Hepatic and extrahepatic malignancies in primary sclerosing cholangitis, J. Hepatol. 36 (2002) 321–327.

[48] K. Burak, P. Angulo, T.M. Pasha, K. Egan, J. Petz, K.D. Lindor, Incidence and Risk Factors for Cholangiocarcinoma in Primary Sclerosing Cholangitis, Am. J. Gastroenterol. 99 (2004) 523–526. doi:10.1016/S0168-8278(01)00288-4.

[49] U. Broome, R. Olsson, L. Lööf, G. Bodemar, R. Hultcrantz, A. Danielsson, et al., Natural history and prognostic factors in 305 Swedish patients with primary sclerosing cholangitis, Gut. 38 (1996) 610–615.

[50] L. Okolicsanyi, L. Fabris, S. Viaggi, N. Carulli, M. Podda, G. Ricci, Primary sclerosing cholangitis: clinical presentation, natural history and prognostic variables: an Italian multicentre study. The Italian PSC Study Group, Eur J Gastroenterol Hepatol. 8 (1996) 685–691.

[51] K.M. Boberg, A. Bergquist, S. Mitchell, A. Pares, F. Rosina, U. Broome, et al., Cholangiocarcinoma in primary sclerosing cholangitis: risk factors and clinical

presentation, Scand. J. Gastroenterol. 37 (2002) 1205–1211.

[52] C.Y. Ponsioen, S.M.E. Vrouenraets, W. Prawirodirdjo, R. Rajaram, E.A.J. Rauws, C.J.J. Mulder, et al., Natural history of primary sclerosing cholangitis and prognostic value of cholangiography in a Dutch population, Gut. 51 (2002) 562–566.

[53] S.A. Ahrendt, H.A. Pitt, A. Nakeeb, A.S. Klein, K.D. Lillemoe, A.N. Kalloo, et al., Diagnosis and management of cholangiocarcinoma in primary sclerosing cholangitis, J. Gastrointest. Surg. 3 (1999) 357–67– discussion 367–8.

[54] M. Jaiswal, N.F. LaRusso, R.A. Shapiro, T.R. Billiar, G.J. Gores, Nitric oxide-mediated inhibition of DNA repair potentiates oxidative DNA damage in cholangiocytes, Gastroenterology. 120 (2001) 190–199.

[55] G.M. Hirschfield, T.H. Karlsen, K.D. Lindor, D.H. Adams, Primary sclerosing cholangitis, Lancet. 382 (2013) 1587–1599. doi:10.1016/S0140-6736(13)60096-3.

[56] K.N. Lazaridis, N.F. LaRusso, Primary Sclerosing Cholangitis, N. Engl. J. Med. 375 (2016) 1161–1170. doi:10.1056/NEJMra1506330.

[57] J. Park, L. Tadlock, G.J. Gores, T. Patel, Inhibition of interleukin 6-mediated mitogen-activated protein kinase activation attenuates growth of a cholangiocarcinoma cell line, Hepatology. 30 (1999) 1128–1133. doi:10.1002/hep.510300522.

[58] H. Takikawa, T. Manabe, Primary sclerosing cholangitis in Japan--analysis of 192 cases, J. Gastroenterol. 32 (1997) 134–137.

[59] K. Taniguchi, L.-W. Wu, S.I. Grivennikov, P.R. de Jong, I. Lian, F.-X. Yu, et al., A gp130–Src–YAP module links inflammation to epithelial regeneration, Nature. 519 (2015) 57–62. doi:10.1038/nature14228.

[60] E. Björnsson, P. Angulo, Cholangiocarcinoma in Young Individuals With and Without

Primary Sclerosing Cholangitis, Am. J. Gastroenterol. 102 (2007) 1677–1682. doi:10.1007/s10620-005-2927-8.

[61] A.K. Singal, C.M. Stanca, V. Clark, L. Dixon, C. Levy, J.A. Odin, et al., Natural history of small duct primary sclerosing cholangitis: a case series with review of the literature, Hepatol Int. 5 (2011) 808–813. doi:10.1007/s12072-011-9260-4.

[62] H. Song, K.K. Mak, L. Topol, K. Yun, J. Hu, L. Garrett, et al., Mammalian Mst1 and Mst2 kinases play essential roles in organ size control and tumor suppression, Proceedings of the National Academy of Sciences. 107 (2010) 1431–1436. doi:10.1073/pnas.0911409107.

[63] D. Yamada, S. Rizvi, N. Razumilava, S.F. Bronk, J.I. Davila, M.D. Champion, et al., IL-33 facilitates oncogene-induced cholangiocarcinoma in mice by an interleukin-6-sensitive mechanism, Hepatology. 61 (2015) 1627–1642. doi:10.1002/hep.27687.

[64] N. Chalasani, A. Baluyut, A. Ismail, A. Zaman, G. Sood, R. Ghalib, et al., Cholangiocarcinoma in patients with primary sclerosing cholangitis: a multicenter case-control study, Hepatology. 31 (2000) 7–11. doi:10.1002/hep.510310103.

[65] D. Zhou, C. Conrad, F. Xia, J.-S. Park, B. Payer, Y. Yin, et al., Mst1 and Mst2 Maintain Hepatocyte Quiescence and Suppress Hepatocellular Carcinoma Development through Inactivation of the Yap1 Oncogene, Cancer Cell. 16 (2009) 425–438. doi:10.1016/j.ccr.2009.09.026.

[66] M.B. Valle, E. Björnsson, B. Lindkvist, Mortality and cancer risk related to primary sclerosing cholangitis in a Swedish population-based cohort, Liver International. 21 (2011) n/a–n/a. doi:10.1111/j.1478-3231.2011.02614.x.

[67] J. Li, N. Razumilava, G.J. Gores, S. Walters, T. Mizuochi, R. Mourya, et al., Biliary

repair and carcinogenesis are mediated by IL-33-dependent cholangiocyte proliferation, J. Clin. Invest. 124 (2014) 3241–3251. doi:10.1172/JCI73742.

[68] A. Bergquist, H. Glaumann, B. Persson, U. Broome, Risk factors and clinical presentation of hepatobiliary carcinoma in patients with primary sclerosing cholangitis: a case-control study, Hepatology. 27 (1998) 311–316. doi:10.1002/hep.510270201.

[69] A. Bergquist, H. Glaumann, P. Stål, G.S. Wang, U. Broome, Biliary dysplasia, cell proliferation and nuclear DNA-fragmentation in primary sclerosing cholangitis with and without cholangiocarcinoma, J. Intern. Med. 249 (2001) 69–75.

