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Review Article

Novel Approach to Bile Duct Damage in Primary Biliary Cirrhosis: Participation of Cellular Senescence and Autophagy

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Primary biliary cirrhosis (PBC) is characterized by antimitochondrial autoantibodies (AMAs) in patients' sera and histologically by chronic nonsuppurative destructive cholangitis in small bile ducts, eventually followed by extensive bile duct loss and biliary cirrhosis. The autoimmune-mediated pathogenesis of bile duct lesions, including the significance of AMAs, triggers of the autoimmune process, and so on remain unclear. We have reported that cellular senescence in biliary epithelial cells (BECs) may be involved in bile duct lesions and that autophagy may precede the process of biliary epithelial senescence in PBC. Interestingly, BECs in damaged bile ducts show characteristicsof cellular senescence and autophagy in PBC. A suspected causative factor of biliary epithelial senescence is oxidative stress. Furthermore, senescent BECs may modulate the microenvironment around bile ducts by expressing various chemokines and cytokines called senescence-associated secretory phenotypes and contribute to the pathogenesis in PBC.

1. Introduction

Primary biliary cirrhosis (PBC) is a chronic, progressive cholestatic liver disease that affects usually middle-aged women and occasionally leads to liver failure and liver transplantation [1–5]. Autoimmune pathogenesis is suggested in PBC [1–4], because PBC is serologically characterized by a high titer of serum antimitochondrial autoantibodies (AMAs) and by an increased level of immunoglobulin M (IgM). PBC-specific antinuclear antibodies (ANAs), such as anti-gp210 are also detected in some patients [1, 2, 6–9]. AMAs are present in about 95% of patients with PBC, with disease specificity close to 100%. An inner lipoyl domain of the E2-component of pyruvate dehydrogenase (PDC-E2) and other 2-oxo-acid dehydrogenases is a major epitope for both B-cell and CD4 and CD8 T-cell response [9–12]. PBC is characterized histologically by the cholangitis of small bile ducts (chronic nonsuppurative destructive cholangitis; CNSDC), eventually followed by the extensive loss of small bile ducts and biliary cirrhosis [2, 3, 13]. Therefore, a major target of autoimmune-mediated injury has been thought to be biliary epithelial cells (BECs) in PBC.

There has been considerable progress in elucidating the immunopathological features [9–12], genetic factors [14–17], and environmental factors such as infectious agents and xenobiotics [5, 18–20] in the pathogenesis of PBC. The most accepted hypothesis states that PBC results from a combination of multiple genetic factors (susceptible genetic background) and superimposed environmental triggers. In this scenario, adaptive, both humoral and cellular (CD4 and CD8 T cells), and innate immunity have been proposed as coplayers in immune-mediated liver damage; however, the etiology and pathogenesis of PBC remain unclear. In particular, the significance of AMAs and autoantigen-specific T-cell response in the pathogenesis of bile duct lesions remains unknown. One hypothesis for a BEC-specific autoimmune reaction is a unique property of apoptosis in BECs, in which there is exposure of autoantigen to the effectors of the immune system [4, 21–23].

We have recently reported that cellular senescence and autophagy may be involved in bile duct lesions in PBC [24–28]. These two cellular processes may be related to autoimmune mechanism such as AMAs and the autoantigen-specific T cell and play a role to cause autoimmune-mediated

bile duct lesions in PBC. Recent studies have disclosed that autophagy plays an important role in innate immune responses and possibly autoimmunity [29–31]. Furthermore, it is plausible that senescent BECs modulate microenvironment around bile duct by expressing senescence-associated secretory phenotypes (SASPs) including various chemokines and contribute to the pathogenesis of bile duct lesions in PBC [32]. In this paper, we will focus on cellular senescence and autophagy in BECs in PBC and their possible involvement in the progression of diseases.

2. Cellular Senescence in the Damaged Small Bile Ducts in PBC

2.1. What Is Cellular Senescence? Cellular senescence is defined as a condition in which a cell no longer has the ability to proliferate. Senescent cells remain metabolically active, even though they are irreversibly arrested at the G1 phase of the cell cycle and do not respond to various external stimuli. Cellular senescence can be triggered by a number of cellular stresses including telomere dysfunction. Other causes include oxidative stress, nontelomeric DNA damage, epigenetic derepression of the INK4a/ARF locus, and oncogenic activation [33]. Several features, such as increased activity of senescence-associated β-galactosidase (SA-β-gal) (Figure 1), shortened telomeres, increased expression of $p16^{INK4}$ and $p21^{WAF1/CIP}$, and histological changes (Figure 1), are known to characterize cellular senescence [34–36]. Cellular senescence is a potent tumor suppression mechanism as well as apoptosis [33, 37]. Senescent cells are also seen in aged or damaged tissues, and they may decline tissue regeneration capacity with age [33]. Cellular senescence may play a role in limiting wound-healing responses following tissue damage [38]. Recent studies have disclosed that cellular senescence is involved in the pathophysiology of various chronic liver diseases, including chronic viral hepatitis and hepatocarcinogenesis [24, 26, 27, 38–44].

2.2. Bile Duct Lesion in PBC. "Chronic nonsuppurative destructive cholangitis (CNSDC)" is a characteristic bile duct lesion in PBC (Figure 1(a)) [2, 3, 13, 45]. Bile duct damage in early PBC mainly affects the septal and larger interlobular bile ducts, while the smaller interlobular ducts remain intact until later. The BECs in the affected bile ducts show irregular shape and arrangement with infiltration of mononuclear cells. The presence of epithelioid granuloma around the affected bile duct is also a feature of PBC. Bile duct loss eventually progresses and chronic cholestasis developes gradually. Hepatitis activity of varying degrees is frequently imposed on the liver at the same time. We proposed a new histological staging and grading system of PBC for comprehensive analysis of the histological progression of PBC (staging) toward extensive bile duct loss, chronic cholestasis and cirrhosis, and also the immune-mediated necroinflammatory activity of small bile ducts and hepatocytes [46].

2.3. Biliary Epithelial Senescence in Damaged Small Bile Ducts in PBC. BECs in damaged small bile ducts in PBC show senescent features, such as the expression of SA-β-gal and the increased expression of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ (Figure 1) [24–27]. Furthermore, a significant decrease in telomere length was observed in BECs in the damaged small bile ducts and bile ductules in PBC compared with normal-looking bile ducts and bile ductules in PBC, chronic viral hepatitis, and normal livers, when examined using quantitative fluorescence *in situ* hybridization [25]. γH2AX DNA damage foci were detected in BECs in damaged small bile ducts and bile ductules in PBC but were absent in BECs in control livers. The expression of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ increased corresponding to telomere shortening and γH2AX DNA damage foci in the damaged small bile ducts in PBC [25]. Taken together, telomere shortening and the accumulation of DNA damage coinciding with increased expressions of $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ in the damaged bile ducts characterize biliary cellular senescence and may play a role in subsequent progressive bile duct loss in PBC [24–27]. Interestingly, chronic liver allograft rejection, which is characterized by bile duct loss similar to PBC, also shows similar biliary epithelial senescence [24, 40].

2.4. How Does Cellular Senescence Result in Bile Duct Loss?

The exact mechanism how cellular senescence of BECs cause bile duct loss in PBC is not clear. Cellular senescence is supposed to impair tissue integrity and cause persistent inflammation [47]. After cellular senescence occurs in injured BECs, these senescent cells are thought to remain *in situ* and not to be replaced by normal cells, although nonsenescent BECs proliferate in response to injury [48]. Therefore, it is plausible that the senescent BECs are prone to further injuries, accentuating inflammation by SASP, which is likely to be followed by bile duct loss in PBC. The fate of senescent BECs remains to be clarified: whether senescent BECs are removed by necrosis, apoptosis, or anoikis. Another possibility is that bile duct loss may be due to impaired function of hepatic stem/progenitor cells in PBC. Cellular senescence is also seen in bile ductular cells in a ductular reaction (DR), which is thought to harbor hepatic stem/progenitor cells in PBC [24, 25]. The impaired proliferation of hepatic stem/progenitor cells may fail to replace the damaged BECs in small bile ducts, subsequently cause bile duct loss.

