Chapter 2
The Role of Cholangiocyte Cell Death in the Development of Biliary Diseases

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Abbreviations

ADPKD	Autosomal dominant polycystic kidney disease
AEA	Anandamide
AKT	Protein kinase B
AMA	Antimitochondrial antibodies
BA	Biliary atresia
BDL	Bile duct ligation
CBDL	Common bile duct ligation
CCA	Cholangiocarcinoma
CD	Cluster of differentiation
DEN	Diethylnitrosamine
FADD	Fas-associated death domain
FFA	Free fatty acid
HSC	Hepatic stellate cells
IFN-γ	Interferon- γ
IL	Interleukin
JNK	c-Jun N-terminal kinase
LMBDL	Left median bile duct ligation

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NAFLD	Nonalcoholic fatty liver disease
NF-kB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer
NOD	Nucleotide-binding oligomerization domain
PAMPs	Pathogen-associated molecular patterns
pANCA	Peri-neutrophil cytoplasmic antibody
PBC	Primary biliary cholangitis
PKD	Polycystic kidney disease
PNPLA3	Patatin-like phospholipase domain-containing protein 3
PRR	Pattern-recognition receptors
PSC	Primary sclerosing cholangitis
PUMA	p53 upregulated modulator of apoptosis
STAT3	Signal transducer and activator of transcription 3
TLRs	Toll-like receptors
TNF	Tumor necrosis factor
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
UDCA	Ursodeoxycholic acid

2.1 Introduction

The liver is comprised primarily of two types of epithelia: hepatocytes and cholangiocytes. Hepatocytes make up approximately 70% of the endogenous population, whereas cholangiocytes constitute only 3–5% (Glaser et al. 2009). Cholangiocytes are heterogeneous, varying in size, shape, and function, and are generally categorized as either small or large based upon both their morphology and functionality. While both kinds of cholangiocytes line the biliary tree, large cholangiocytes are more columnar in shape and line larger bile ducts, whereas small cholangiocytes tend to be cuboidal and are found in smaller bile ducts (Maroni et al. 2015). In addition, small cholangiocytes are less specialized with a higher nucleus/cytoplasm ratio. Large cholangiocytes have a smaller nucleus/cytoplasm ratio and are ciliated, allowing them to act as both chemo- and mechanosensors (Maroni et al. 2015). Together, their primary role is to secrete, modify, and transport the bile into the duodenum. Cholangiocytes also facilitate repair following hepatic injury, sense, and respond to inflammatory signals and are the target of cholangiopathies. Effective targeted therapies for this subset of biliary diseases are lacking; therefore, it is critical to establish a thorough understanding of the biological pathways and mechanisms of cholangiocyte death that may play a role in the pathogenesis of cholestatic liver diseases.

2.2 Cholangiopathies

Cholangiopathies are diseases of the biliary epithelium and commonly include primary sclerosing cholangitis (PSC), primary biliary cholangitis (PBC), biliary atresia, polycystic liver disease, and cholangiocarcinoma (Lazaridis and LaRusso 2015).

Cholangiopathies can be categorized, but are not limited to the following groups: autoimmune, drug- or toxin-induced, ischemic, idiopathic, infectious, and genetic (O'Hara et al. 2013). The five major cholangiopathies – PSC, PBC, autosomal dominant polycystic kidney disease (ADPKD), biliary atresia, and cholangiocarcinoma – affect both genders and all ages worldwide.

2.3 Primary Sclerosing Cholangitis

PSC is a chronic, progressive disease that results in periductular inflammation and fibrosis that progresses to end-stage liver diseases (Primary Biliary Cirrhosis 2016). Unlike other cholestatic liver diseases, PSC affects both the small and large bile ducts with 20% of small-duct PSC presenting patients progressing to large-duct PSC (Primary Biliary Cirrhosis 2016). Additionally, the majority of PSC patients will also have inflammatory bowel disease (Trivedi and Chapman 2012). PSC predominantly strikes males (66%) more than females and ranges from adolescence to advanced ages with the average age of a PSC patient being 41 years at diagnosis (Primary Biliary Cirrhosis 2016). Currently there are no effective treatments that halt disease progression, and liver transplantation is often necessary (Hirschfield et al. 2013; Karlsen et al. 2010). Complicating the need for liver transplantation is the reoccurrence of the disease, the predominance toward hepatobiliary cancers and secondary autoimmune disorders.

