Impact of Aging on Liver Cells and Liver Disease: Focus on the Biliary and Vascular Compartments

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The aging process is represented by the time-dependent decay in physiologic functions of living beings. Major interest has been focused in recent years on the determinants of this progressive condition due to its correlative relationship with the onset of diseases. Several hallmark features have been observed in aging, such as genetic alterations, mitochondrial impairment, and telomere shortening. At the cellular level, a senescent phenotype has been identified in response to aging that is characterized by a flat appearance, proliferative arrest, and production of specific molecules. The net effect of these cells in the course of diseases is an argument of debate. In fact, while the onset of a senescent phenotype may prevent tumor spreading, these cells appear to support pathological processes in some conditions. Several studies are now focused on clarifying the specific molecular pathways of aging/senescence in different cells, tissues, or organs. Biliary and vascular components, within the liver, have emerged as important determinants of some form of liver disease. In this review we summarize the most recent achievements on aging/senescence, focusing on the biliary and vascular liver system. *Conclusion:* Several findings, in both preclinical animal models and on human liver specimens, converge in supporting the presence of specific aging hallmarks in the diseases involving these hepatic compartments. (*Hepatology Communications* 2021;5:1125-1137).

Aging recapitulates the several processes impairing pathophysiological functions and homeostasis of a living organism over time. In aged organisms, including humans, several biochemical and cellular systems undergo a progressive time-dependent impairment such as mitochondrial respiration, stem cell reserves, and genome repair function.[1] The possibility to counteract the deleterious effects of aging requires an in-depth knowledge of different intracellular mechanisms involved in this process. In fact, several studies have focused on this target during the past few decades.[2] In 1939, the first observations of a prolonged life span in rodents maintained at a low caloric intake[3] were made; and we are now using senolytic treatment in clinical trials.[4] Moreover, possible individual aging patterns have been recently suggested in humans by multiomic analysis.[5] Aging effects on the liver appear to be less relevant than those on heart, kidney or brain, possibly due to the specific enhanced regenerative properties of the liver.[6,7] However, important hepatic deleterious changes occur with time, also demonstrated in the clinical setting observed by suboptimal outcomes of liver-transplanted patients with hepatitis

Abbreviations: Bcl-xL, B-cell lymphoma, extra-large; BDL, bile duct ligated; CCL2, chemokine (C-C motif) ligand 2; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; FoxA2, forkhead box A2; HCV, hepatitis C virus; HGF, hepatocyte growth factor; HSC, hepatic stellate cell; IL, interleukin; KC, Kupffer cell; LSEC, liver sinusoidal endothelial cell; Mdr2$^{-/-}$, multidrug-resistant knockout; NK-1R, neurokinin-1 receptor; PBC, primary biliary cholangitis; PBP, peribiliary vascular plexus; PSC, primary sclerosing cholangitis; SASP, senescence-associated secretory phenotype; SA-β-gal, senescence-associated-β-galactosidase; SCF, stem cell factor; Sec, secretin; SP, substance P; SR, secretin receptor; TGF-β1, transforming growth factor beta 1; VEGF, vascular endothelial growth factor; α-CGRP, α-calcitonin gene-related peptide.

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C virus (HCV), when older donors were used.[8] In this review, we discuss the specific aging-related biliary and vascular liver phenotypes, including those observed at the cellular/molecular level and with regard to liver diseases.