2.5. Oxidative Stress Is a Potential Factor Inducing Cellular Senescence. Cellular senescence can be triggered by a number of cellular stresses, including telomere dysfunction, oxidative stress, nontelomeric DNA damage, epigenetic derepression of the INK4a/ARF locus, and oncogenic stress [33, 39]. The possible association of oxidative stress is suggested to be involved in the pathogenesis of cellular senescence in PBC [24, 26, 27]. For example, $p21^{WAF1/Cip1}$, activated/phosphorylated ATM, and an oxidative stress marker, 8-OHdG, were frequently and extensively coexpressed in the nuclei of CNSDC in PBC, and their expressions were

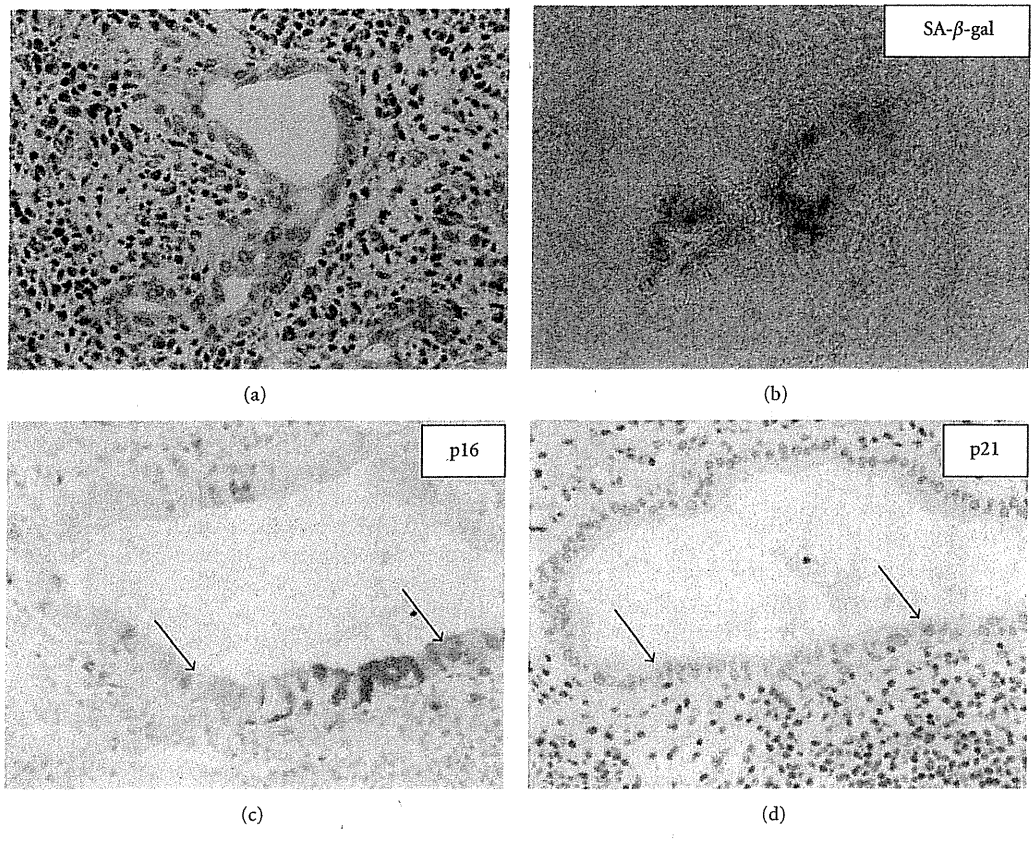

FIGURE 1: Biliary epithelial senescence in PBC. BECs in small bile ducts involved in chronic nonsuppurative destructive cholangitis (CNSDC) show histological features of senescence, such as cytoplasmic eosinophilia, cellular and nuclear enlargement, and uneven nuclear spacing (a). SA-β-gal activity is detected in BECs in PBC (b). Senescent markers, $p21^{WAF1/Cip1}$ and $p16^{INK4a}$, were expressed in BECs in damaged small bile ducts in PBC (c) and (d). Immunostaining for $p21^{WAF1/Cip1}$ and $p16^{INK4a}$. Original magnification: ×400.

correlated [26]. Cell culture study suggests that oxidative stress and proinflammatory cytokines, such as IFN-β, IFN-γ, and TNF-α, which induce ROS generation, activate the ATM/p53/$p21^{WAF1/Cip1}$ pathway, followed by biliary epithelial senescence [49]. The expression of polycomb group protein Bmi1 is significantly decreased in damaged bile ducts in PBC, coordinating with the increased expression of $p16^{INK4a}$ [27]. The decreased expression of Bmi1 is induced by oxidative stress, followed by the increased expression of $p16^{INK4a}$ in cultured BECs [27]. Since an antioxidant, N-acetylcysteine can inhibit cellular senescence induced by oxidative stress and proinflammatory cytokines [49], antioxidants may have therapeutic implications in PBC.

3. Cellular Senescence in Ductular Reaction (DR) in PBC

DR is a reactive lesion at the portal tract interface composed of increased bile ductules with an accompanying complex of stromal and inflammatory cells [50]. DR is thought to harbor hepatic stem/progenitor cells [50]. We investigated the pathological significance of DR in chronic liver diseases, including PBC, with respect to cellular senescence [24, 25, 51]. The expression of senescence-associated markers ($p16^{INK4a}$ and $p21^{WAF1/Cip1}$) was frequently expressed in ductular cells in the advanced stage of chronic liver diseases, especially in PBC. Double immunostaining disclosed that neural cell adhesion molecules (NCAM) were frequently coexpressed in ductular cells showing senescence-associated markers ($p16^{INK4a}$ and $p21^{WAF1/Cip1}$) and cell cycle G1-phase marker (cyclin D) (Figure 2) [51]. These findings suggest that DR is heterogeneous in cell kinetics and the expression of NCAM and that some ductular cells in DR in chronic liver diseases were at G1 arrest and undergoing cellular senescence. Such senescent cells may be involved in the progression of fibrosis of these diseases, particularly in PBC [51]. This study raises the possibility that NCAM can be used as a cellular senescent marker developing in DRs. Furthermore, our recent study revealed that CCL2 expressed by senescent BECs can induce the cell migration of hepatic stellate cells (HSCs), which may play a role in the periportal fibrosis in chronic advanced liver diseases [52].