2.3.1 Primary Biliary Cholangitis

PBC is a chronic inflammatory cholestatic disease of autoimmune origins that affects the small interlobular and septal bile ducts. Without treatment, PBC will progress to cirrhosis and end-stage liver failure (Corrigan and Hirschfield; Czul and Levy 2016). It presents more frequently in women than men, and individuals with a diagnosis before 50 years of age have a 50% chance that treatment options will fail (Corrigan and Hirschfield). Approximately 30% of PBC patients will also be diagnosed with additional autoimmune disorders, the most common being Sjögren's syndrome (17.4%) and Raynaud's syndrome (12.5%) (Primary Biliary Cirrhosis 2016). Currently the treatment of choice is ursodeoxycholic acid (UDCA) which aids in slowing the disease progression and delays transplantation necessity or death (Qian and Dinghang 2014). Due to the pruritus that accompanies PBC, antihistamines will often be prescribed to improve the patient's quality of life. In addition, anti-inflammatories may help reduce the ductal inflammation allowing for better bile flow (Primary Biliary Cirrhosis 2016).

2.3.2 Autosomal Dominant Polycystic Kidney Disease

ADPKD is an inherited renal disorder characterized by the development and growth of cysts in the kidneys, liver, pancreas, and spleen (Ali et al. 2015; Srivastava and Patel 2014; Tong et al. 2015). ADPKD is the most common form of genetic renal failure in adults. ADPKD can result from two types of genetic mutations: ADPKD-1 sufferers carry a genetic mutation on the gene-encoding protein polycystin-1 found on chromosome 16, while ADPKD-2 is caused by a gene mutation on chromosome 4 that encodes the protein polycystin-2 (Erickson et al. 2008; Tong et al. 2015). ADPKD-1 mutations tend to be more severe with patients entering end-stage renal disease at a younger median age. It also accounts for 85% of clinical cases overall (Ali et al. 2015; Srivastava and Patel 2014). Both mutations result in the development of cysts, resembling grape clusters and primarily filled with serous fluid, blood, and urine. These cysts eventually compromise normal renal function through crowding and compression of the renal tubes (Srivastava and Patel 2014). Within the liver, cysts arise from cholangiocytes and ultimately replace healthy liver tissue to a point where they may compress adjacent organs, leading to discomfort and anorexia (Gordon 2015). Consequently many patients remain asymptomatic until the sheer size and number of cysts necessitate medical intervention, generally not until adulthood. Treatment options include cyst fenestration, partial hepatectomy, or liver transplantation (Drenth 2010).

2.3.3 Biliary Atresia

Biliary atresia is a congenital disorder that affects more females than males and predominantly children of Asian or African American descent. There are two types of biliary atresia: fetal and perinatal. Of the two, fetal biliary atresia poses a greater threat to the infant as it is often accompanied by defects of the heart, spleen, or intestines. (Hartley et al. 2009). Models of biliary atresia and livers from affected children express increased levels of proapoptosis molecules such as caspase 1 and 4 and TNFα (Petersen and Davenport 2013). While there are no known hereditary components, several theories have been proposed to explain this phenomenon, including viral or bacterial infections, autoimmunity, or dysregulated prenatal liver and bile duct development. A Kasai procedure is often required to restore enteric drainage of bile; however, many of these patients will go on to require hepatic transplantation or die from complications of the disease (Hartley et al. 2009).