[70] K.A. Fleming, K.M. Boberg, H. Glaumann, A. Bergquist, D. Smith, O.P. Clausen, Biliary dysplasia as a marker of cholangiocarcinoma in primary sclerosing cholangitis, J. Hepatol. 34 (2001) 360–365.

[71] J.T. Lewis, J.A. Talwalkar, C.B. Rosen, T.C. Smyrk, S.C. Abraham, Precancerous bile duct pathology in end-stage primary sclerosing cholangitis, with and without cholangiocarcinoma, Am. J. Surg. Pathol. 34 (2010) 27–34. doi:10.1097/PAS.0b013e3181bc96f9.

[72] M. Cadamuro, G. Nardo, S. Indraccolo, L. Dall'Olmo, L. Sambado, L. Moserle, et al., Platelet-derived growth factor-D and Rho GTPases regulate recruitment of cancer-associated fibroblasts in cholangiocarcinoma, Hepatology. 58 (2013) 1042–1053. doi:10.1002/hep.26384.

[73] M. Cadamuro, M. Vismara, S. Brivio, A. Furlanetto, M. Strazzabosco, L. Fabris, JNK signaling activated by Platelet-Derived Growth Factor D (PDGF-D) stimulates secretion of Vascular Endothelial Growth Factor-C (VEGF-C) by cancer-associated fibroblasts to promote lymphangiogenesis and early metastatization in

cholangiocarcinoma, Digestive and Liver Disease. 47 (2015) e22–e23. doi:10.1016/j.dld.2015.01.052.

[74] P.J. Roberts, C.J. Der, Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer, Oncogene. 26 (2007) 3291–3310. doi:10.1038/72799.

[75] S.E.K. MD, M.A. Emily G Barr Fritcher CT ASCP, M.B. Campion, J.S.V.C. ASCP, B.R.K. PhD, K.C.H.M. PhD, et al., Biliary dysplasia in primary sclerosing cholangitis harbors cytogenetic abnormalities similarto cholangiocarcinoma, Human Pathology. 45 (2014) 1797–1804. doi:10.1016/j.humpath.2014.05.008.

[76] N.W. Werneburg, J.-H. Yoon, H. Higuchi, G.J. Gores, Bile acids activate EGF receptor via a TGF-α-dependent mechanism in human cholangiocyte cell lines, AJP: Gastrointestinal and Liver Physiology. 285 (2003) G31–G36. doi:10.1152/ajpgi.00536.2002.

[77] J.-H. Yoon, G.-Y. Gwak, H.-S. Lee, S.F. Bronk, N.W. Werneburg, G.J. Gores, Enhanced epidermal growth factor receptor activation in human cholangiocarcinoma cells, J. Hepatol. 41 (2004) 808–814. doi:10.1016/j.jhep.2004.07.016.

[78] J.-H. Yoon, H. Higuchi, N.W. Werneburg, S.H. Kaufmann, G.J. Gores, Bile acids induce cyclooxygenase-2 expression via the epidermal growth factor receptor in a human cholangiocarcinoma cell line, Gastroenterology. 122 (2002) 985–993.

[79] B.K. Chung, G.M. Hirschfield, Immunogenetics in primary sclerosing cholangitis, Curr. Opin. Gastroenterol. 33 (2017) 93–98. doi:10.1097/MOG.0000000000000336.

[80] C.E. Trussoni, J.H. Tabibian, P.L. Splinter, S.P. O'Hara, Lipopolysaccharide (LPS)-Induced Biliary Epithelial Cell NRas Activation Requires Epidermal Growth Factor

Receptor (EGFR), PLoS ONE. 10 (2015) e0125793. doi:10.1371/journal.pone.0125793.

[81] E. Liaskou, E.K.K. Henriksen, K. Holm, F. Kaveh, D. Hamm, J. Fear, et al., High-throughput T-cell receptor sequencing across chronic liver diseases reveals distinct disease-associated repertoires, Hepatology. 63 (2016) 1608–1619. doi:10.1002/hep.28116.

[82] B.K. Chung, B.T. Guevel, G.M. Reynolds, D.B.R.K. Gupta Udatha, E.K.K. Henriksen, Z. Stamataki, et al., Phenotyping and auto-antibody production by liver-infiltrating B cells in primary sclerosing cholangitis and primary biliary cholangitis, Journal of Autoimmunity. (2016). doi:10.1016/j.jaut.2016.10.003.

[83] S. Kobayashi, N.W. Werneburg, S.F. Bronk, S.H. Kaufmann, G.J. Gores, Interleukin-6 contributes to Mcl-1 up-regulation and TRAIL resistance via an Akt-signaling pathway in cholangiocarcinoma cells, Gastroenterology. 128 (2005) 2054–2065.

[84] W.G. Haigh, S.P. Lee, Identification of Oxysterols in Human Bile and Pigment Gallstones, Gastroenterology. 121 (2001) 118–123. doi:10.1053/gast.2001.25513.

[85] P.J. Trivedi, G.M. Hirschfield, Treatment of autoimmune liver disease: current and future therapeutic options, Ther Adv Chronic Dis. 4 (2013) 119–141. doi:10.1177/2040622313478646.

[86] J.-H. Yoon, A.E. Canbay, N.W. Werneburg, S.P. Lee, G.J. Gores, Oxysterols induce cyclooxygenase-2 expression in cholangiocytes: implications for biliary tract carcinogenesis, Hepatology. 39 (2004) 732–738. doi:10.1002/hep.20125.

[87] R. Kuver, Mechanisms of oxysterol-induced disease: insights from the biliary system, Clinical Lipidology. 7 (2012) 537–548. doi:10.2217/clp.12.53.

[88] J.R. Dwyer, N. Sever, M. Carlson, S.F. Nelson, P.A. Beachy, F. Parhami, Oxysterols are novel activators of the hedgehog signaling pathway in pluripotent mesenchymal cells, J. Biol. Chem. 282 (2007) 8959–8968. doi:10.1074/jbc.M611741200.

[89] L.M. Sollid, B. Jabri, Triggers and drivers of autoimmunity: lessons from coeliac disease, Nat. Rev. Immunol. 13 (2013) 294–302. doi:10.1038/nri3407.