4. Autophagy in Damaged Small Bile Ducts in PBC

4.1. What Is Autophagy? Autophagy, or cellular selfdigestion, is a cellular pathway that results from various cellular

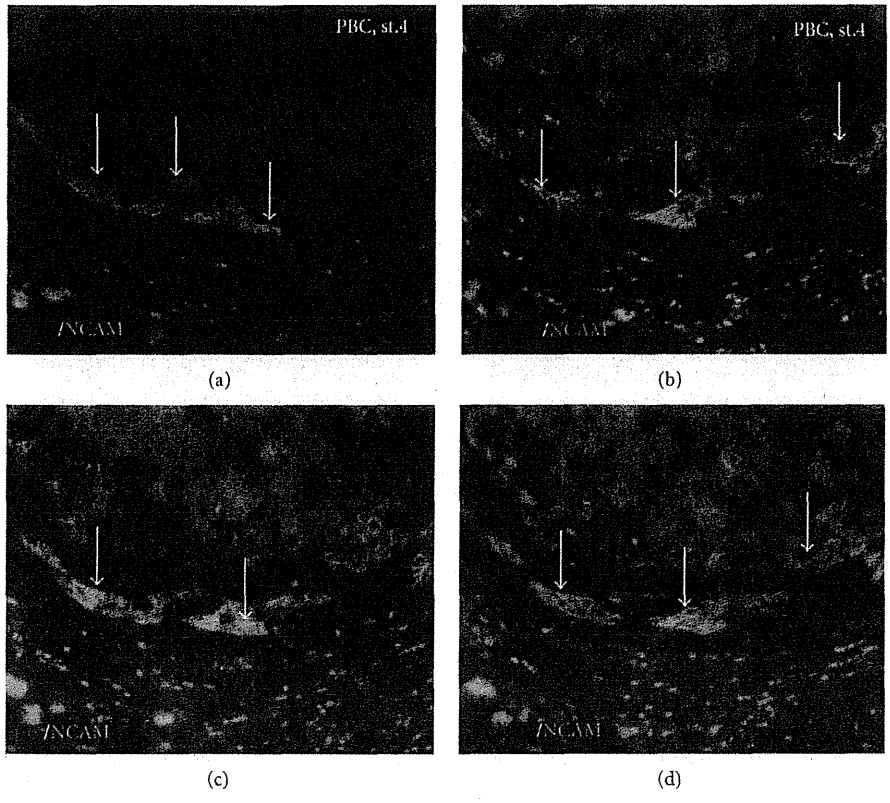

FIGURE 2: Double immunostaining for senescence markers ($p16^{INK4a}$ or $p21^{WAF1/Cip1}$) and cell cycle markers (G1-phase, cyclin D; S-phase, cyclin A) (red) and NCAM (green) in PBC, stage 4. (a), (c) The expression of senescent markers $p16^{INK4a}$ and $p21^{WAF1/Cip1}$ is seen in NCAM-positive ductular cells (arrows) in PBC, stage 4. (b), (d) Most NCAM-positive ductular cells (arrows) express cyclin D, whereas there is no cyclin A expression in DRs in PBC, stage 4. Original magnification ×400.

stresses, such as nutrient starvation, anoxia, and activation of the endoplasmic reticulum stress pathway [53, 54]. Three types of autophagy, macroautophagy, microautophagy, and chaperone-mediated autophagy, have been classified, and macroautophagy is the major type [53–55]. It is becoming evident that macroautophagy (hereafter referred to as autophagy) is important for development, differentiation, survival, homeostasis, and also many pathological processes. Autophagy occurs physiologically at low basal levels in cells to perform homeostatic functions such as protein and organelle turnover. It is rapidly upregulated through an inhibition of mammalian target of rapamycin (mTOR) when cells need to generate intracellular nutrients and energy, for example, in starvation [53–55]. Microtubule-associated protein-light chain 3β (LC3), a homologue of autophagy-related protein 8 (Apg8p), which is essential for autophagy and associated with autophagosome membranes after processing, is a widely used marker of autophagy [56, 57].

4.2. Cellular Senescence, Apoptosis, and Autophagy. An appropriate cellular stress response is critical for maintaining tissue integrity and function and for preventing diseases [58]. Cellular senescence, apoptosis, and autophagy are cellular responses to stress, correlating with each other [58]. Cellular stresses cause adaptation, repair, autophagy, apoptosis, or cellular senescence in cells [58]. These cell fate decisions are critical to dealing with the emergence of damaged and potentially dangerous cells that can cause cancer. Interestingly, a recent study disclosed that autophagy is induced during and facilitates the process of senescence [56]. Cellular senescence can be a failsafe program against a variety of cellular insults, as well as apoptosis. Cellular senescence is a typical delayed stress response involving multiple effector mechanism, in contrast, cytotoxic signals converge to a common mechanism in apoptosis. With the onset of cellular senescence cells can remain viable within tissues for long periods; resistance to apoptosis is a characteristics of senescent cells [41, 59].

4.3. Biliary Epithelial Autophagy in PBC. We have reported the upregulated autophagy in the damaged small bile ducts along with cellular senescence in PBC [28] (Figure 3). LC3, a commonly used marker of autophagy, was characteristically expressed in cytoplasmic vesicles in bile duct lesions in PBC [28]. Autophagic marker LC3 was coexpressed with senescent markers $p21^{WAF1/Cip1}$ and $p16^{INK4a}$ in damaged bile ducts in PBC [28]. The inhibition of autophagy reduced stress-induced cellular senescence in cultured cells with stress [28]. This finding is in consistent with a recent study in which the involvement of autophagy is reported in the process of

senescence [56]. Taken together, biliary epithelial autophagy may mediate the process of biliary epithelial senescence in bile duct lesions in PBC and it may be involved in the pathogenesis of bile duct lesions in PBC.

4.4. Autophagy and Autoimmune-Mediated Processes in PBC. An unsolved problem is how autophagy and cellular senescence are involved in the autoimmune-mediated processes such as AMA and other PBC-related autoantigens in PBC. Regarding apoptosis, it has been reported that BECs manifest unique features during apoptosis and that the combination of AMA and BECs apoptotic bodies (apotopes) could activate innate immune response with involvement of some inflammatory cytokines [21]. This study provides a mechanism for the biliary specificity of PBC and the involvement of AMA in autoimmune pathogenesis [21]. Recent studies reveal a crucial role for the autophagy pathway and proteins in immunity and inflammation [29–31]. The autophagy pathway and autophagy proteins may function as a central fulcrum that balances the beneficial and harmful effects of the host response to infection and other immunological stimuli [31]. Autophagy proteins function in adaptive immunity, including in the development and homeostasis of the immune system and in antigen presentation [31]. Furthermore, autophagy proteins play a role in both the activation and inactivation of innate immune signaling [30, 31]. On the contrary, it is demonstrated that autophagy is regulated by immune-signaling molecules, such as toll-like receptors (TLRs), IFN-γ, and NF-κB [30, 31].

The dysfunctional autophagy related to the regulation of immunity may contribute also to chronic inflammatory diseases and probably autoimmune diseases. A well-characterized link is between mutations in autophagy regulators and Crohn's disease, a chronic inflammatory bowel disease, in which autophagy proteins, ATG16L1, NOD2, and IRGM are reported as susceptibility genes [60]. Abnormal autophagy/autophagy protein may also result in inflammatory autoimmune disease, although not yet proven. Autophagy-related processing of self-proteins provides a source of immunostimulatory molecules and autoantigens, that is, by MHC-class II presentation of cytosolic antigens and control of T-cell homeostasis [61–63]. It is of interest that genomewide association studies (GWAS) have linked several single nucleotide polymorphisms (SNPs) in ATG5, an autophagy protein, to systemic lupus erythematosus (SLE) susceptibility [64, 65]. SLE is a representative multisystem autoimmune disease characterized by an enormous array of autoantibodies such as ANAs and autoimmune responses against self-antigens generated from dying cells. To date, it is unclear how such SNPs affect the expression level and function of ATG5. Interestingly, in mice, the lack of ATG5-dependent negative thymic selection generates autoimmunity and multiorgan inflammation [66]. The autoimmunity and inflammation associated with SLE may be caused by loss of other ATG5-dependent effects, such as regulation of IFN and proinflammatory cytokine secretion, clearance of dying cells [67], and dendritic cell antigen presentation [68]. Taken together, a link between SLE pathogenesis and ATG5 mutation or mutation of other autophagy genes is plausible, although not yet proven.

Similar to SLE, it is possible that a dysfunctional autophagic process of BECs may play a role in autoimmune pathogenesis, for example, the immune tolerance breakdown of autoantigens, in PBC, although this is only speculative at this moment. Recent genetic studies of PBC including GWAS identified, in a reproducible fashion, genetic associations between PBC and human leukocyte antigen as well as polymorphisms in the genes encoding IL-12 α-chain and IL-12 receptor β-chain [14, 15]. GWAS also identified interferon regulatory factor 5 (IRF5)-transportin 3 (TNPO3), 17q12-21, MMEL1, and SPIB as new PBC susceptibility loci [14, 15]. These immune-related genes may be associated with dysfunction autophagy in PBC, although there have been no identified autophagy proteins such as ATG5 and ATG16L1 as PBC susceptibility genes. In fact, IRF5 plays a key role in the innate immunity response as part of the TLR signaling pathway and mediates apoptosis induced by tumor necrosis factor-related apoptosis-inducing ligand [69, 70]. Interestingly, IRF5 loci have been reported as associated loci with several autoimmune diseases including SLE and Sjogren's syndrome [71, 72]. Therefore, IRF5 might be related to dysfunctional autophagy in PBC, although not yet reported.

