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Cellular Senescence in Cholestatic Liver Injury

Sato K[3]*, Meng F[1,2,3], Glaser S[1,2,3] and Alpini G[1,2,3]

[1]Research, Central Texas Veterans Health Care System, USA

[2]Baylor Scott & White Digestive Disease Research Center, Baylor Scott & White Health Care, USA

[3]Department of Medical Physiology, Texas A&M University College of Medicine, USA

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***Corresponding author:** Keisaku Sato, Department of Medical Physiology, Texas A&M University Health Science Center, College of Medicine, Department of Medical Physiology, 1901 South 1st Street, Bldg. 205, 1R58 Temple, TX, 76504, Tel: 254-743-2502; Email: sato@medicine.tamhsc.edu

Abstract

Cholestatic liver diseases such as primary biliary cholangitis and primary sclerosing cholangitis are characterized by bile duct inflammation and damage followed by obstruction or impaired bile flow leading to liver fibrosis. Cholangiocytes are the target of therapies of cholangiopathies. Although bile duct hyperplasia caused by elevated cholangiocyte proliferation is characteristic during cholestatic liver injury, accumulating evidence shows that enhanced cellular senescence in cholangiocytes is an important factor for pathophysiology of cholangiopathies. Senescent cholangiocytes secrete elevated levels of proinflammatory cytokines and chemokines that activate hepatic stellate cells or myofibroblasts leading to fibrogenesis in the liver. Previous studies suggest that endotoxin or lipopolysaccharide derived from gut bacteria may be a key factor for cholangiocyte senescence in cholangiopathies. This review summarizes current understandings for functional roles of cellular senescence in cholangiocytes during cholestatic liver injury.

Keywords: Cellular Senescence; Cholestatic liver injury; Cholangiocyte

Abbreviations: BA: Biliary Atresia; BDL: Bile Duct Ligation; IL: Interleukin; LPS: Lipopolysaccharide; PBC: Primary Biliary Cholangitis; PSC: Primary Sclerosing Cholangitis; SA-β-gal: Senescence-associated β-galactosidase; SASP: Senescence-Associated Secretory Phenotype; TAA: Thioacetamide; SAHF: Senescence-Associated Heterochromatin Foci.

Introduction

Cellular senescence is a state of cells that is caused by replication stress and/or various DNA or cellular damage. It is characterized by strong and irreversible cell cycle arrest [1-3].

Senescent cells also show morphological change, increased activity of senescence-associated β-galactosidase (SA-β-gal), elevated expression of senescence-associated genes, such p16 and p21, and senescence-associated heterochromatin foci (SAHF),

which are specialized domains of heterochromatin that lead to suppression of proliferation-promoting genes [4]. Senescent cells are functionally and metabolically active, and they secrete elevated or different compositions of secretome, which is referred as senescence-associated secretory phenotype (SASP), compared to normal cells including extracellular vesicles, proinflammatory cytokines, chemokines, growth factors, and profibrotic factors [4-6]. For more information about mechanisms of cellular senescence, see recent reviews [7-9].

Recent studies have shown that cellular senescence can be identified in cholangiopathies or cholestatic liver injuries, such as primary biliary cholangitis (PBC), primary sclerosing cholangitis (PSC), and biliary atresia (BA). Cholangiocytes are bile duct epithelial cells and they are a primary target of therapies for cholestatic liver diseases. Accumulating evidence suggests that cellular senescence in cholangiocytes may play a key role in pathophysiology of cholangiopathies [10,11]. This review summarizes current understandings of cholangiocyte senescence during cholestatic liver injuries.

Association Between Cholangiocyte Senescence and Cholestatic Liver Injury

Cholangiopathies, such as PBC and PSC, are characterized by activated proliferating cholangiocytes that lead to bile duct hyperplasia or ductular reaction. Cholangiocytes are quiescent at normal conditions but are activated by various mediators including cytokines, hormones, or bile acids that are secreted in the liver at disease conditions [12,13]. Cholangiocytes are heterogeneous cells, small and large cholangiocytes, depending on the diameter of bile ducts (<15 μm or >15 μm diameter for small or large cholangiocytes, respectively) [14,15]. Small and large cholangiocytes have different functions as well as protein expressions *in vivo* [16-19]. In experimental animal models of cholestatic liver injuries using bile duct ligation (BDL), large but not small cholangiocytes proliferated in BDL rats [20]. During acute carbon tetrachloride feeding, large cholangiocytes were damaged showing elevated apoptosis, but small cholangiocytes started proliferation [21]. Kamimoto, *et al*. have performed three-dimensional tracking of labeled cholangiocytes during thioacetamide (TAA)-induced liver injury *in vivo* and have found that some cholangiocytes but not all are proliferative contributing to dynamic remodeling of biliary tree, and those proliferative cholangiocytes are scattered in the biliary epithelial tissue [22]. These studies show that cholangiocytes are heterogeneous in cell functions and proliferation during liver damage.