[90] M. El Khatib, A. Kalnytska, V. Palagani, U. Kossatz, M.P. Manns, N.P. Malek, et al., Inhibition of hedgehog signaling attenuates carcinogenesis in vitro and increases necrosis of cholangiocellular carcinoma, Hepatology. 57 (2013) 1035–1045. doi:10.1002/hep.26147.

[91] D. Komichi, S. Tazuma, T. Nishioka, H. Hyogo, K. Chayama, Glycochenodeoxycholate plays a carcinogenic role in immortalized mouse cholangiocytes via oxidative DNA damage, Free Radic. Biol. Med. 39 (2005) 1418–1427. doi:10.1016/j.freeradbiomed.2005.07.005.

[92] S. Rizvi, J.E. Eaton, G.J. Gores, Primary Sclerosing Cholangitis as a Premalignant Biliary Tract Disease: Surveillance and Management, Clinical Gastroenterology and Hepatology. 13 (2015) 2152–2165. doi:10.1016/j.cgh.2015.05.035.

[93] B.P. Lewis, C.B. Burge, D.P. Bartel, Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets, Cell. 120 (2005) 15–20. doi:10.1016/j.cell.2004.12.035.

[94] L.P. Garo, G. Murugaiyan, Contribution of MicroRNAs to autoimmune diseases, Cellular and Molecular Life Sciences. 73 (2016) 2041–2051. doi:10.1007/s00018-016-2167-4.

[95] P.D. Robbins, A. Dorronsoro, C.N. Booker, Regulation of chronic inflammatory and

immune processes by extracellular vesicles, J. Clin. Invest. 126 (2016) 1173–1180. doi:10.1172/JCI81131.

[96] F. Bernuzzi, F. Marabita, A. Lleo, M. Carbone, M. Mirolo, M. Marzioni, et al., Serum microRNAs as novel biomarkers for primary sclerosing cholangitis and cholangiocarcinoma, Clin. Exp. Immunol. 185 (2016) 61–71. doi:10.1111/cei.12776.

[97] M. Krawczyk, A. Höblinger, F. Mihalache, F. Grünhage, M. Acalovschi, F. Lammert, et al., Digestive and Liver Disease, Digestive and Liver Disease. 45 (2013) 612–615. doi:10.1016/j.dld.2012.12.017.

[98] N. Oishi, M.R. Kumar, S. Roessler, J. Ji, M. Forgues, A. Budhu, et al., Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma, Hepatology. 56 (2012) 1792–1803. doi:10.1002/hep.25890.

[99] C.-N. Yeh, W.-H. Weng, G. Lenka, L.-C. Tsao, K.-C. Chiang, S.-T. Pang, et al., cDNA microarray profiling of rat cholangiocarcinoma induced by thioacetamide, Mol Med Rep. 8 (2013) 350–360. doi:10.3892/mmr.2013.1516.

[100] A. Strasser, The role of BH3-only proteins in the immune system, Nat. Rev. Immunol. 5 (2005) 189–200. doi:10.1038/sj.cdd.4401204.

[101] L. Meng, M. Quezada, P. Levine, Y. Han, K. McDaniel, T. Zhou, et al., Functional Role of Cellular Senescence in Biliary Injury, The American Journal of Pathology. 185 (2015) 602–609. doi:10.1016/j.ajpath.2014.10.027.

[102] R.V. Sionov, S.A. Vlahopoulos, Z. Granot, Regulation of Bim in Health and Disease, Oncotarget. 6 (2015) 23058–23134. doi:10.18632/oncotarget.5492.

[103] J.M. Loo, A. Scherl, A. Nguyen, F.Y. Man, E. Weinberg, Z. Zeng, et al., Extracellular

metabolic energetics can promote cancer progression, Cell. 160 (2015) 393–406. doi:10.1016/j.cell.2014.12.018.

[104] K.-S. Yoo, W.T. Lim, H.S. Choi, Biology of Cholangiocytes: From Bench to Bedside, Gut and Liver. 10 (2016) 687–698. doi:10.5009/gnl16033.

[105] E. Contardi, G.L. Palmisano, P.L. Tazzari, A.M. Martelli, F. Falà, M. Fabbi, et al., CTLA-4 is constitutively expressed on tumor cells and can trigger apoptosis upon ligand interaction, Int. J. Cancer. 117 (2005) 538–550. doi:10.1002/ijc.21155.

[106] A. Celli, F.G. Que, Dysregulation of apoptosis in the cholangiopathies and cholangiocarcinoma, Semin. Liver Dis. 18 (1998) 177–185. doi:10.1055/s-2007-1007153.

[107] D.M. Pardoll, The blockade of immune checkpoints in cancer immunotherapy, Nat Rev Cancer. 12 (2012) 252–264. doi:10.1038/nrc3239.

[108] M. Vétizou, J.M. Pitt, R. Daillère, P. Lepage, N. Waldschmitt, C. Flament, et al., Anticancer immunotherapy by CTLA-4 blockade relies on the gut microbiota, Science. 350 (2015) 1079–1084. doi:10.1126/science.aad1329.

[109] E. Melum, T.H. Karlsen, E. Schrumpf, A. Bergquist, E. Thorsby, K.M. Boberg, et al., Cholangiocarcinoma in primary sclerosing cholangitis is associated with NKG2D polymorphisms, Hepatology. 47 (2008) 90–96. doi:10.1002/hep.21964.

[110] L.L. Lanier, NKG2D Receptor and Its Ligands in Host Defense, Cancer Immunology Research. 3 (2015) 575–582. doi:10.1158/2326-6066.CIR-15-0098.

[111] K. Wiencke, Primary sclerosing cholangitis is associated to an extended B8-DR3 haplotype including particular MICA and MICB alleles, Hepatology. 34 (2001) 625–630. doi:10.1053/jhep.2001.27543.

[112] M. Forsbring, E.S. Vik, B. Dalhus, T.H. Karlsen, A. Bergquist, E. Schrumpf, et al., Catalytically impaired hMYH and NEIL1 mutant proteins identified in patients with primary sclerosing cholangitis and cholangiocarcinoma, Carcinogenesis. 30 (2009) 1147–1154. doi:10.1093/carcin/bgp118.

[113] E. Marouli, M. Graff, C. Medina-Gomez, K.S. Lo, A.R. Wood, T.R. Kjaer, et al., Rare and low-frequency coding variants alter human adult height, Nature. 542 (2017) 186–190. doi:10.1038/nature21039.

[114] N. Kanno, G. LeSage, S. Glaser, D. Alvaro, G. Alpini, Functional heterogeneity of the intrahepatic biliary epithelium, Hepatology. 31 (2000) 555–561. doi:10.1002/hep.510310302.