5. Senescence-Associated Secretory Phenotypes (SASPs) in PBC

5.1. What Are SASPs? An increasing body of work described the change in the cellular secretosome in senescent cells. Senescent cells play an important role in modulating the microenvironment by secreting biological active molecules, senescence-associated secretory phenotypes (SASPs). SASPs include diverse proinflammatory factors such as cytokines (IL-6, IL-1 and so on) and chemokines (CXCL8/IL-8, CCL2/monocyte chemotactic protein-1 (MCP)-1) and so on), growth factors and profibrogenic factors [73–77]. Previous studies have shown that BECs express a number of profibrogenic proinflammatory and chemotactic factors (e.g., IL-1, IL-6, CXCL8/IL-8, and CCL2/MCP-1) [78–81]. These factors can attract and activate inflammatory cells and also stellate cell lineage in humans with biliary disorders and in animal models of biliary fibrosis. Taken together, these cytokines and chemokines previously reported in PBC may belong to SASPs [73–77].

5.2. SASPs May Play a Role in the Pathogenesis of PBC. The upregulation of several cytokines and chemokines in damaged bile ducts in PBC has been reported [79, 80, 82], and these factors may represent SASPs, as described above [73–77]. We have recently reported that the involvement of senescent BECs in modulation of the inflammatory microenvironment around affected small bile ducts in PBC (Figure 4) [32]. In this study, we have shown that the expression of CCL2 and CX3CL1 was significantly higher in BECs in inflamed and damaged small bile ducts in PBC, than in noninflamed bile ducts and control livers

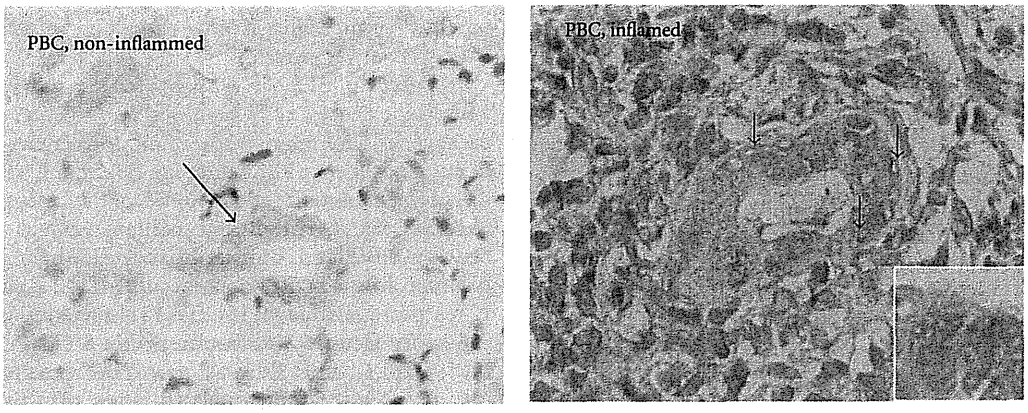

FIGURE 3: Biliary epithelial autophagy in PBC. (a) The expression of autophagy marker LC3 was not observed in BECs in noninflammed bile ducts (arrow) in PBC. (b) The expression of autophagy marker LC3 was detected in intracytoplasmic vesicles (arrows) in BECs involved in inflamed and damaged small bile ducts in PBC. Immunostaining for LC3. Original magnification, ×400 (inset, ×1000).

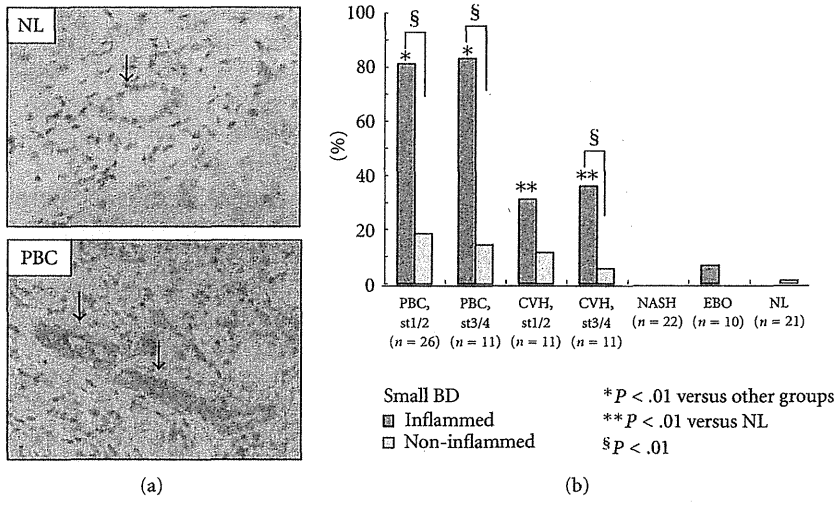

FIGURE 4: Increased expression of CCL2 in inflamed and damaged bile ducts in PBC. (a) The expression of CCL2 was absent or faint in biliary epithelial cells (BECs) in the small bile duct (arrow) in normal liver (top). CCL2 was extensively expressed in the membrane and cytoplasm of damaged and senescent BECs (arrows) in the early stage of PBC (bottom). Immunostaining for CCL2. Original magnification, ×400. (b) The expression of CCL2 was significantly more frequent and intense in inflamed small bile ducts in PBC, when compared with noninflamed small bile ducts in PBC and small bile ducts in control livers ($P < .01$). CVH: chronic viral hepatitis; NASH: nonalcoholic steatohepatitis; EBO: extrahepatic biliary obstruction; NL: normal liver.

(Figure 4). The expression of CCL2 and CX3CL1 was colocalized with the expression of senescent markers in damaged bile ducts in PBC [32]. In culture study, senescent BECs induced by cellular stresses expressed a significantly higher level of chemokines. Furthermore, senescent BECs significantly accelerated the migration of RAW264.7 cells, and neutralizing antibodies against CCL2 and CX3CL1 blocked in part the migration induced by senescent BECs [32]. These findings suggest that senescent BECs may play an important role in the pathogenesis of bile duct lesion in PBC by the accentuated inflammatory microenvironment through recruiting monocytes and other inflammatory cells via SASP (Figure 5). SASPs in senescent BECs in PBC may contribute to activation of the innate immune system around injured bile ducts. Furthermore, it raises the possibility that once biliary senescence develops, the change in the tissue microenvironment wrought by the SASP may induce senescence of surrounding BECs another types of cells in appositive feedback loop (Figure 5).

The mechanisms that initiate and maintain SASPs have not been clarified, so far [33, 73–75]. It is plausible that these stresses may induce SASPs via a common mechanism in the senescent state, because various cellular stresses, such as oxidative stress and serum deprivation, induce SASPs in senescent BECs [32].

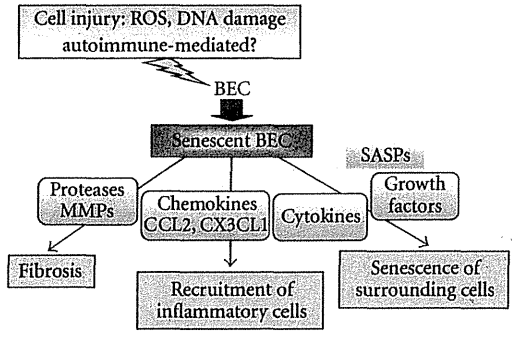

FIGURE 5: Possible regulation of microenvironment by senescent BECs expressing SASPs in PBC. Senescent BECs may function in modulation of the inflammatory microenvironment by recruiting monocytes and possibly other inflammatory cells by secreting chemokines and cytokines as SASPs. Senescent BECs may also participate in the induction of senescence in surrounding cells and progression of fibrosis via SASPs.

6. Summary

PBC is thought to result from a combination of multiple genetic factors and superimposed environmental triggers and apparently belongs to the "complex disease" category like most polygenic autoimmune diseases. Even though mitochondrial autoantigens and B-cell and T-cell autoepitopes have been well characterized in PBC, the pathogenesis of characteristic bile duct lesion and the exact role of AMA still remain to be elucidated. In this paper, we focused on a possible involvement of two novel cellular processes, autophagy and cellular senescence in BECs in bile duct lesions in PBC. Autophagy is expected to be a promising cellular mechanism involved in the autoimmune mechanism together with apoptosis. Cellular senescence may play a role in the immunopathology of BECs by expressing SASPs in PBC. Further studies are needed to disclose the autoimmune pathogenesis of PBC.