Liver Aging and Cellular Senescence

Liver aging is associated with impaired proliferative and metabolic functions. With regard to this latter point, evidence suggests that changes occurring in the liver over time may have a role in susceptibility to nonalcoholic fatty liver disease (NAFLD).[9] Linked to the concept of tissue aging, cellular senescence has been recognized as the most important age-associated phenotypic change.[10] Cellular senescence is an irreversible halt of cell cycle progression; thus, the cell is no longer able to proliferate but can remain metabolically active.[11] This process was first described in the early 1960s in diploid cell cultures, in which the exhaustion of replicative cell capacity over time led to a senescent phenotype characterized by growth arrest.[12] Cellular senescence, together with autophagy and apoptosis, participates in the network of the possible cellular response to stress; in contrast, autophagy (a process removing damaged intracellular organelles or molecules) has been suggested to promote senescent transformation in some cases.[13] Although the onset of senescence is postulated to be a protective mechanism against malignant transformation during aging, the secretion of several molecular factors, termed senescence-associated secretory phenotype (SASP), is triggered by these cells.[14] SASP contribute to several biological processes such as angiogenesis, tissue inflammation, and repair. At the same time, SASPs are able to maintain and to diffuse (in the neighboring cells) the senescence phenotype through autocrine and paracrine mechanisms.[14,15] Although the correlation between tissue aging and cellular senescence may be, in part, supported by the possible accumulation of genomic injuries and/or mutational signals during time, the major role in this process appears to be played by telomere shortening.[16] This time-dependent reduction of telomeres, which is a loss of a maximum of 200 kD at the end of any replicative cycle, in the end determines apoptosis or the onset of the senescence phenotype.[17] This process is generally regarded as the form of replicative senescence; however, a premature senescence may also arise in particular conditions of cellular stress and damage.[14] Finally, telomere shortening and/or

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cellular senescence has been consistently reported in human liver diseases,[18,19] suggesting their possible role in these maladies that present a worst outcome in aged livers.

Biliary and Vascular Compartment Interplay

The anatomical relationship between the biliary and the vascular component within the liver was already described by the identification, by means of scanning electron microscopy coupled with liver casting, of the peribiliary-vascular-plexus (PBP).[10,20] A graphical representation of the relationship between PBP and biliary tract is depicted in Fig. 1. Originating from lateral branches of the hepatic artery, the PBP reaches the sinusoids with small direct anastomotic vessels or interconnecting with vessels of the portal vein, and becoming narrow when surrounding smaller ducts. A physiological cooperation occurs between the biliary tract and PBP, as the latter supports cholangiocyte nutrition and recirculation of biliary components back again to the liver.[21] The strict relationship between the biliary compartment and PBP is further confirmed by the parallel proliferation of these two different systems during injury, as observed in bile duct ligated (BDL) rats.[21] Moreover, cross-talk occurs between the biliary and vascular compartment during normal, developmental, or pathological condition, primarily through vascular endothelial growth factor (VEGF) and angiopoietin 1 and 2.[21-24] With regard to VEGF, this is produced by cholangiocytes and supports their growth with an autocrine mechanism. Moreover, in a rodent model, this mediator is capable of counteracting PBP vanishing induced by hepatic artery ligation.[21] Taken together these data support the idea of a unique physiologic system in which biliary and vascular functions are integrated.

Biliary Epithelium

The biliary epithelium is lined by cholangiocytes, which contribute to qualitative and quantitative changes in bile secretion/composition, before its release in the duodenum.[25] Bile acid–independent ductal bile secretion is sustained primarily by the activity of secretin (Sec)/secretin receptor (SR) pathway