[115] P.S. Vestentoft, P. Jelnes, B.M. Hopkinson, B. Vainer, K. Møllgård, B. Quistorff, et al., Three-dimensional reconstructions of intrahepatic bile duct tubulogenesis in human liver, BMC Dev. Biol. 11 (2011) 56. doi:10.1186/1471-213X-11-56.

[116] A.I. Masyuk, T.V. Masyuk, N.F. LaRusso, Cholangiocyte primary cilia in liver health and disease, Dev. Dyn. 237 (2008) 2007–2012. doi:10.1002/dvdy.21530.

[117] F.P. Lemaigre, Mechanisms of liver development: concepts for understanding liver disorders and design of novel therapies, Gastroenterology. 137 (2009) 62–79. doi:10.1053/j.gastro.2009.03.035.

[118] J. Fevery, C. Verslype, G. Lai, R. Aerts, W. Van Steenbergen, Incidence, diagnosis, and therapy of cholangiocarcinoma in patients with primary sclerosing cholangitis, Dig Dis Sci. 52 (2007) 3123–3135. doi:10.1007/s10620-006-9681-4.

[119] E. Björnsson, R. Olsson, A. Bergquist, S. Lindgren, B. Braden, R.W. Chapman, et al., The natural history of small-duct primary sclerosing cholangitis, Gastroenterology. 134

(2008) 975–980. doi:10.1053/j.gastro.2008.01.042.

[120] G. Alpini, S.S. Glaser, Y. Ueno, L. Pham, P.V. Podila, A. Caligiuri, et al., Heterogeneity of the proliferative capacity of rat cholangiocytes after bile duct ligation, Am. J. Physiol. 274 (1998) G767–75.

[121] G.D. LeSage, A. Benedetti, S. Glaser, L. Marucci, Z. Tretjak, A. Caligiuri, et al., Acute carbon tetrachloride feeding selectively damages large, but not small, cholangiocytes from normal rat liver, Hepatology. 29 (1999) 307–319. doi:10.1002/hep.510290242.

[122] M. Strazzabosco, L. Fabris, Development of the bile ducts: essentials for the clinical hepatologist, J. Hepatol. 56 (2012) 1159–1170. doi:10.1016/j.jhep.2011.09.022.

[123] X.-M. Chen, S.P. O'Hara, N.F. LaRusso, The immunobiology of cholangiocytes, Immunol. Cell Biol. 86 (2008) 497–505. doi:10.1038/icb.2008.37.

[124] X.-M. Chen, S.P. O'Hara, J.B. Nelson, P.L. Splinter, A.J. Small, P.S. Tietz, et al., Multiple TLRs are expressed in human cholangiocytes and mediate host epithelial defense responses to Cryptosporidium parvum via activation of NF-kappaB, J. Immunol. 175 (2005) 7447–7456.

[125] T. Yokoyama, A. Komori, M. Nakamura, Y. Takii, T. Kamihira, S. Shimoda, et al., Human intrahepatic biliary epithelial cells function in innate immunity by producing IL-6 and IL-8 via the TLR4-NF-kappaB and -MAPK signaling pathways, Liver International. 26 (2006) 467–476. doi:10.1097/01.LAB.0000080606.96797.A5.

[126] J. Han, R.J. Ulevitch, Limiting inflammatory responses during activation of innate immunity, Nat Immunol. 6 (2005) 1198–1205. doi:10.1038/ni1274.

[127] T.D. Gilmore, Introduction to NF-kappaB: players, pathways, perspectives, Oncogene. 25 (2006) 6680–6684. doi:10.1038/sj.onc.1209954.

[128] S. Devkota, Y. Wang, M.W. Musch, V. Leone, H. Fehlner-Peach, A. Nadimpalli, et al., Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10−/− mice, Nature. (2012). doi:10.1038/nature11225.

[129] S.I. Sayin, A. Wahlstrom, J. Felin, S. Jäntti, H.-U. Marschall, K. Bamberg, et al., Gut microbiota regulates bile acid metabolism by reducing the levels of tauro-beta-muricholic acid, a naturally occurring FXR antagonist, Cell Metab. 17 (2013) 225–235. doi:10.1016/j.cmet.2013.01.003.

[130] J.H. Tabibian, S.P. O'Hara, C.E. Trussoni, P.S. Tietz, P.L. Splinter, T. Mounajjed, et al., Absence of the intestinal microbiota exacerbates hepatobiliary disease in a murine model of primary sclerosing cholangitis, Hepatology. 63 (2016) 185–196. doi:10.1002/hep.27927.

[131] J.M. Ridlon, D.J. Kang, P.B. Hylemon, J.S. Bajaj, Bile acids and the gut microbiome, Curr. Opin. Gastroenterol. 30 (2014) 332–338. doi:10.1097/MOG.0000000000000057.

[132] G. Kakiyama, W.M. Pandak, P.M. Gillevet, P.B. Hylemon, D.M. Heuman, K. Daita, et al., Modulation of the fecal bile acid profile by gut microbiota in cirrhosis, J. Hepatol. 58 (2013) 949–955. doi:10.1016/j.jhep.2013.01.003.

[133] S.E. McGarr, J.M. Ridlon, P.B. Hylemon, Diet, anaerobic bacterial metabolism, and colon cancer: a review of the literature, J Clin Gastroenterol. 39 (2005) 98–109.

[134] U. Broome, A. Scheynius, R. Hultcrantz, Induced expression of heat-shock protein on biliary epithelium in patients with primary sclerosing cholangitis and primary biliary cirrhosis, Hepatology. 18 (1993) 298–303.

[135] H.P. Dienes, A.W. Lohse, G. Gerken, P. Schirmacher, H. Gallati, H.F. Löhr, et al., Bile duct epithelia as target cells in primary biliary cirrhosis and primary sclerosing

cholangitis, Virchows Arch. 431 (1997) 119–124.

[136] D.H. Adams, B. Eksteen, Aberrant homing of mucosal T cells and extra-intestinal manifestations of inflammatory bowel disease, Nat. Rev. Immunol. 6 (2006) 244–251. doi:10.1038/nri1784.

[137] J.-H. Yoon, N.W. Werneburg, H. Higuchi, A.E. Canbay, S.H. Kaufmann, C. Akgul, et al., Bile acids inhibit Mcl-1 protein turnover via an epidermal growth factor receptor/Raf-1-dependent mechanism, Cancer Res. 62 (2002) 6500–6505.