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Review Article

Caroli's Disease: Current Knowledge of Its Biliary Pathogenesis Obtained from an Orthologous Rat Model

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Caroli's disease belongs to a group of hepatic fibropolycystic diseases and is a hepatic manifestation of autosomal recessive polycystic kidney disease (ARPKD). It is a congenital disorder characterized by segmental saccular dilatations of the large intrahepatic bile duct and is frequently associated with congenital hepatic fibrosis (CHF). The most viable theory explaining its pathogenesis suggests that it is related to ductal plate malformation. The development of the polycystic kidney (PCK) rat, an orthologous rodent model of Caroli's disease with CHF as well as ARPKD, has allowed the molecular pathogenesis of the disease and the therapeutic options for its treatment to be examined. The relevance of the findings of studies using PCK rats and/or the cholangiocyte cell line derived from them to the pathogenesis of human Caroli's disease is currently being analyzed. Fibrocystin/polyductin, the gene product responsible for ARPKD, is normally localized to primary cilia, and defects in the fibrocystin from primary cilia are observed in PCK cholangiocytes. Ciliopathies involving PCK cholangiocytes (cholangiociliopathies) appear to be associated with decreased intracellular calcium levels and increased cAMP concentrations, causing cholangiocyte hyperproliferation, abnormal cell matrix interactions, and altered fluid secretion, which ultimately result in bile duct dilatation. This article reviews the current knowledge about the pathogenesis of Caroli's disease with CHF, particularly focusing on studies of the mechanism responsible for the biliary dysgenesis observed in PCK rats.

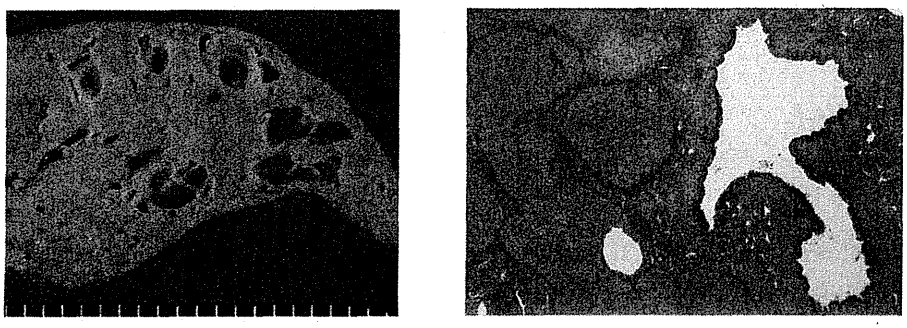
1. Introduction

Caroli's disease belongs to a group of hepatic fibropolycystic diseases [1, 2]. It is a congenital disorder characterized by a biliary abnormality consisting of segmental saccular dilatations of the large intrahepatic bile duct. It is frequently associated with varying degrees of portal fibrosis, corresponding to congenital hepatic fibrosis (CHF). Caroli initially described two variants of the biliary abnormality with and without CHF (Caroli's syndrome and Caroli's disease), and the form without CHF is quite rare.

A significant proportion of Caroli's disease cases involving CHF are transmitted in an autosomal recessive manner and are associated with autosomal recessive polycystic kidney disease (ARPKD). The incidence of ARPKD is 1 in 20,000 live births [3]. Renal failure may be present at birth, and the disease presentation is not limited to the neonatal period; it can be diagnosed in childhood or even adolescence or adulthood [4]. These late-presenting cases typically display less severe kidney disease, but more commonly involve liver disease complications.

Caroli's disease is a developmental anomaly, and the most viable theory explaining its pathogenesis is that it is related to ductal plate malformation at different levels of the intrahepatic biliary tree [5]. Intrahepatic bile ducts develop from bipotential liver progenitor cells that are in contact with the mesenchyme of the portal vein, which form from the ductal plates [6]. The ductal plates are then remodeled into mature tubular ducts. The ductal plate remodeling process begins from the larger ducts to the smaller peripheral ducts. The heredity factors causing Caroli's disease can exert their influence not only during the early embryological period in which large intrahepatic duct formation occurs, but also during the later development of the more proximal interlobular ducts involved in CHF.

The molecular pathogenesis of Caroli's disease is incompletely understood. Human and experimental data have suggested several potential mechanisms that could lead to cyst



(a) (b)

FIGURE 1: The liver of a Caroli's disease patient with CHF. Multiple cystic dilatations of the intrahepatic bile ducts are grossly (a) and histologically (b) visible. Hematoxylin-eosin staining (b).

formation in fibropolycystic liver diseases including those of Caroli's disease patients: (i) increased cell proliferation and apoptosis; (ii) enhanced fluid secretion; (iii) abnormal cell-matrix interactions; (iv) alterations in cell polarity; and (v) abnormal ciliary structure or function [7]. To study the cyst pathogenesis of ARPKD, different experimental animal models including cpk, bpk, and orpk mice and several types of knockout mice have been used [8–12]. Among them, the polycystic kidney (PCK) rat is an orthologous model of ARPKD that represents the phenotype of the slowly progressive form of ARPKD and is also a novel animal model of Caroli's disease with CHF [13].

This article reviews our current knowledge of the pathogenesis of Caroli's disease with CHF, particularly focusing on studies about the mechanism responsible for the biliary dysgenesis observed in the PCK rat. First, the clinicopathological and genetic aspects of Caroli's disease with CHF are described. In the following section, Caroli's disease refers to the form of the disease associated with CHF, since Caroli's disease without CHF is rare.

2. Caroli's Disease

2.1. Clinical Features. Renal involvement is encountered in up to 60% of patients with Caroli's disease [14]. Hepatic manifestations of ARPKD are present in 15–45% of patients and include an enlarged liver, portal hypertension, or abnormal findings on hepatic imaging [15]. A few rare cases of Caroli's disease have occurred in the setting of autosomal dominant polycystic kidney disease (ADPKD) [16].

The clinical manifestations of Caroli's diseases are related to both biliary abnormalities and portal hypertension due to CHF [14, 17, 18]. Its clinical progression and presentation are highly variable, and symptoms may appear late in life.

Bile ducts dilatation induces a predisposition to bile stagnation, leading to the formation of lithiases. Bacterial cholangitis occurs frequently and may be complicated by hepatic abscess formation and sepsis. Recurrent cholangitis dominates the clinical course and is the principal cause of morbidity and mortality. After cholangitis occurs, a large number of patients die within 5–10 years [14]. Secondary biliary cirrhosis can occur due to biliary obstruction.

Portal hypertension due to CHF may lead to ascites and esophageal variceal hemorrhaging. Splenomegaly and hepatomegaly are common. Children with Caroli's disease usually display earlier symptom onset and a more rapidly progressive disease because of the combined effects of cholangitis and portal hypertension.

Caroli's disease may progress to cholangiocarcinoma. The occurrence of cholangiocarcinoma has been reported in 7–14% of patients [19]. A rare case of cholangiocarcinoma arising in CHF has also been reported [20].

The laboratory findings of Caroli's disease are nonspecific. Transaminase levels may be slightly elevated. A complete blood count might reveal thrombocytopenia and leukopenia if portal hypertension and hypersplenism are present. An elevated white blood cell count and increased serum alkaline phosphatase or direct bilirubin levels could indicate cholangitis. BUN and creatinine values should also be measured to detect any associated renal disease.

2.2. Pathology. The biliary abnormalities of Caroli's disease are characterized by progressive and segmental saccular or cystic dilatation of the intrahepatic bile duct (Figure 1). The disease might be limited to one lobe of the liver, most commonly the left lobe. Histologically, the dilated ducts are lined by the biliary epithelium, which may be hyperplastic and ulcerated. In patients with cholangitis, an acute and chronic inflammatory cell infiltrate is seen around the dilated bile ducts. In the presence of CHF, dense portal fibrosis is observed, and the fibrotic region often contains variable numbers of abnormally shaped bile ducts and hypoplastic portal vein branches. The hepatic parenchyma is subdivided by the overgrowth of portal fibrous tissue, while no parenchymal regenerative activity is evident, which allows the condition to be differentiated from cirrhotic regenerative nodules.