2.3.4 Cholangiocarcinoma

Cholangiocarcinoma is a rare malignancy of the biliary epithelium that commonly presents late in the disease progression. The malignancy is difficult to treat due to the advanced stage at presentation and is often fatal with an overall 5-year survival rate of less than 15% (Ciombor and Goff 2013). Cholangiocarcinomas are categorized by the tumor's origins, either arising from the bile ducts lying within the liver (intrahepatic CCA) or outside the liver (extrahepatic CCA) (Keller and Schub 2016). Difficulties in the treatment of cholangiocarcinoma stem from the fact that it is most often diagnosed at an advanced stage limiting available treatment options. To date, the most palliative option is liver resection, which carries additional risks of bile leakage, abdominal abscesses, and liver failure (Ciombor and Goff 2013).

2.4 Animal Models

Currently there are many commercially available live animal models of cholestatic liver disease that include mechanical, chemical, genetically modified, and parasitic etiologies (Primary Biliary Cirrhosis 2016). Bile duct ligation (BDL) (mechanical), Mdr2$^{-/-}$ mice (genetic modification), IL-12p35$^{-/-}$ (genetic modification), and dnTGFβRII (genetic modification) will be discussed in further detail. BDL is a well-studied mechanical representation of liver fibrosis used to model cholestatic liver disease. This is achieved through surgical ligation of the common bile duct, which impedes the flow of bile, inducing a strong fibrotic response after 21–28 days (Tag et al. 2015). Recent years have seen the technical advancements of partial BDL or complete BDL with surgical reanastomosis. Bile is a necessary component of the digestive system to emulsify fats and digest lipids while excreting bilirubin and additional wastes. Blockage of the common bile duct causes toxic buildup of waste products resulting in inflammatory cascades and upregulation of fibrotic gene expression. This model has the added benefit of being highly reproducible.

Another reliable animal model of fibrosis is the generation of Mdr2 (Abcb4)$^{-/-}$ mice, which, as a result of a canalicular phospholipid flippase deficiency, spontaneously develop liver injury and present with features of PSC. The resultant cascade of events includes bile leakage into the portal tract due to a disruption of both the tight junctions and basement membranes of the biliary epithelium (Popov et al. 2005). It is also thought that the presence of these phospholipids emulsifies certain molecules such as bile acids, reducing their overall toxicity.

PBC, like biliary atresia, is notoriously difficult to mimic in its pathogenesis. Previous models demonstrate the development of the hallmark antimitochondrial antibodies (AMA) yet lack in their replication of the trademark fibrosis or cirrhosis,

the underlying cause for liver transplantation in PBC patients (Popov 2013). Recently, a double-mutant mouse generated by crossing two PBC mouse models, dnTGFβRII and IL-12p35$^{-/-}$, to generate an IL-12p35$^{-/-}$; dnTGFβRII strain, features both circulating AMA and periportal fibrosis (Popov 2013). This advancement represents an outstanding opportunity for researchers to test novel therapies and targets in order to gain insight into this devastating disease.

Two rat models with ADPKD are utilized: one where the animals exhibit spontaneous manifestations of polycystic kidney disease (PKD) through heritable traits and the other where genetic modifications mimic the mutation of human orthologous genes (Erickson et al. 2008). In the spontaneous hereditary model Han:SPRD-Cy, rats form renal cysts as a result of a missense mutation. This mutation shares the human PKD phenotype through non-orthologous genes. Homozygous rats will have initial cysts discoverable in the neonate stage, whereas the heterozygotes develop the disease at a much slower rate. PKD rats carry a mutation orthologous to the ADPKD gene in humans, and these animals express this gene mutation in the kidney, liver, and pancreas (Erickson et al. 2008). Both the PKD and Cy/+ strains have an approximate life span of 1.5 years.

Historically, several animal models have been developed to mimic biliary atresia, from lampreys and zebra fish to rhesus monkeys and mice. Lampreys have been found to naturally develop degeneration of the biliary system. Although it has proven to be useful for the study of bile duct transport, it lacks the disease pathology of human biliary atresia. Zebra fish are advantageous for studying biliary atresia because of their rapidly developing gastrointestinal system and clear elucidation of biliary development. Rhesus monkeys provided the first evidence that viral infections play a role in the etiology of biliary atresia when infant monkeys with biliary pathology tested positive for reovirus type 3. Hepatic viral-induced mice have provided further details into the pathogenic pathways, but to date no individual animal model has been discovered that truly mimics the disease pathology of biliary atresia in human infants (Petersen 2012).