[138] G. Ichim, J. Lopez, S.U. Ahmed, N. Muthalagu, E. Giampazolias, M.E. Delgado, et al., Limited mitochondrial permeabilization causes DNA damage and genomic instability in the absence of cell death, Molecular Cell. 57 (2015) 860–872. doi:10.1016/j.molcel.2015.01.018.

[139] X. Liu, Y. He, F. Li, Q. Huang, T.A. Kato, R.P. Hall, et al., Caspase-3 Promotes Genetic Instability and Carcinogenesis, Molecular Cell. 58 (2015) 284–296. doi:10.1016/j.molcel.2015.03.003.

[140] J.-C. Nault, J. Zucman-Rossi, Genetics of Hepatobiliary Carcinogenesis, Semin. Liver Dis. 31 (2011) 173–187. doi:10.1055/s-0031-1276646.

[141] N. Ishimura, Trail induces cell migration and invasion in apoptosis-resistant cholangiocarcinoma cells, AJP: Gastrointestinal and Liver Physiology. 290 (2005) G129–G136. doi:10.1152/ajpgi.00242.2005.

[142] K. Takeda, Y. Kojima, K. Ikejima, K. Harada, S. Yamashina, K. Okumura, et al., Death receptor 5 mediated-apoptosis contributes to cholestatic liver disease, Proceedings of the National Academy of Sciences. 105 (2008) 10895–10900. doi:10.1073/pnas.0802702105.

[143] M.M. Lovric, C.J. Hawkins, TRAIL treatment provokes mutations in surviving cells, Oncogene. 29 (2010) 5048–5060. doi:10.1038/onc.2010.242.

[144] J.-P. Coppé, K. Kauser, J. Campisi, C.M. Beauséjour, Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence, J. Biol. Chem. 281 (2006) 29568–29574. doi:10.1074/jbc.M603307200.

[145] A. Krtolica, S. Parrinello, S. Lockett, P.Y. Desprez, J. Campisi, Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging, Proc. Natl. Acad. Sci. U.S.a. 98 (2001) 12072–12077. doi:10.1073/pnas.211053698.

[146] S.P. O'Hara, J.H. Tabibian, P.L. Splinter, N.F. LaRusso, The dynamic biliary epithelia: molecules, pathways, and disease, J. Hepatol. 58 (2013) 575–582. doi:10.1016/j.jhep.2012.10.011.

[147] F. Sampaziotis, M. Cardoso de Brito, P. Madrigal, A. Bertero, K. Saeb-Parsy, F.A.C. Soares, et al., Cholangiocytes derived from human induced pluripotent stem cells for disease modeling and drug validation, Nat. Biotechnol. 33 (2015) 845–852. doi:10.1038/nbt.3275.

[148] P. Fickert, M.J. Pollheimer, U. Beuers, C. Lackner, G. Hirschfield, C. Housset, et al., Characterization of animal models for primary sclerosing cholangitis (PSC), J. Hepatol. 60 (2014) 1290–1303. doi:10.1016/j.jhep.2014.02.006.

[149] J. Albores-Saavedra, D.E. Henson, L.H. Sobin, The WHO Histological Classification of Tumors of the Gallbladder and Extrahepatic Bile Ducts. A commentary on the second edition, Cancer. 70 (1992) 410–414.

[150] L.J. Layfield, T.D. Wax, J.G. Lee, P.B. Cotton, Accuracy and morphologic aspects of pancreatic and biliary duct brushings, Acta Cytol. 39 (1995) 11–18.

[151] K.M. Boberg, P. Jebsen, O.P. Clausen, A. Foss, L. Aabakken, E. Schrumpf, Diagnostic benefit of biliary brush cytology in cholangiocarcinoma in primary sclerosing cholangitis, J. Hepatol. 45 (2006) 568–574. doi:10.1016/j.jhep.2006.05.010.

Figure Legends

Figure 1. Several studies support a model of primary sclerosing cholangitis-associated cholangiocarcinoma whereby inflammatory epithelial damage leads to sequential progression from normal non-dysplastic epithelium to low grade dysplasia (LGD), high grade dysplasia (HGD) and ultimately to invasive cholangiocarcinoma. In the clinical setting, presence of dysplastic changes in the biliary epithelium is commonly determined based on biliary brush cytology obtained from the bile ducts during endoscopic retrograde cholangiography. Shown are cytological and histological images and generally accepted cytological criteria to classify biliary brush specimens into the following categories: (i) non-dysplastic, (ii) LGD and (iii) HGD. The main criteria for invasiveness are histological (tumor growth beyond the basement membrane), as there are no clear-cut cytological criteria able to distinguish between HGD and carcinoma [149-151]. Cytological and histological images kindly provided by pathologists Peter Jebsen and Henrik M. Reims at Department of Pathology, Oslo University Hospital Rikshospitalet (Oslo, Norway).

Figure 2. Suggested surveillance procedure for cholangiocarcinoma (CCA) in patients with primary sclerosing cholangitis (PSC). Abbreviations: AFP, alpha-fetoprotein; ERCP, endoscopic retrograde cholangiopancreatography, FISH; fluorescent *in situ* hybridization; MDT, multidisciplinary team, MRI/MRCP, magnetic resonance imaging/magnetic resonance cholangiopancreaticography; US, ultrasound.

Figure 3. Genetic findings highlight potential disease pathways in the etiopathogenesis of primary sclerosing cholangitis (PSC) and development of cholangiocarcinoma (PSC-CCA). PSC is a complex idiopathic cholangiopathy involving the interaction of genetic and undetermined environmental risk factors (dietary, microbiota, toxins). PSC risk loci identified by genome-wide association studies (GWAS) mainly point towards immune cell pathways (*ATXN2*, *BACH2*, *BCL2L11*, *CCDC88B*, *CD28*, *CD226*, *CCL20*, *CLECK16A*, *CTLA4*, *FOXP1*, *GPR35*, *HDAC7*, *IL2*, *IL2RA*, *IL21*, *MST1*, *NFKB1*, *PRKD2*, *PSMG1*, *RIC8B*, *RFX4*, *SH2B3*, *SIK2*, *SOCS1*, *TNFRSF14*, *TCF4*, *UBASH3A*) however several putative risk genes may be involved in the transformation of cholangiocytes (*ATXN2*, *CD226*, *FOXP1*, *MUTYH*, *NEIL1*, *NFKB1*, *STRN4*) and promote the development of PSC-CCA.