The mechanism of the development of cholangiocarcinoma in Caroli's disease remains unclear. In chronic biliary diseases, it has become evident that cholangiocarcinoma

arising in the large bile ducts undergoes a multistep carcinogenic process, and biliary intraepithelial neoplasia (BilIN) is considered to be the precursor lesion [21]. BilIN is frequently seen in patients with hepatolithiasis, and it can be encountered in the livers of Caroli's disease patients. Biliary papillomatosis has also been observed in Caroli's disease [22]. Thus, cholangiocarcinoma in Caroli's disease probably arises despite a multistep carcinogenic process that is closely related to chronic epithelial damage.

2.3. Pathological Studies. There is limited data available from pathological molecular studies using human liver tissues from patients with Caroli's disease and/or CHF. Cholangiocytes from the livers of patients with Caroli's liver have been shown to overexpress vascular endothelial growth factor (VEGF), its receptors (VEGFR-1 and VEGFR-2), and angiopoietin-2 [23]. VEGF expression on cholangiocytes positively correlates with microvascular density around the bile ducts, suggesting that it has a proliferative effect on cholangiocyte growth by inducing the production of an abundant vascular supply. VEGF may also stimulate bile duct dilatation through the induction of cholangiocyte proliferation via an autocrine effect [24]. In addition, the activation of the mammalian target of rapamycin (mTOR) pathway has been implicated in the overgrowth of cholangiocytes in Caroli's disease [25].

Cholangiocyte overgrowth is linked to abnormalities in cell cycle progression and also to microRNA expression. The progression of cells through the cell cycle is controlled by a family of dual specificity phosphatases, Cdc25, that activate cyclin-dependent kinases. The biliary epithelium of CHF overexpresses Cdc25A protein (an isoform of Cdc25), which is accompanied by the downregulation of a microRNA (miR15a) [26].

Around intrahepatic bile ducts, basement membrane components such as laminin and type IV collagen, the major basal laminar components are degraded in Caroli's disease [27]. These findings indicate that the reduction of laminin and type IV collagen expression in the basement membrane, a supportive structure of intrahepatic bile ducts exacerbates the observed bile duct dilatation. The degradation of laminin and type IV collagen around bile ducts is also observed in foci of cholangiocarcinoma in situ arising in Caroli's disease, indicating that once cholangiocarcinoma in situ develops in the biliary epithelia of Caroli's disease patients, it tends to transform into invasive carcinoma [27].

In most types of chronic liver disease, activated hepatic stellate cells play major roles in hepatic fibrosis. However, necroinflammatory changes and the activation of hepatic stellate cells are not as marked in CHF as those seen in ordinary chronic liver diseases such as chronic viral hepatitis. The fact that abundant connective tissue growth factor (CTGF) is retained by heparin sulfate proteoglycans (HSPG) in the fibrous portal tracts could be responsible for the unresolved hepatic fibrosis observed in CHF [28]. Portal mononuclear cells and endothelial cells expressing CTGF and/or HSPG tend to collect around proliferated bile ducts in CHF, providing a possible explanation for the mechanism of the fibrosis that characterizes CHF.

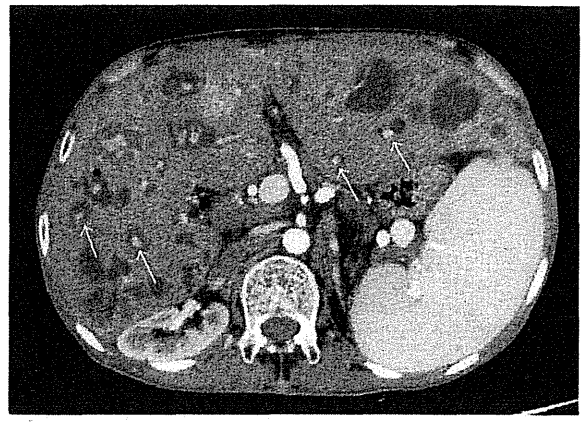


FIGURE 2: Dynamic CT reveals multiple cystic dilatations of the intrahepatic bile ducts in a patient with Caroli's disease with CHF. The arrows indicate the central dot sign.

2.4. Diagnosis. Caroli's disease is diagnosed by imaging studies showing nonobstructive, saccular, or fusiform dilatations of the intrahepatic bile ducts [29–31]. Ultrasonography and endoscopic retrograde cholangiopancreatography are the traditional methods of diagnosis. However, magnetic resonance cholangiopancreatography is emerging as the most useful diagnostic modality. On sonography, Caroli's disease presents as intrahepatic cystic anechoic areas in which fibrovascular bundles and linear bridges or septa may be present. The fibrovascular bundles are composed of the portal veins and hepatic arteries, which can be demonstrated by Doppler ultrasonography and are recognized as the central dot sign on CT with contrast enhancement (Figure 2). Overlapping imaging findings are often detected, which reflect its underlying pathology and associated complications, including fibrosis, ductal dilatation, cholangitis, lithiasis, and malignancy [32]. A liver biopsy is rarely required to make a diagnosis of Caroli's disease.

2.5. Treatment. Treatment for Caroli's disease is largely supportive and is directed toward treating the biliary infection and complications associated with portal hypertension [15]. Cholangitis, hepatic abscesses, and sepsis should be treated aggressively with appropriate antibiotics. Infections are particularly difficult to eradicate in the presence of bile stasis and intrahepatic lithiasis. Recurrent bouts of cholangitis can lead to end-stage liver disease.

Common bile duct stones may require endoscopic sphincterotomy and stone extraction, while the extraction of intrahepatic stones is difficult. Partial hepatectomy may be curative when the disease is confined to a single lobe of the liver [33]. Ursodeoxycholic acid has been used to treat intrahepatic lithiasis, which probably acts by increasing bile flow and decreasing bile stasis [34].

Variceal bleeding can be treated endoscopically with sclerotherapy or band ligation. A selective shunting procedure can provide relief from the complications associated with portal hypertension.

(a) (b)

FIGURE 3: The liver of a PCK rat. The gross (a) and histological (b) appearance of the adult rat liver closely resembles those of patients with Caroli's disease with CHF (Figure 1). Hematoxylin-eosin staining (b).

Liver transplantation is regarded as the ultimate treatment for patients who suffer recurrent bouts of biliary infection and those who also have complications related to portal hypertension [35]. Patient survival is reported to be excellent, and graft survival is comparable to or better than that of patients who have received transplants for other diseases [36]. Since the inheritance of the disease seems to occur in an autosomal recessive manner, it is important to provide genetic counseling to the patient's family.

2.6. Genetics. ARPKD is caused by mutations in a single gene, *PKHD1*, which has been localized to chromosome 6p21.1-p12. The gene consists of 86 exons and has a number of alternatively spliced transcripts. Its longest open reading frame contains 67 exons, which encode a 4074 amino acid protein called fibrocystin or polyductin [37, 38].

PKHD1 exhibits a high level of allelic heterogeneity, and more than 300 mutations have been described throughout *PKHD1*. A clear genotype/phenotype correlation has been described in ARPKD, with two truncating mutations associated with the most severe phenotype, while one or two missense changes are associated with milder disease [39]. The genetic basis for the differences between ARPKD with and without CHF has not been fully elucidated [40]. Mutations in *PKHD1* have also been identified in patients with Caroli's disease. *PKHDL1*, a homologous gene that is not involved in renal cystic disease has also been described [41].

2.7. Fibrocystin/Polyductin. Fibrocystin is a receptor-like membrane-associated protein. Structural predictions indicate that it has a large extracellular region with multiple copies of the TIG domain (an immunoglobulin-like fold), a single transmembrane region, and a short cytoplasmic tail. Based on its similarity with other TIG-containing proteins such as the hepatocyte growth factor receptor MET, fibrocystin is suggested to function as a receptor or ligand, since secreted forms can be generated from alternatively spliced transcripts [42]. In addition, the promoter might be directly regulated by hepatocyte nuclear factor-1β [43].