Although elevated cholangiocyte proliferation leading to bile duct hyperplasia is characteristic during cholestatic liver injuries, previous studies have demonstrated that cellular senescence in cholangiocytes is also characteristic in cholangiopathies. Sasaki, *et al*. have analyzed liver tissue samples from PBC patients and have found that small bile ducts in early stage of PBC patients show highly elevated cellular senescence compared to those of healthy individuals detected by SA-β-gal activity and expression of senescence markers p16 and p21 [23]. Tabibian, *et al*. have also analyzed liver samples of PBC and PSC patients and have demonstrated that cholangiocytes in liver tissues of PBC and PSC patients have elevated expression levels of senescence markers including p16 as well as SASP markers including interleukin (IL)-6, IL-8, and CCL2 that are proinflammatory cytokines causing biliary inflammation [24].

In studies using experimental animal models of cholestatic liver injuries, $Mdr2^{-/-}$ mice are commonly used as a model of PSC [25-27]. Isolated cholangiocytes from $Mdr2^{-/-}$ mice showed elevated expression levels of p16 and p21 compared to cholangiocytes from wild-type mice [28]. Cultured cholangiocytes isolated from PSC patients showed higher SA-β-gal activity, lower cell proliferation, and elevated expression of IL-6 and IL-8 compared to control normal human cholangiocytes [29]. Another study has also demonstrated that cultured cholangiocytes isolated from PSC patients are senescent showing elevated SA-β-gal activity and p16 expression [30]. These studies indicate that some cholangiocytes are not proliferative but senescent showing SASP characteristics during PBC or PSC progression, and this may contribute to disease conditions in the liver or progression of cholangiopathies.

The Cause of Cholangiocyte Senescence in Cholestatic Liver Injury

Although detailed mechanisms for pathophysiology of cholangiocyte senescence in cholangiopathies are not fully understood to date, a potential key mediator is bacteria-associated endotoxin or lipopolysaccharide (LPS). LPS is a strong senescence inducer and stimulation with LPS introduces cellular senescence in various tissues and cells [31-33]. Sasatomi, *et al*. have analyzed liver specimen from PBC and PSC patients and have identified accumulated endotoxin in cholangiocytes of these patients using a monoclonal antibody against lipid A [34]. Fluorescence intensity of lipid A in cholangiocytes was over ten times higher in PBC or PSC patients compared to

healthy individuals [34]. Another study has demonstrated that PBC patients have elevated small bowel permeability compared to healthy individuals detected by lactulose-mannitol test [35]. Endotoxin/LPS are a strong inducer of inflammatory responses by activating Kupffer cells leading to inflammation and fibrogenesis in the liver [36]. Although short-term LPS stimulation induces cell proliferation and proinflammatory cytokine production in cholangiocytes *in vitro*, [37,38] long-term LPS stimulation induces cellular senescence in cholangiocytes *in vitro* [24]. Senescent cholangiocytes induced by LPS stimulation secreted elevated proinflammatory cytokines [24].

During cholestatic liver injuries, cholangiocytes become senescent and secrete various cytokines such as IL-6 and TGF-β1 [28-39]. Kupffer cells or bone marrow-derived macrophages in the liver also produce those cytokines when activated at disease conditions [36]. TGF-β1 is a key factor for fibrogenesis because it activates hepatic stellate cells that are the major source of extracellular matrix proteins leading to liver fibrosis [40,41]. Figure 1 summarizes functional roles of senescent cholangiocytes in cholangiopathies.