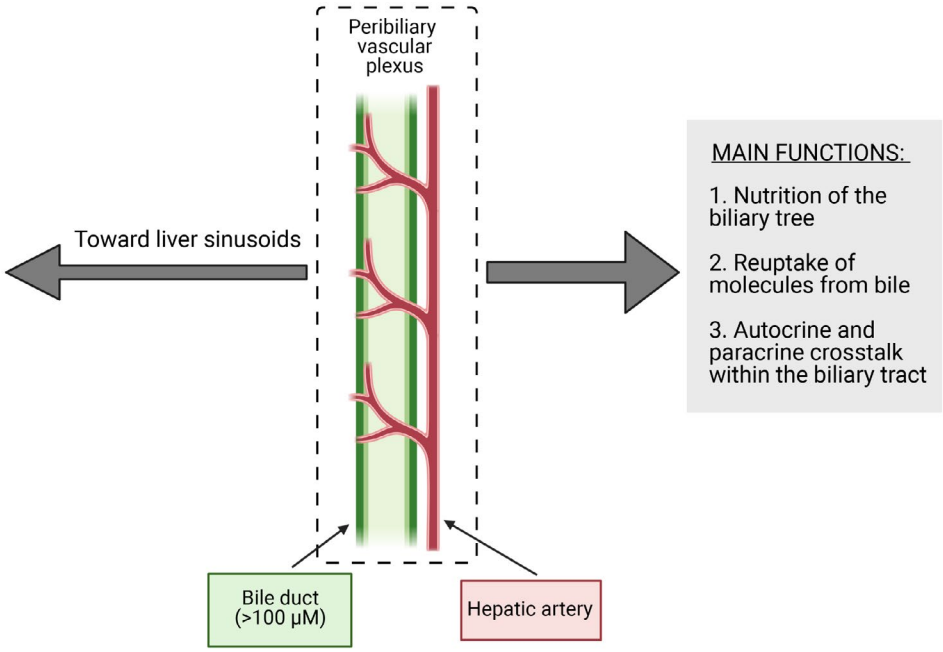

FIG. 1. Schematic representation of biliary tract and peribiliary vascular plexus assembly. The main functions of peribiliary vascular plexus, in supporting biliary tract activities, are reported in the gray box on the right side. (This figure was made with BioRender.com under a purchased license agreement.)

within these cells.[25,26] Following stimulation with its ligand, SR activates cyclic adenosine monophosphate/protein kinase A/cystic fibrosis transmembrane conductance regulator/Cl^-/HCO_3^- exchanger signaling, thus simulating a bicarbonate-enriched choleresis.[27] However, different from other liver cells, numerous hormones and hormone receptors are expressed by cholangiocytes, which regulate not only the secretive, but also the proliferative, response in normal as well as in pathological conditions. Moreover, several other organic molecules, such as bile acids, angiogenic factors or neuropeptides, modulate the activities of the biliary epithelium, suggesting cholangiocytes to be the main liver collectors of signals coming from circulating molecules.[28] Finally, bile duct cells are composed of two different cellular types: small cholangiocytes and large cholangiocytes, lining small and large ducts, respectively.[29] Large cholangiocytes are those promoting the main physiologic activities of biliary tract such as secretion, response to hormones, and mediators.[29] In contrast, small cholangiocytes are considered a quiescent population that is able to proliferate and acquire the large cholangiocytes phenotype when these are damaged.[29]

Aging of the Biliary Epithelium

Age-related impairment of cholangiocytes has emerged as an important field of research in the last decade. The evaluation of the aging processes may, in fact, shed light on the mechanisms of adult chronic cholestatic liver diseases. Among the latter, primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are the most represented in humans.[30] PBC, the most frequent autoimmune liver disease with a prevalence of approximately 30/100,000, generally affects middle age women and is characterized by the presence of circulating anti-mitochondrial antibodies.[31] From a pathological point of view, the disease presents as a lymphocytic cholangitis that may evolve in ductopenia and fibrosis. PSC is less frequent, with a male-to-female ratio of 2:1. Abundant deposition of scar tissue, determining strictures of the biliary tract, is the main hallmark of the disease.[32] Both PBC and PSC are conditions affected by a significant morbidity and mortality, whereas a definitive pharmacological treatment for severe cases has not been identified so far. This perspective, among the other molecular aspects also related to the aging process, deserves interest.

In the context of biliary damage, modulation of biliary senescence during cholestatic damage has become of increasing interest. Indeed, enhanced biliary senescence, specifically in ductular reactive cells, has been found in chronic liver diseases, particularly in PBC.[11] Additionally, enhanced biliary senescence correlates with fibrosis progression in chronic liver diseases.[11] Telomere shortening, which was stated previously as a key aspect of senescence, has been found in human PBC samples,[33] and the effect of biliary senescence on PBC outcomes has been largely studied.[34-36] Cholangiocytes isolated from patients with PSC show exacerbated senescence and SASP marker expression compared with other cholestatic diseases.[19] Similarly, in a 3D organoid model using PSC cholangiocytes, these "cholangioids" displayed senescent and SASP features, which promoted macrophage recruitment and number.[37] The role of senescence/SASP in biliary injury has been demonstrated; thus, evaluation of targeting this axis for therapeutic use is of great interest.