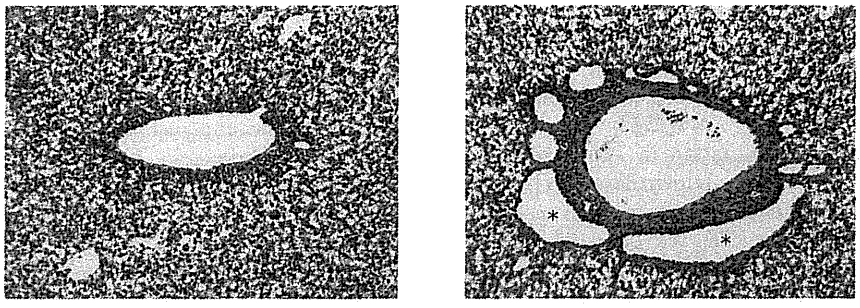
PKHD1 is preferably expressed in the kidneys with lower levels observed in the liver, pancreas, and lungs [37]. Similarly, fibrocystin is expressed in the cortical and medullary collecting ducts of the kidney as well as the biliary and pancreatic ducts [44], in which its distribution is consistent with the disease's phenotype. Fibrocystin is localized to primary cilia as well as to the basal body of epithelial cells and colocalizes with polycystin-2, the gene product responsible for ADPKD [42, 45]. Fibrocystin is also expressed in the normal ductal plate as well as in ductal plate malformations including CHF and colocalizes with stem cell markers in some ductal plate cells [46].

Fibrocystin can undergo notch-like processing, resulting in the release of the ectodomain from primary cilia [47]. Other studies have demonstrated cleavage of the fibrocystin ectodomain as well as the generation of a cytoplasmic fragment that translocates to the nucleus [48]. Such proteolytic cleavage can be elicited by the stimulation of intracellular calcium release or protein kinase C activation. Fibrocystin and polycystin-2 may act in a common molecular pathway to regulate calcium responses in the epithelia [49]. The structure and homologies of fibrocystin suggest that it plays a role in the regulation of cellular adhesion, repulsion, and proliferation and/or the regulation and maintenance of renal collecting tubules and bile ducts, but its exact role in normal and cystic epithelia remains unknown.

3. The PCK Rat

The PCK rat is derived from a Crj:CD (Sprague-Dawley) rat strain, originating in Japan [50]. The polycystic disease it suffers from is inherited in an autosomal recessive manner, and this model has a spontaneous mutation in its *Pkhd1* gene, an ortholog of human *PKHD1* [37]. The model has been rederived and is commercially available from Charles River Laboratories (Wilmington, MA).

In the livers of PCK rats, multiple segmental and saccular dilatations of the intrahepatic bile duct are observed (Figure 3). In addition, ductal plate malformations are evident in the livers of PCK rat fetuses (Figure 4), and the ductal dilatation spreads throughout the liver and increases in degree with age. The overgrowth of portal connective tissue progresses after delivery. All of the gross and histological



(a) (b)

FIGURE 4: Ductal plate malformation in the fetal liver of a PCK rat. Compared with a normal fetal rat (a), dilatation of the ductal plate is evident in the PCK liver (b, asterisks). Hematoxylin-eosin staining (a, b).

features of the PCK liver correspond to Caroli's disease with CHF.

To explore the mechanism of biliary dysgenesis suffered by PCK rats, a cholangiocyte cell line has been developed from the intrahepatic bile ducts of the PCK rat [51, 52]. The cholangiocytes of the PCK rat exhibit a higher rate of proliferation, with a doubling time of approximately half that of normal cholangiocytes, and maintain their biliary features during passaging.

Hereafter, based on the results of studies using PCK rats and/or the cholangiocyte cell line derived from them, the recent developments in our understanding of the cellular and molecular pathogeneses of the biliary dysgenesis observed in PCK rats are reviewed.

3.1. Proliferation and Apoptosis. Two key signaling pathways, 3′,5′-cyclic adenosine monophosphate (cAMP) activated B-Raf/MEK/ERK and AKT/mTOR/S6K/S6, have been implicated in the increased proliferation of PCK cholangiocytes.

Epidermal growth factor (EGF) and its receptor (EGFR) play important roles in promoting cholangiocyte proliferation. PCK cholangiocytes are hyperresponsive to EGF, and the increase in their proliferation is accompanied by activation of the MEK5/ERK5 pathway [51]. The phosphorylation of ERK1/2 is also increased in PCK cholangiocytes [53]. In contrast to other mouse models of ARPKD, EGFR is not overexpressed or mislocalized to the apical membrane in the PCK cholangiocytes [51, 54].

The cAMP levels of the PCK cholangiocytes are also increased [55]. These elevated cAMP levels stimulate cholangiocyte proliferation via downstream effectors and exchange proteins activated by cAMP (Epac1 and Epac2 isoforms) and protein kinase A (PKA) [56]. Hyperproliferation of the PCK cholangiocytes in response to PKA stimulation is associated with decreased intracellular calcium levels, and the restoration of calcium levels blocks PKA-dependent proliferation via the PI3K/AKT pathway. In addition, PCK cholangiocyte hyperproliferation is accompanied by the overexpression of Cdc25A protein and the downregulation of miR15a [26, 57]. miR15a overexpression in PCK cholangiocytes decreases Cdc25A levels, inhibits cell proliferation, and reduces cyst growth, indicating a potential therapeutic strategy for the disease.

The signaling pathway composed of AKT/mTOR/S6K/S6 is activated in PCK cholangiocytes (Ren XS et al., unpublished data). In the PCK rat liver, apoptosis of the biliary epithelium is less frequent than in normal rats until 1 week after delivery but is more common than in normal rats at 3 weeks after delivery [13]. Thus, dysregulated cell kinetics may be involved in the biliary abnormalities associated with PCK rats.

3.2. Fluid Secretion. Activated cAMP pathways can also lead to increased fluid secretion. Indeed, bile secretion is increased in the PCK rats compared with that in age-matched normal rats [58]. In normal cholangiocytes, the water channel aquaporin-1, the chloride channel cystic fibrosis transmembrane conductance regulator (CFTR), and the anion exchanger AE2 regulate ion-driven water transport. In PCK cholangiocytes, aquaporin-1, CFTR, and AE2 are overexpressed and show abnormal cellular localization, which could account for their altered fluid secretion [59].

3.3. Cell-Matrix Interactions. As is true in human Caroli's disease, the matrix proteins of the basement membranes of the intrahepatic bile ducts are degraded in PCK rats, and the biliary epithelium sits on the basement membrane and displays abnormal decreases in laminin and type IV collagen expression [27]. Since PCK cholangiocytes overexpress plasminogen and the tissue-type plasminogen activator, the generation of excessive amounts of plasmin and the subsequent plasmin-dependent lysis of extracellular matrix molecules may contribute to the progressive bile duct dilatation observed.

3.4. Primary Cilia and Ciliopathies. Cholangiocytes are ciliated cells, and cholangiocyte cilia extend from the apical plasma membrane into the bile duct lumen [60]. Cholangiocyte primary cilia are mechanosensory, osmosensory, and chemosensory organelles that can detect changes in bile flow and osmolarity and transduce them into intracellular signals. Changes in flow are communicated to other cellular response

elements via changes in intracellular calcium and cAMP concentrations. Increased flow causes a cilium-dependent rise in intracellular calcium followed by a decrease in the cAMP concentration via a calcium-inhibitable adenyl cyclase.

In PCK rats, a splicing mutation in *Pkhd1* results in structural and functional ciliary abnormalities [61]. Fibrocystin is normally localized to primary cilia, whereas defects in fibrocystin from primary cilia are observed in PCK cholangiocytes [62]. Defects in ciliary structure and their integrated sensory/transducing functions appear to result in decreased intracellular calcium and increased cAMP concentrations, causing cholangiocyte hyperproliferation, abnormal cell-matrix interactions, and altered fluid secretion. These modifications can lead to abnormalities in biliary tree differentiation, ultimately resulting in bile duct dilatation.