Cholangiocarcinomas arise from chronic cholestasis, which ultimately contributes to the overall hepatocellular injury. One way to effectively model this pathogenesis is through a combination of left median bile duct ligation (LMBDL) and diethylnitrosamine (DEN). Due to the catastrophic insult suffered by the liver after common bile duct ligation (CBDL), many animals display high mortality with severe liver damage or greater survival rates associated with a significant decrease in liver damage (Yang et al. 2011). Intraperitoneally injected diethylnitrosamine, a known carcinogen, initiates liver cancer formation, and when coupled with the LMBDL procedure, a model of cholestasis results in a working model of human cholangiocarcinoma which effectively mimics the disease progression and chronic cholestasis and fibrosis (Yang et al. 2011).

2.5 Cell Death

2.5.1 Innate Immunity in Cholangiocytes

The liver's unique histological structure provides the basis for its role as the first line of defense against xenobiotics and infection (Ishibashi et al. 2009). The functional unit of the liver is called the hepatic lobule consisting of a hexagonal-like sheet of cells surrounding a central vein with portal triads at the periphery. Blood rich in metabolites, toxins, hormones, and immune cells returning from splanchnic and mesenteric circulation enters the liver from the portal vein and flows into the sinusoids lined by fenestrated endothelial cells. Here hepatocytes, along with Kupffer cells, hepatic stellate cells (HSCs), endothelial cells, and various lymphocytes, coordinate immune surveillance and detoxify as the blood flows to the central vein. Additionally, hepatocytes and cholangiocytes secrete and modify the bile into bile canaliculi, which flows the opposite direction from the central vein to the portal triad and is drained by the biliary tree into the gallbladder and duodenum (Gao et al. 2008; Turcotte and Jarnagin 2015).

Cholangiocytes play a major role in hepatobiliary innate immunity. As opposed to the fenestrated endothelial cells lining the sinusoid, cholangiocytes maintain an epithelial barrier through regulation of tight junction proteins. Cholangiocytes secrete antimicrobial peptides such as defensins and secretory IgA as well as mucosal layer that protects the epithelial surface (Chen et al. 2008). Cholangiocytes also express primary cilia that extend into the biliary lumen whose function is both mechanical and sensory. Defects in primary cilia are associated with ADPKD and BA (Chu et al. 2012). Furthermore, cholangiocytes express pattern-recognition receptors (PRR) that recognize pathogen-associated molecular patterns (PAMPs) to initiate a localized, nonspecific inflammatory response (Fig. 2.1). Examples of PRR include surface Toll-like receptors (TLRs) and cytoplasmic NOD and RIG receptors (Gao et al. 2008; Turcotte and Jarnagin 2015).

PAMP recognition by these receptors can lead to programmed gene expression changes and coordinated defense against pathogens through secretion of inflammatory cytokines (TNF-α, IFN-γ, IL-1, IL-6, and TGF-β) and recruitment of immune cells (Kupffer cells, neutrophils, and various lymphocytes) (Gao et al. 2008; Turcotte and Jarnagin 2015). Cholangiocytes also respond in various ways to cytokines secreted by other cells including, but not limited to, changes in proliferation, apoptosis, cytotoxicity, and expression of surface adhesion ligands (Chen et al. 2008). It is widely believed that adhesion ligands can facilitate direct cell-to-cell cytotoxicity between cholangiocytes and immune cells.