Figure 4. Cholangiocyte heterogeneity is associated with distinct phenotypes of primary sclerosing cholangitis (PSC). Small cholangiocytes found most often in the intrahepatic ducts show a flattened or cuboidal morphology, large nucleus to cytoplasm ratio and microvilli that extend towards the biliary lumen. Large cholangiocytes located mainly in the extrahepatic bile ducts, appear columnar, have a small nucleus to cytoplasm ratio and project a primary cilium into the bile duct lumen. Cholangiocarcinoma (PSC-CCA) develops more frequently in small duct PSC compared to large duct PSC in patients and may reflect may reflect differential responses to chronic cholangiocyte activation.

Figure 5. Primary sclerosing cholangitis (PSC) involves a cycle of immune-mediated damage to the cholangiocytes leading to fibrosis and cholangiocarcinoma (PSC-CCA). Activated T cells and macrophage trigger the apoptosis and senescence of cholangiocytes resulting in the production of pro-inflammatory cytokines and chemokines that act on hepatic stellate cells and lead to liver fibrosis. Cholangiocyte activation can induce the upregulation of human leukocyte antigen (HLA) molecules but it is unknown if activated cholangiocytes directly stimulate T cell expansion and antibody production of B cells as disease-relevant antigens in PSC are unknown. Chronic cholangiocyte activation in PSC can lead to the development of PSC-CCA, which restricts the passage of bile through the bile ducts and commonly metastasizes to the liver, peritoneum, intra-abdominal lymph nodes and lungs.

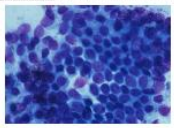
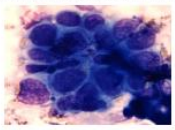
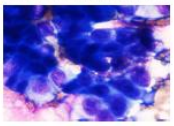
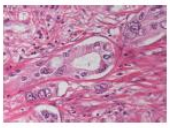
	PSC without dysplasia	Low-grade dysplasia	High-grade dysplasia	Invasive cancer
Cytological/ histological appearance				
Diagnostic criteria	**Normal/irregular non-dysplastic epithelium** Cytological criteria: Sheets of cells in monolayer with even, relatively dense chromatin pattern and no nucleoli.	**Low-grade dysplasia** Cytological criteria: Sheets and clusters of cells with nuclear overlap, smooth nuclear shape and moderately increased nuclear/cytoplasmatic ratio. No dissociation of single cells. Mild clumping of nuclear chromatin. Small but visible nucleoli.	**High-grade dysplasia** Cytological criteria: Clusters of atypical cells with nuclear overlap and crowding. Marked increase in nuclear/cytoplasmatic ratio. Nuclear membrane irregular with signs of moulding. Nuclei with course chromatin and prominent nucleoli.	**Invasive cancer** Histological criteria: Tumour growth beyond the basement membrane of the bile duct.