DATA FROM EXPERIMENTAL MODELS

As previously discussed, the main cellular response to the aging process is represented by the onset of a senescent phenotype. Morphologically these cells are larger, with increased cytoplasm/nuclear ratio, and present an increased number of vacuoles[14] The tumor suppressor retinoblastoma protein and p-53 are considered the main molecular routes for the induction of senescent phenotypes.[38] A cellular increase of cyclin-dependent kinase inhibitors is found in senescent phenotype. Among these, p21 and p16 are usually considered as appropriate markers to establish the rate of senescence of a specific tissue in normal and pathological conditions. In addition, levels of senescence-associated-β-galactosidase (SA-β-gal), an enzyme present in lysosomes, has been generally regarded as an important biochemical indicator of senescence and has been used widely in research.[39] Senescent hallmarks have been studied in preclinical models of both PBC and PSC. One study focused on Forkhead box A2 (FoxA2) activity during experimental cholestasis

in BDL and multidrug-resistant knock-out (*Mdr2*$^{-/-}$) mice.[40] FoxA2 is regarded as an important regulator of cell differentiation, and the model of cholestasis is characterized by the decrease of this molecular mediator.[41] In this study, an inverse relationship was observed between FoxA2 expression and hallmarks of senescence such as p16 expression or SA-β-gal staining in liver section. Interestingly, FoxA2 restoration obtained by small cholangiocyte cell therapy determined an important reduction of senescent phenotype.[40] These data were extended by a study examining the role of substance P (SP), a tachykinin family neuropeptide, in the same preclinical models of murine cholestasis (BDL and *Mdr2*$^{-/-}$).[42] The interest regarding SP and biliary tract came by observations demonstrating (1) decreased cholangiocarcinoma (CCA) cells growth and (2) BDL-induced biliary proliferation, after inhibition or genetic suppression of the specific SP receptor neurokinin-1 (NK-1R).[43,44] When senescent features were examined in both cholangiocytes and hepatic stellate cells (HSCs) coming from cholestatic rodent, these were increased in bile duct cells, while the opposite behavior (less senescence, more cellular activity) was observed in HSCs, thus resulting in increased collagen deposition and fibrosis. Moreover, in this study, the SP/NK-1R pathway played a major role in differential modulation of cholangiocyte–HSC senescence balancing, as inhibition of NK-1R increased biliary tract viability and reduced profibrotic processes. Although the specific mechanism linking SP/NK-1R to differential cellular senescence remains undetermined, this research showed that possible regulation of senescent phenotype in different cells might be a possible target for therapy.[45] The relationship of the main secretory and proliferative cholangiocyte pathway, the Sec/SR axis was also examined with regard to cellular senescence in preclinical models of cholestasis. In BDL mice, knockout of the SR gene reduces hyperplasia[46] and inhibition of Sec/SR axis; in *Mdr2*$^{-/-}$ mice, it ablates the transforming growth factor beta 1 (TGF-β1)–mediated enhanced liver fibrosis.[47] Changes in cellular senescence processes were evaluated in a study examining the double SR$^{-/-}$/*Mdr2*$^{-/-}$ knockout mice.[48] The Sec/SR system exhibited effects similar to those observed with regard to SP. In fact, genetic ablation of SR greatly reduced fibrosis and proliferation, down-regulating cholangiocyte senescence and up-regulating the senescent phenotype of HSCs. This view was corroborated by data from the BDL model of Sec$^{-/-}$/SR$^{-/-}$ mice.[47] Again, the differential effect of Sec/SR on cholangiocytes–HSC senescence was confirmed; moreover, these findings were extended, suggesting that Sec-related effects were achieved by TGF-β1/TGF-β1R stimulation.[47] In the last few years, other biomolecules such as α-Calcitonin gene-related peptide (α-CGRP), stem cell factor (SCF), or vimentin have been demonstrated to participate in experimental cholestatic injury, differentially regulating cholangiocyte and HSC senescence. In the α-CGRP$^{-/-}$ mouse model, the typical senescence phenotypic distribution (cholangiocytes^{+}; HSCs^{-}) observed with BDL and in parallel with cholestatic damage was reverted.[49] Accordingly, several senescence markers were decreases in cholangiocytes, such as p16, p21, chemokine (C-C motif) ligand 2 (CCL2), and plasminogen activator inhibitor 1 (PAI-1). SCF signaling also modulates experimental cholestatic biliary damage, as demonstrated by reduction of cholangiocytes injury/senescence when its axis is ablated.[50] Similar results are also obtained by targeting vimentin. Its inhibition improves biliary damage and cellular senescence, possibly interfering with the epithelial to mesenchymal transition (EMT) process of bile duct cells, a mechanism linked to the onset of fibrosis.[51] Finally, the direct targeting of senescent cellular machinery has been attempted, in experimental systems, to improve pathological features. Inhibition of the cyclin-dependent kinase inhibitor p16 has been obtained in a model of cholestasis by the administration of p16 morpholino. This strategy decreased senescence, secretion of SASP, and ductular reaction in *Mdr2*$^{-/-}$ mice.[52] In another experimental approach, B-cell lymphoma, extra-large (Bcl-xL) inhibition determined apoptosis of both senescent cholangiocytes and activated fibroblast, thus reducing liver damage in a *Mdr2*$^{-/-}$ rodent model.[53] In contrast, Bcl-xL overexpression, in the course of cholestatic injury, was related to ETS proto-oncogene factor 1 and p300 cooperation.[54] In conclusion, the data coming from experimental studies support the hypothesis of a unifying model linking ductular reaction, EMT, fibrosis, and the differential cholangiocyte/HSC cellular senescence in an integrated machinery that determines chronic cholestatic liver diseases. This picture may be implemented in the future including also the role of mast cells. During liver injury, mast cells surround the biliary tract and play a critical role