Natural killer (NK) cells are a major player in innate immunity and are implicated in cholangiopathies (Gao et al. 2008). Recent evidence revealed that cholangiocytes express surface CD1d ligands that recruit NKT cells (a subtype of NK cells) and initiate cell death (Schrumpf et al. 2015). NK cells are crucial in fighting viruses, intracellular pathogens, and tumors because a) they do not express antigen receptors like T and B cells and b) they target cells with decreased expression of

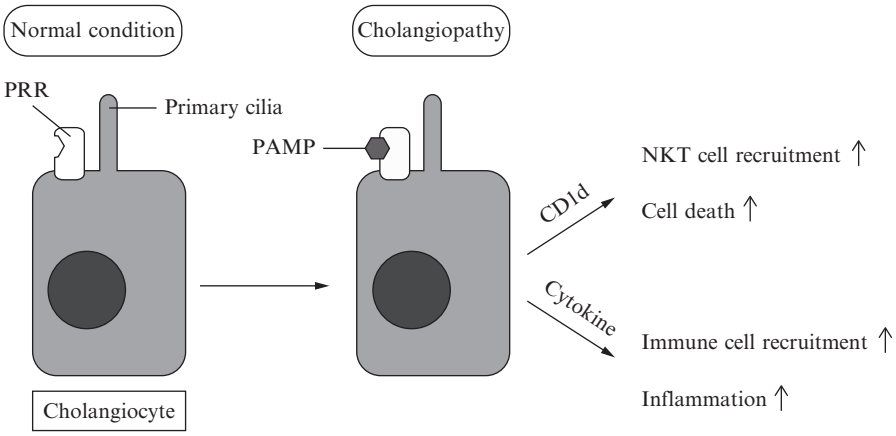

Fig. 2.1 Cholangiocyte innate immunity in cholangiopathies. Cholangiocytes express primary cilia and pattern-recognition receptors (*PRR*) as part of the innate immune system. Defects in primary cilia or uncontrolled activation of PRR can lead to cholangiopathies

MHC class I molecules (presumably due to evasive actions of the disease state) (Vivier et al. 2008). NK cells can also have a protective effect through clearing of profibrotic, proinflammatory cells. NK cells target activated hepatic stellate cells (HSCs) for cell death, but not quiescent HSCs (Gao et al. 2008), and are responsible for clearing self-recognizing T cells and dendritic cells (Tian et al. 2013).

Cholangiocytes also play a role in maintaining homeostasis between innate and adaptive immunity. Both cytokine secretion and cognate interaction via MHC class II molecules can drive activation and differentiation of various T and B lymphocytes. Dysregulation of this balance can lead to cholangiopathies. PBC is characterized by the presence of antimitochondrial autoantibodies (AMA) that drive sustained TLR signaling, increased proinflammatory Th1 and Th17 and decreased Treg phenotypes, and increased cholangiocyte cytotoxicity and HSC activation (Syal et al. 2012; Wang et al. 2016). PSC has a similar pathophysiology however with a few key differences. For instance, p-ANCA autoantibodies predominate instead of AMA, and cholangiocyte senescence is more prolonged (Nakanuma et al. 2015).

2.5.2 Apoptosis in Cholangiocytes

Cholangiopathies are rare disorders with no common genetic defects, yet chronic inflammation seems to be a universal contributing factor. Apoptosis is an important regulatory mechanism to keep cellular transformation in check through the removal of damaged cells and reduction in malignant progression (Humphreys et al. 2010). Apoptosis also serves to maintain homeostasis and promote liver tissue regeneration

as apoptotic bodies release growth signals that in turn stimulate the progenitor cells to initiate proliferation (Guicciardi et al. 2013). Apoptosis is the process by which a cell exposes a phosphatidylserine on the outer leaflet while condensing its chromatin and fragmenting its DNA. As a result, the cell shrinks and blebs and is reduced to smaller membrane-enclosed organelles called apoptotic bodies which are then marked for phagocytosis (Guicciardi et al. 2013). This fragmentation and signaling cascade, through a family of caspases, act upon various molecules to regulate normal cellular turnover and maintenance during times of stress.