Fig. 1

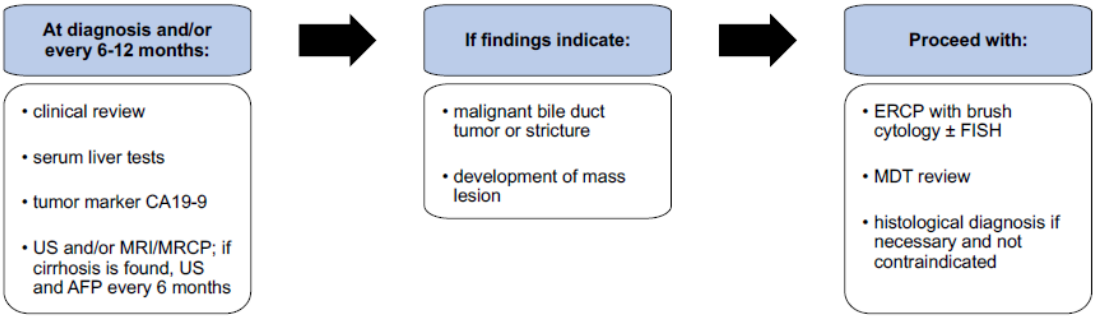


Fig. 2

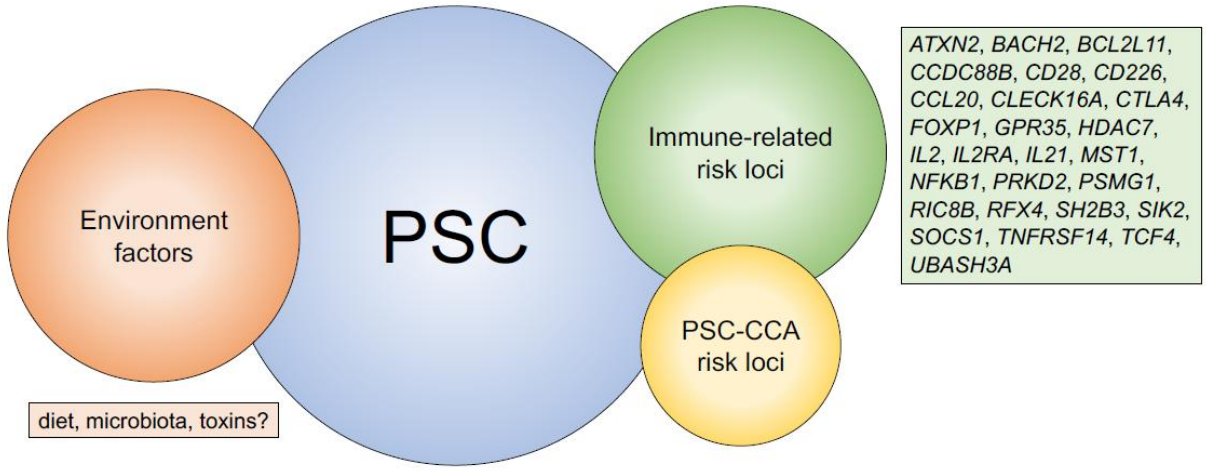


Fig. 3

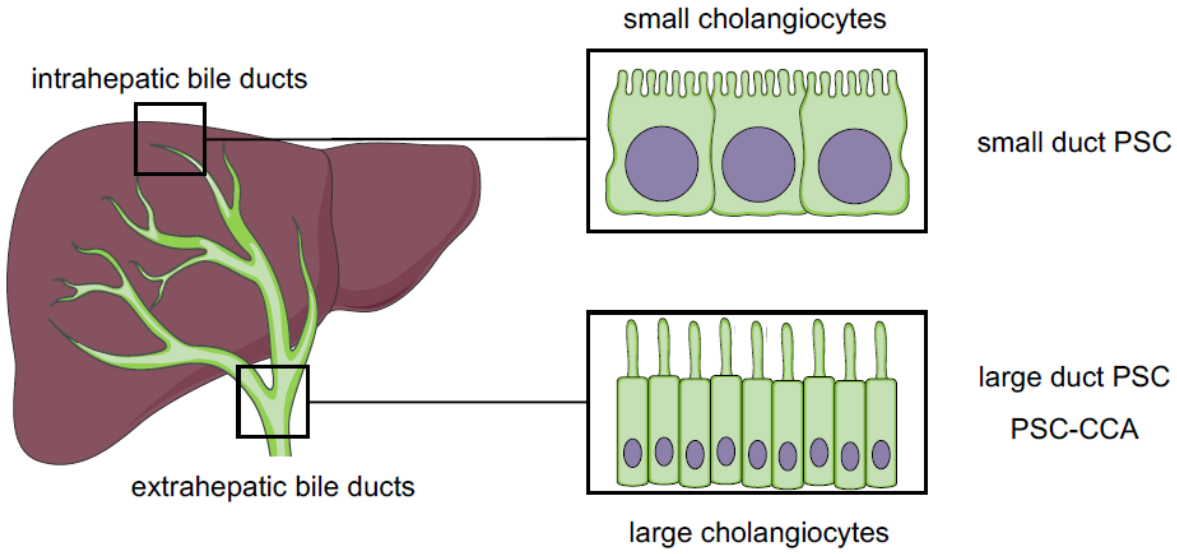


Fig. 4

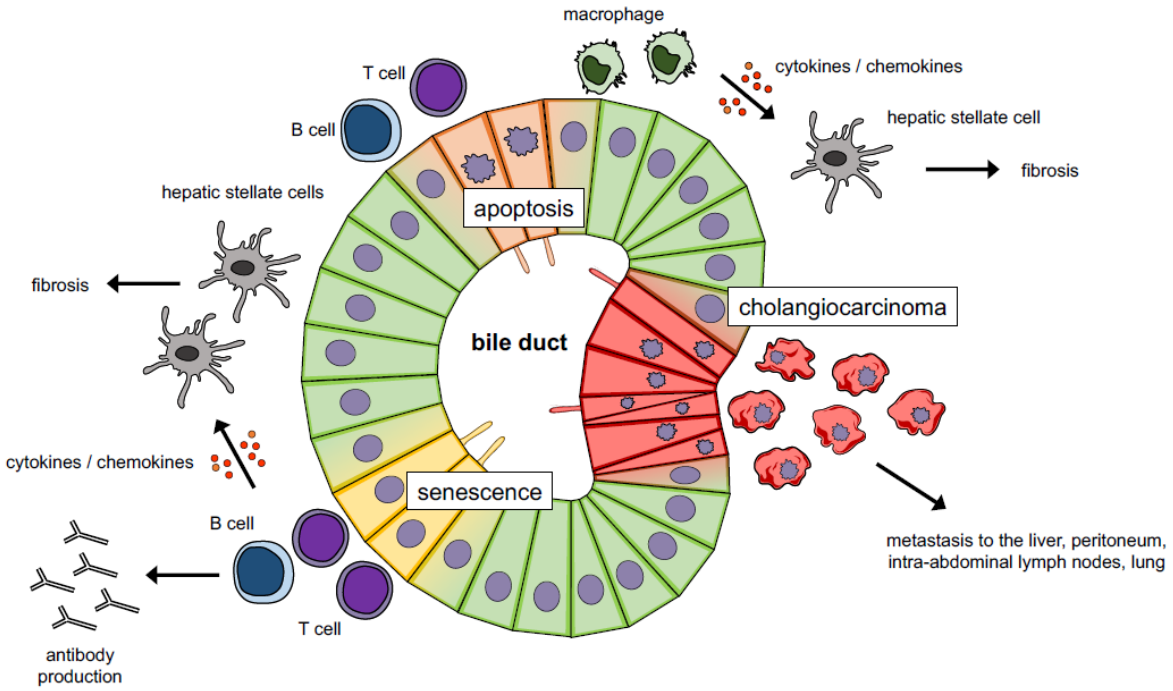


Fig. 5

Table 1. Non-HLA genome-wide significant risk loci ($P \leq 5 \times 10^{-8}$) in primary sclerosing cholangitis.

Putative non-HLA candidate gene(s) associated with PSC. Suspected functions of the candidate genes are given but are based on circumstantial evidence as definitive interactions linking PSC-associated loci to specific genes and protein functions are lacking.

Risk locus	Candidate risk gene(s)	Reference(s)	Putative pathophysiological implication of candidate gene(s)
1p36	*MMEL1, TNFRSF14*	Folseraas *et al*. (2012)	*MMEL1*: Membrane metallo-endopeptidase family. Little is known about its function. Potentially involved in neuropeptide degradation. *TNFRSF4*: Acts as a molecular switch modulating T cell activation and regulation of immune tolerance.
2q13	*BCL2L11*	Melum *et al*. (2011)	Encodes BCL-2-like protein (BCL2-L-11) which interacts with other BCL-2 protein family members and acts as apoptopic activator. Critical for apoptosis and termination of inflammatory responses in B and T cells, granulocytes and macrophages. Absence of BCL2-L-11 promotes autoimmunity, whereas early induction of BCL2-L-11 may lead to chronic inflammation and tumor progression.
2q33	*CD28, CTLA4*	Liu *et al*. (2013)	*CD28*: Co-stimulatory receptor that by binding B7 ligands (CD80 and CD86) is crucial for T cell activation, survival and proliferation. Liver-infiltrating T cells in patients with PSC observed to downregulate CD28 and produce higher levels of IFN-γ and TNF-α. CTLA-4: Major negative regulator of T-cell development and function by binding to CD80 and CD86 in competition with CD28. Blockade of CTLA4 blockade has demonstrated gut microbiota-dependent anticancer effects.
2q36	*CCL20*	Ellinghaus *et al*. (2016)	Ligand for chemokine receptor CCR6. Highly expressed in the liver and in peripheral blood lymphocytes. Recruits CCR6+ lymphocytes to liver. CCL20 is expressed on cholangiocytes and putatively recruits IL-17-secreting CCR6+ T cells to the biliary epithelium.
2q37	*GPR35*	Ellinghaus *et al*. (2013)	Member of G-protein-coupled metabolite-sensing receptors. Expressed on several immune cells and subsets of gut epithelium. Putatively act as receptors for kynurenic acid and chemokine ligand 17. Kynurenic acid is a metabolite in the tryptophan pathway and is present in high concentrations in bile. Synthesis of kynurenic acid is rate-limited by the immune-regulatory enzyme indoleamine 2,3-dioxygenase. Participates in regulation of IL-4 release from natural killer T cells.
3p13	*FOXP1*	Ji *et al.* (2017)	Member of forkhead box transcription factor family. Relative high expression in the gut. Maintains quiescence in CD3+ CD4+ T cells. Involved in regulation of CD4+ proliferation and differentiation. May act as tumor suppressor.
3p21	*MST1*	Melum *et al*. (2011)	Serine-threonine kinase. Participates in the Hippo signaling pathway. High expression in liver. Involved in cell morphogenesis, proliferation, apoptosis and tumor suppression in the liver. Affects hepatocyte quiescence and liver size. Suppresses hepatocellular carcinoma through