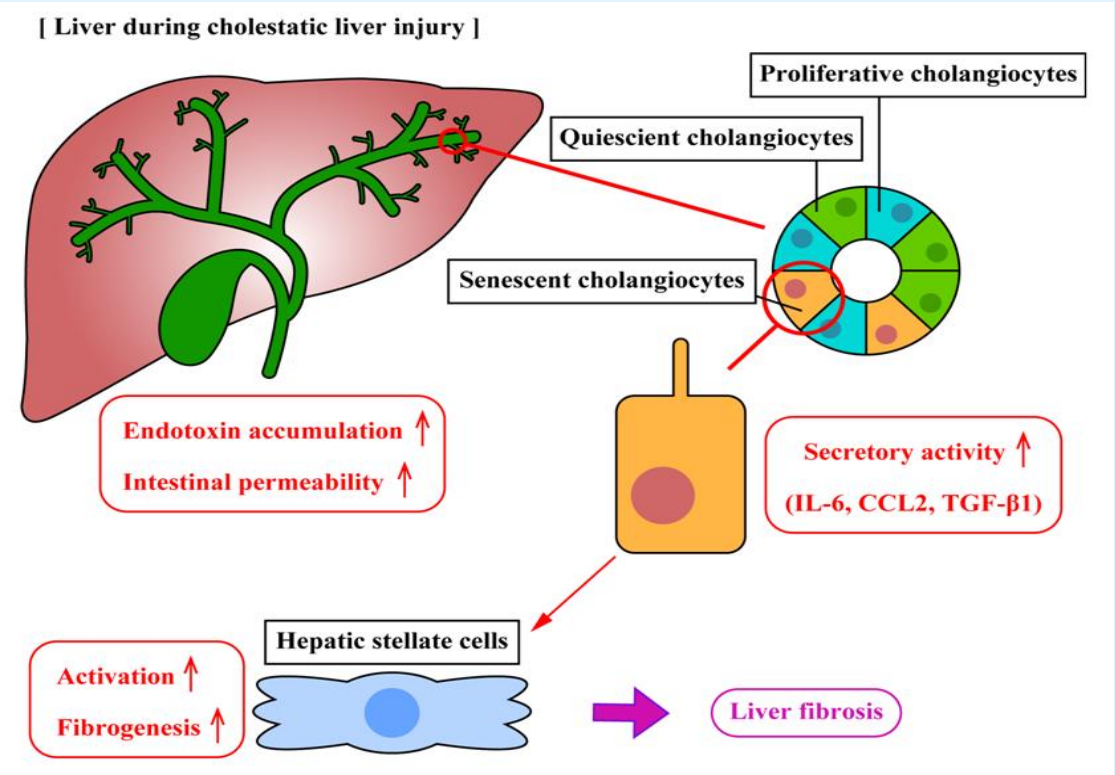

Figure 1: During cholangiopathies, intestinal permeability is elevated and bacteria-derived endotoxin/LPS are accumulated in cholangiocytes. Some cholangiocytes are proliferative but others are senescent. Senescent cholangiocytes show SASP characteristics with elevated secretion of cytokines and fibrotic factors such as IL-6 and TGF-β1. TGF-β1 activates hepatic stellate cells and/or myofibroblasts leading to fibrogenesis in the liver.

Conclusion and Future Perspectives

Current studies suggest that some cholangiocytes are proliferative but others are senescent and these senescent cholangiocytes may play a key role in pathophysiology of liver inflammation and fibrosis during cholestatic liver injuries via secretion of SASP such as IL-6 and TGF-β1. Cholangiocyte senescence is a potential therapeutic target to manage or cure liver conditions in cholangiopathies. Moncsek, *et al*. has found that Bcl-xL is an important factor for fibroblast activation by senescent cholangiocytes, and has demonstrated that inhibition of Bcl-xL attenuates liver fibrosis in Mdr2$^{-/-}$ mice [42].

Cellular senescence in cholangiopathies is a relatively new research aspect in this field. Majority of current studies has identified cholangiocyte senescence by SA-β-gal activity assay using liver sections, detection of gene expression for p16 and/or p21 using liver tissues or isolated cholangiocytes, or detection of SASP such as IL-6 or TGF-β1 by PCR or ELISA. Results obtained by those techniques, however, could vary depending on the stage of senescence in cholangiocytes [4]. Although further studies are required to elucidate detailed mechanisms and signaling pathways involved in cellular senescence in cholangiocytes, novel therapies could be developed by targeting senescent cholangiocytes.

In conclusion, cellular senescence in cholangiocytes is associated with liver damage and fibrosis in cholestatic liver injuries, and may be a potential target for novel therapies.

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