Cholangiocytes undergo apoptosis through a variety of molecular pathways. Common pathways include Fas (CD95), TRAIL-R1/2, TNFR1/2, and CD40 (Fig. 2.2). In addition to the aforementioned receptors, there is evidence that autophagy and lipoapoptosis are also key players in the apoptotic regulatory system. Fas (CD95) receptors, a member of the TNF receptor superfamily, are widely expressed in the liver at low levels, which dramatically increase during inflammation, via its ligand FasL (CD178). In PBC, there is cross-linking of the ligand, resulting a progressive loss of the bile ducts (Guicciardi et al. 2013). Furthermore, there is evidence that both hepatocytes and cholangiocytes form regenerative clusters that overexpress death receptor Fas and mito-inhibitory protein glypican 3 (Hattoum et al. 2013). Additionally, previous research has shown that anandamide (AEA), an endocannabinoid, is both antiproliferative and proapoptotic. Cannabinoid receptors, Cb1 and Cb2, are found in cells of the central nervous system, various peripheral tissues, the gastrointestinal tract, and the immune system. However, blocking these G-protein-coupled receptors does not alter the effects of AEA on cholangiocarcinomas. This is due, in part, to the reaction of AEA with other receptor-independent microdomains that recruits lipid rafts, along with Fas and FasL, in the outer leaflet of the plasma membrane. Disruption of these rafts with methyl-β-cyclodextrin mitigated the AEA suppression of cellular proliferation (DeMorrow et al. 2007).

Another indispensable member of the TNF receptor superfamily is the TNF-related apoptosis-inducing ligand, or TRAIL, additionally noted as death receptor 4/5. In healthy cells, proapoptotic pathways are blocked, and TRAIL activates NF-kB and JNK to promote cellular differentiation and proliferation (Ishimura et al. 2006). However, in cholestatic liver disease, such as human PSC and PBC, TRAIL expression and apoptosis are significantly increased causing cholangiocyte cell death and cholangitis (Takeda et al. 2008). In this pathway, the ligand binds to the death receptors TRAIL-R1/2 and forms a trimeric structure. It is this structure that recruits the Fas-associated death domain (FADD) and procaspases 8 and 10. Through self-activation apoptosis is initiated (Ishimura et al. 2006).

Tumor necrosis factor alpha (TNF-α) is a necessary immunological cytokine that plays a critical role in the inflammatory response of the innate immune system. It is produced in response to viruses, parasites, bacterial endotoxins, and various cytokines. Its course of action is determined by its cell surface receptors, TNF-α receptor 1 (TNF-αR1) and TNF-α receptor 2 (TNF-αR2). TNF-αR1 is widely distributed

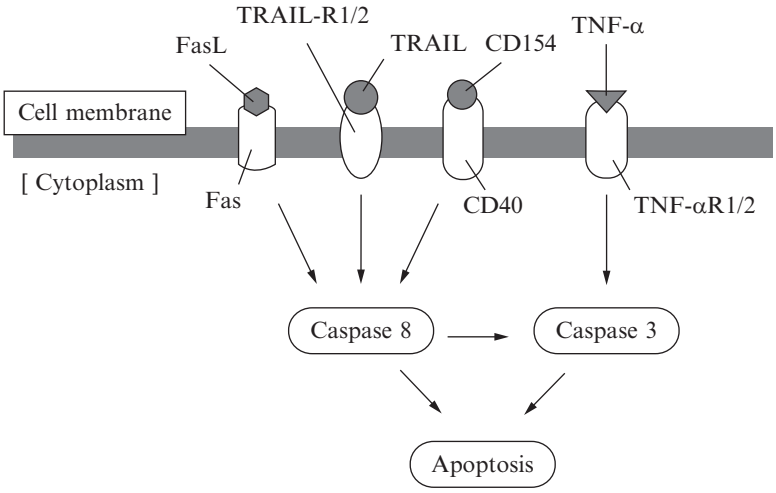

Fig. 2.2 Extrinsic apoptosis signaling in cholangiocytes. Various external signaling pathways that lead to caspase cascade and apoptosis

and responsible for the majority of the TNF-α cellular responses, including apoptosis (Idriss et al. 2014). Previous research has found that this pathway operates through caspase 3, a common end point caspase in the apoptosis cascade. Generally, cholangiocytes are markedly resistant to TNF-α and proliferate in response to ductal secretions of secretin in an autocrine and paracrine manner following injury. However, cholestatic injury, a primary feature of cholangiopathies, sensitizes cholangiocytes to TNF-α signaling in a way that alters ductular secretions and increases apoptosis (Alpini et al. 2003).