			inactivation of the YAP1 oncogene. Genetic variants in *MST1* are associated to risk of sporadic cholangiocarcinoma.
4q24	*NFKB1*	Ellinghaus *et al*. (2016)	Member of NF-kappaB family. Part of the pleiotropic NF-kappaB transcription factor family. Involved in the regulation of multiple biological processes including inflammation, tumorigenesis and apoptosis.
4q27	*IL2, IL21*	Liu *et al*. (2013)	*IL2*: Cytokine with broad range of roles in the immune system, among other crucial for T-cell proliferation and regulatory T-cell activity. Liver derived T cells from PSC patients produce low levels of IL-2 compared to other autoimmune liver diseases and healthy controls. *IL21*: Cytokine that regulates the activity of multiple target cells (including macrophages, natural killer cells, B- and T-cells) in the innate- and adaptive immune response.
6q15	*BACH2*	Liu *et al*. (2013)	Transcription factor that regulates B-cell differentiation. Inhibits differentiation of effector-memory T cells. Implicated in the anti-viral innate immune response.
10p15	*IL2RA*	Srivastava *et al*. (2012)	Encodes the IL-2 receptor subunit alpha (CD25), which binds to CD122 and forms the high-affinity receptor for IL-2. Binds IL-2 and mediates its signaling effects. *IL2RA*-deficient mice develop T-cell infiltrates in the liver and colon resulting in portal inflammation and colitis.
11q13	*CCDC88B*	Ji *et al.* (2017)	Member of the hook-related protein family and regulates the maturation and effector functions of T cells.
11q23	*SIK2*	Liu *et al*. (2013)	Serine-threonine kinase that can phosphorylate HDAC7 (see below). Affects IL-10 in macrophages. Liver-specific SIK2 knockdown in mice results in steatosis, insulin resistance and inflammation.
12q13	*HDAC7*	Liu *et al*. (2013)	Class IIa histone deacetylase. Highly expressed in CD4+CD8+ thymocytes and involved in TCR-dependent differentiation in the thymus. Dephosphorylation of HCDA7 results in HDAC7-accumluation in the nucleus and suppression of genes essential for cytotoxic T lymphocyte function.
12q23	*RFX4, RIC8B*	Ellinghaus *et al*. (2016)	RFX4: Transcription factor of the regulatory factor family. Potentially involved in the regulation of immune and infectious responses. RIC8B: G-alpha-binding protein that catalyzes cAMP production.
12q24	*SH2B3, ATXN2*	*Liu et al. (2013)*	*SH2B3: Negative regulator of cytokine signaling and cell proliferation. Suppresses normal B progenitor cells by downregulating JAK-STAT signaling. SH2B3 associations have been reported in autoimmune liver diseases (PSC, PBC and AIH).* *ATXN2: Expressed in hepatocytes and specific neuron populations. Involved in EGFR trafficking and potentially plays a role in insulin resistance.*
16q12	*CLEC16A, SOCS1*	Ellinghaus *et al*. (2016)	*CLEC16A*: Regulation of B-cell function and autophagy of mitochondria. *SOCS1*: Negative regulator of cytokine signaling and thymocyte development. Maintains regulatory T-cell integrity and function. Deficiency of SOCs leads to fatal neonatal inflammatory disease.

18q21	*TCF4*	Ellinghaus *et al*. (2013)	Transcription factor that regulates plasmacytoid dendritic cells and early B- and T-cell development.
18q22	*CD226*	Liu *et al*. (2013)	Co-stimulatory surface molecule expressed on T cells and natural killer cells, enhancing cytotoxic function. Involved in a broad range of pathological processes including autoimmunity and cancer development.
19q13	*PRKD2, STRN4*	Liu *et al*. (2013)	*PRKD2*: Member of protein kinase D family and involved in regulation of cell proliferation and cytokine production via the NF-kappaB pathway. Required for the production of IL-2 and IFN-γ after TCR engagement in peripheral T cells. Can phosphorylate HDAC7 (see above) which leads to nuclear export and inhibition of HDAC7-mediated transcriptional function. *STRN4*: Involved in cell differentiation, transformation and apoptosis by regulating protein phosphatase 2A. STRN4 silencing suppresses malignant features of cancer cells.
21q22	*PSMG1*	Liu *et al*. (2013)	Chaperone protein. May be involved in altering the function of the ubiquitin-proteasome system and formation of the immunoproteosome.
21q22	*UBASH3A*	Ji *et al*. (2017)	Member of protein tyrosine phosphatase family. Negatively regulates T cells by facilitating apoptosis.

Highlights

- Genetic studies primarily highlight immune pathways as risk factors in primary sclerosing cholangitis (PSC).

- Activation of cholangiocytes by infection, microbial products, bile acids or immune-mediated inflammation triggers the production of cytokines/chemokine and growth factors involved in both biliary damage and repair.

- Persistent cholangiocyte activation in PSC induces the transition of senescent cholangiocytes to an irreversible senescence-associated secretory phenotype (SASP) that promotes liver fibrosis and development of PSC-associated cholangiocarcinoma (PSC-CCA).

- Biliary tract carcinogenesis in PSC is considered a multi-step process involving the progression from cholangiocyte metaplasia to low grade dysplasia (LGD), high grade dysplasia (HGD) and ultimately invasive PSC-CCA.