Other calcium channels such as Trpv4 are present in cholangiocyte cilia, and the activation of Trpv4 leads to increased intracellular calcium levels and reduces the hyperproliferative phenotype of PCK cholangiocytes [63].

In the liver, mutations in genes encoding ciliary-associated proteins cause a broad spectrum of genetically heterogeneous disorders, which are referred to as ciliopathies [64]. Since cholangiocytes are the only epithelial cells in the liver that possesses primary cilia, conditions affecting the liver are more appropriately called cholangiociliopathies [65].

3.5. Cholangitis. As PCK rats age, chronic suppurative cholangitis becomes a frequent histologic finding [66]. Although the clinical significance of cholangitis due to biliary infection is well recognized in Caroli's disease, the impact of biliary infection on its pathogenesis and progression is poorly understood.

Lipopolysaccharides (LPS) induce VEGF expression in PCK cholangiocytes via toll-like receptor 4 expressed on the cells, which is accompanied by the activation of NF-κB (Ren XS et al., unpublished data). Both LPS and VEGF increase the proliferation of PCK cholangiocytes, suggesting that LPS-induced overexpression of VEGF in the biliary epithelium leads to hypervascularity around the bile ducts, and concurrently, LPS and VEGF act as cell proliferative factors for cholangiocytes. Thus, biliary infection may exacerbate biliary cystogenesis through the induction of VEGF in the biliary epithelia of PCK rats.

Cholangitis is frequently associated with goblet cell metaplasia of the biliary epithelium in PCK rats. LPS induces upregulated CDX2 expression followed by aberrant mucus core protein-2 expression via the activation of NF-κB in PCK cholangiocytes, which accounts for the development of intestinal metaplasia in the setting of biliary infection [66]. Although recurrent cholangitis probably leads to the development of cholangiocarcinoma in some patients with Caroli's disease, we have not encountered the occurrence of cholangiocarcinoma in PCK rats.

3.6. Hepatic Fibrosis. A mechanism similar to the epithelial-mesenchymal transition (EMT) has been implicated in the hepatic fibrosis observed in PCK rats [67]. In PCK rat liver sections, the intrahepatic bile ducts display two different phenotypes, bile ducts lined by cuboidal-shaped (C-type), and flat-shaped (F-type) cholangiocytes. The flat-shaped cholangiocytes (F-type) show reduced immunohistochemical expression of the biliary epithelial marker cytokeratin19 and positive immunoreactivity for the mesenchymal markers vimentin and fibronectin. Treating PCK cholangiocytes with transforming growth factor-β1 (TGF-β1), a potent inducer of EMT induces the expression of vimentin and fibronectin in vitro, indicating that PCK cholangiocytes acquire mesenchymal features in response to TGF-β1 and participate in progressive hepatic fibrosis by producing extracellular matrix molecules. EMT has been also implicated in the pathogenesis of interstitial fibrosis in the kidneys of PCK rats [68].

In elderly PCK rats, suppurative cholangitis is a frequent histological finding in C-type cholangiocytes, while F-type cholangiocytes are not associated with suppurative cholangitis accompanied by polymorphonuclear leukocyte accumulation in their lumen [67]. In addition, F-type cholangiocytes occasionally show a fibrous scar-like appearance. Recent studies have shown that the majority of dilated intrahepatic bile ducts in PCK rats are initially connected to the biliary tree but over time become separated from it, resulting in true cyst formation [61]. It is speculated that F-type cholangiocytes are derived from the bile ducts that have been disconnected from the biliary tree, which may account for the observation of true biliary cysts in PCK rats.

The renin-angiotensin system is upregulated in the livers of PCK rats [69]. Angiotensin-converting enzyme (ACE) and angiotensin II, as well as their downstream target, the profibrotic mediator TGF-β, are overexpressed in the PCK liver, suggesting that the renin-angiotensin system activation is another important mediator of hepatic fibrosis.

3.7. Therapeutic Approaches. Understanding of the molecular mechanisms of cyst formation and growth has led to the discovery of novel potential therapeutic approaches for fibropolycystic diseases. However, in PCK rats, relatively few therapeutic reagents are effective for both liver and kidney cystogenesis.

Octreotide, a somatostatin analogue known to inhibit cAMP, decreases hepatic cyst volume, the hepatic fibrosis score, and mitotic indices in the PCK liver, and similar effects are observed in the kidneys [55]. Pioglitazone, a peroxisome proliferator activator receptor gamma agonist, inhibits bile duct dilatation and hepatic fibrosis as well as renal cyst growth, which is associated with decreased CFTR expression and reduced cell proliferation [53, 70].

As another example of therapies that are effective for both liver and kidney lesions in PCK rats, the inhibition of Src activity with SKI-606 ameliorates biliary ductal abnormalities and renal cyst formation [71]. The effects of Src inhibition suggest that the Erb2 and B-Raf/MEK/ERK pathways are involved in Src mediated signaling in ARPKD and that this occurs without any reduction in cAMP levels.

The inhibition of renal cAMP production by treatment with a vasopressin V2 receptor antagonist or by increasing water intake to reduce plasma vasopressin decreases cell proliferation and ameliorates kidney cystogenesis with an associated reduction in B-Raf/MEK/ERK activity, leading to

improved renal function in PCK rats [72–74]. However, consistent with the absence of the vasopressin V2 receptor in the liver, it does not have a significant effect on fibropolycystic liver disease. Similarly, Trpv4 activation induces a significant decrease in renal cystic area but causes a nonsignificant decrease in liver cyst formation [63].

ACE inhibition by chronic treatment with lisinopril decreases proliferative and apoptotic pathways in the kidneys of PCK rats, resulting in improved kidney function [75]. Chronic blockade of 20-hydroxyeicosatetraenoic acid (HETE) with a specific inhibitor of the CYP4A and CYP4F enzyme family prevents the formation of 20-HETE, resulting in a significant decrease in renal cyst formation in PCK rats [76]. The activation of calcium-sensing receptors with R-568 reduces the interstitial fibrosis, but not the cystogenesis, of the PCK kidney [77]. However, it is unclear whether these treatments, that is, the inhibition of ACE and 20-HETE synthesis, and the activation of calcium-sensing receptors, are effective treatments for biliary dysgenesis in PCK rats.

Gefitinib, an EGFR tyrosine kinase inhibitor, significantly improves biliary cystogenesis and hepatic fibrosis in PCK rats but has no beneficial effects on renal cyst pathogenesis [78]. In addition, EGFR tyrosine kinase inhibition with EKI-785 and EKB-569 has no effect on biliary dysgenesis in PCK rats, and the kidney lesions are unaffected or rather worsened by the treatment [54]. The inhibition of mTOR with sirolimus does not attenuate the progression of liver or kidney disease in PCK rats, which may be due to intrinsic or acquired sirolimus resistance [79].

4. Conclusions

The development of PCK rats has allowed us to explore the molecular pathogenesis of the disease and potential therapeutic strategies for Caroli's disease and ARPKD. The relevance of findings obtained from studies using PCK rats and/or the cholangiocyte cell line derived from them to the pathogenesis of human diseases is currently being analyzed, and several key signaling pathways have been elucidated. It seems likely that future treatments for Caroli's disease will involve combination therapies affecting several cystogenesis pathways.

Abbreviations

ACE: Angiotensin-converting enzyme
ADPKD: Autosomal dominant polycystic kidney disease
ARPKD: Autosomal recessive polycystic kidney disease
BilIN: Biliary intraepithelial neoplasia
cAMP: 3′,5′-Cyclic adenosine monophosphate
CHF: Congenital hepatic fibrosis
CFTR: Cystic fibrosis transmembrane conductance regulator
CTGF: Connective tissue growth factor
EGF: Epidermal growth factor
EGFR: EGF receptor
EMT: Epithelial-mesenchymal transition
HETE: Hydroxyeicosatetraenoic acid
HSPG: Heparin sulfate proteoglycan
LPS: Lipopolysaccharide
mTOR: Mammalian target of rapamycin
PKA: Protein kinase A
PCK: Polycystic kidney
TGF-β: Transforming growth factor-β
VEGF: Vascular endothelial growth factor
VEGFR: VEGF receptor.

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