CD40 is a major player in cholangiocyte apoptosis through a variety of signaling pathways such as Fas-mediated signaling, STAT3 upregulation, and CD154-bearing Kupffer cells. Fas, or CD95, is a typical death receptor of the TNF family that, in response to cross-linking with its ligand FasL, results in cholangiocyte apoptosis and subsequent bile duct loss. However, research has demonstrated that Fas-FasL cross-linking on its own is not enough to induce apoptosis in either normal or malignant cells. Additionally, Fas has been shown to be downregulated as the tumor moves toward genetic instability. Thus, it appears that CD40, a cell surface glycoprotein, is the main route for apoptosis signaling. In addition to autocrine and paracrine death induction, cholangiocytes are also susceptible to immune-related death through the CD154-expressing Kupffer cells. In chronic liver diseases, circulating macrophages may be activated by bacterial endotoxins or proinflammatory cytokines. These activated macrophages then overexpress CD154 directly inducing apoptosis through binding its cognate receptor, CD40 (Alabraba et al. 2008). Other key players also regulate CD40 signaling in apoptotic

cell death. For example, inhibition of STAT3 yields a 50% reduction in overall cholangiocyte cell death (Ahmed-Choudhury et al. 2006). While this reduction is significant and offers a promising therapeutic target, it also indicates there are other, equally important, players to be explored along the CD40 axis.

In addition to cholangiocyte innate death receptors, nonconventional pharmaceutical treatments have also been found to increase apoptosis in cholangiocarcinoma cell lines. For example, Simvastatin, a cholesterol-lowering drug readily used in the prevention and treatment of atherosclerosis, has been shown to increase apoptosis in cholangiocarcinoma cell lines while simply inhibiting proliferation in normal cholangiocytes with no effect on apoptosis (Miller et al. 2011). Several cholesterol pathway intermediates facilitate proper membrane localization and activity of the Rho family of GTPases, including Rac1. Simvastatin interferes with cholangiocyte's ability to localize Rac1 to cholesterol rafts, presumably through inhibition of these crucial intermediates, subsequently decreasing Rac1 activity and increasing apoptosis. Specifically, simvastatin was shown to increase caspase 3 and caspase 7 activities. These results were completely reversible when the cells were pretreated with cholesterol (Miller et al. 2011). As cholangiocytes are typically quiescent unless under duress, the treatment poses no threat to the healthy epithelial cells (Maroni et al. 2015).

2.5.3 Senescence and Autophagy

Recent studies in biliary physiology have shown that cholangiocytes use senescence and autophagy in response to biliary injury, including fibrosing cholangiopathies, such as PSC and PBC (Nakanuma et al. 2015; Wang 2015). Cellular senescence is a cellular process by which proliferation halts and cells become arrested in the G1 phase of the cell cycle. Senescent cells remain metabolically active; however, they do not respond to external stimuli (Kuilman et al. 2010). Cellular senescence can occur following numerous insults, such as oxidative stress and oncogene activation, and is thought to be a protective mechanism against additional insults (Nakanuma et al. 2015). Senescent cells have also been shown to take on a secretory phenotype, which persistently secrete cytokines, chemokines, growth factors, and profibrotic factors (Kuilman et al. 2010; Lawless et al. 2010).

Autophagy is a regulated program of self-degradation that allows cells to remove long-lived or damaged proteins and/or organelles. When autophagy is activated during cellular stress, organelles and proteins become sequestered in autophagosomes and digested through fusion with lysosomes (Mizushima 2007). These mechanisms can act as a protective response to cellular stress and enable cells to maintain cellular viability and homeostasis (Kroemer et al. 2010). These cellular mechanisms, along with apoptosis, play a critical role in cholangiocyte response to injury. Regulation of these cellular processes occurs through a balance between the tumor suppressor p53 and expression of anti-apoptotic signaling, specifically the AKT/mTOR pathway (Wang 2015). The cross talk between p53 and AKT/mTOR determines whether or not cells survive injury (Nakanuma et al. 2015).

Previous studies have shown that these mechanisms are implicated in PBC and may be associated with the progressive bile duct loss that is characteristic of the disease. For example, cholangiocytes from PBC patients exhibit shortened telomeres and increased expression of SA-β-Gal, $p16^{INK4a}$, and $p21^{WAF1/Cip}$, which are characteristics of senescent cells (Sasaki et al. 2005, 2008b). It is hypothesized that the senescent cholangiocytes in PBC maintain a secretory phenotype, which promotes the release of cytokines and chemokines into the periductular environment, creating an inflammatory reaction resulting in biliary injury (Sasaki et al. 2008a). Additionally, studies have shown that the autophagy markers are upregulated in bile ducts from PBC patients (Sasaki et al. 2012). This includes microtubule-associated protein light chain 3β (LC3) and p62, a protein involved in the ubiquitin-bound cargo to autophagosomes (Wang 2015). Accumulation of p62 is suggestive of deregulated autophagy and insufficient processing of damaged proteins, which could be a contributing factor in the pathogenesis of PBC (Nakanuma et al. 2015).

Additionally, studies have shown that senescence and autophagy may also contribute to the pathogenesis of PSC. Cultured cholangiocytes isolated from PSC patients demonstrate a senescent phenotype, such as overexpression of $p16^{INK4a}$ and SA-β-Gal. These cholangiocytes also express a secretory phenotype, such as in PBC, which may contribute to the recruitment of inflammatory cells and modulation of the periductal environment (Tabibian et al. 2014a, b). Less is known about the role of autophagy in PSC; however, it has been suggested that the deregulated autophagy in respect to processing microbial pathogens from the intestinal flora may contribute to the development of peri-neutrophil cytoplasmic antibody (pANCA), an autoantibody commonly found in PSC patients (Nakanuma et al. 2015; Schwarze et al. 2003). These findings suggest that senescence and autophagy may play a role in the progression of PBC and PSCs, but more research is needed to elucidate the differences between the two etiologies of biliary injury.

2.5.4 Lipoapoptosis

Nonalcoholic fatty liver disease (NAFLD) is not recognized in the cholangiopathy family, but it is the most common chronic liver disease in Western countries (Gautheron et al. 2014). The involvement of cholangiocyte damage in the pathogenesis of NAFLD has not been thoroughly investigated. A recent study has demonstrated that the PNPLA3 rs738409 variant (I148M) that is associated with an increased risk for the development of NAFLD and alcoholic liver disease due to hepatocyte lipotoxicity is also a risk factor for reduced survival in male PSC patients with dominant bile duct stenosis presumably due to lipotoxicity at the cholangiocyte level (Friedrich et al. 2013; Romeo et al. 2008; Tian et al. 2010). A recent in vitro study has demonstrated that saturated FFAs palmitate and stearate induced cholangiocyte lipoapoptosis via caspase activation, nuclear translocation of FoxO3, and increased proapoptotic PUMA expression (Natarajan et al. 2014). These findings may help explain why a subset of NAFLD patients with symptoms of

cholestasis have biliary injury not consistent with what is normally observed in the majority of patients with NAFLD. Further in vivo model studies are necessary to clearly elucidate the role of cholangiocyte lipoapoptosis in the pathogenesis of NAFLD.

2.6 Conclusions and Future Directions

Cholangiocytes play an integral role in the overall function of the liver and its ability to clear toxins, aid in the digestion and absorption of fats, and expel waste through the bile. Cholangiopathies continue to carry a large disease burden due to the lack of effective treatment strategies despite extensive research over the past two decades. The use of transgenic mice to mimic these diseases will hopefully provide more insight into the mechanisms of liver injury. Studies suggest that cholangiocytes use numerous mechanisms to respond to hepatic injury including apoptosis, autophagy, and senescence. A better understanding of the biological functions and cell death pathways of cholangiocytes in both normal and disease states will aid in the development of novel targeted therapies for cholangiopathies.

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