in the contribution by driving a senescent phenotype, thus promoting damage.[55] The main molecular factors, supporting cholangiocyte senescence in experimental models of cholestasis, are summarized in Fig. 2. Correlative findings in human are reported in the following section.

HUMAN STUDIES

Several aspects observed in animal models related to aging, such as telomere shortening, cellular senescence, DNA alteration, mitochondrial impairment and others, have been also observed in human liver.[56] These changes may impair the physiologic defense against liver injury or they could enhance pathological pathways with lipids accumulation, as observed in NAFLD.[57] With regard to cholestatic diseases, for instance, more severe evolution of PBC are usually observed in middle-aged women, with the postmenopausal drop of protective estrogens.[58] However, in addition to unrelated liver aging factors that may worsen cholestatic injury, as in the previous case, specific aging hallmarks have been identified in human liver affected by disease of the biliary tract. Cholangiocytes freshly isolated from patients with PSC are enlarged, with tight junction defects, and stain positive for SA-β-gal (marker of senescence) in nearly 50% of cases.[59] These cells also exhibit an increase production of SASP, such as interleukin (IL) 6 and IL-8. In another study, comparison of liver tissue coming from healthy subjects and patients with PSC, PBC, and HCV demonstrated a maximum increase of markers of cellular senescence (p16) and SASP secretion (IL-6, IL-8, CCL-2, and PAI-1) in patients with PSC, followed by those with PBC.[19] Patients with HCV exhibited cellular senescence features similar to the healthy control. Finally, in the same research, normal human cholangiocytes exposed to different damaging agents within 10 days evolved toward a senescent phenotype with production of SASP. Another study examined the hallmarks of senescence in human PBC liver tissue as a function of clinical outcome and response to therapy.[60] This study demonstrated that (1) the number of senescent cells was proportional to the severity of the disease, and (2) increased expression of p16 was related to an inadequate response to ursodeoxycholic acid standard therapy.

Taken together, these findings suggest that cholangiocyte senescence might be a characteristic feature of

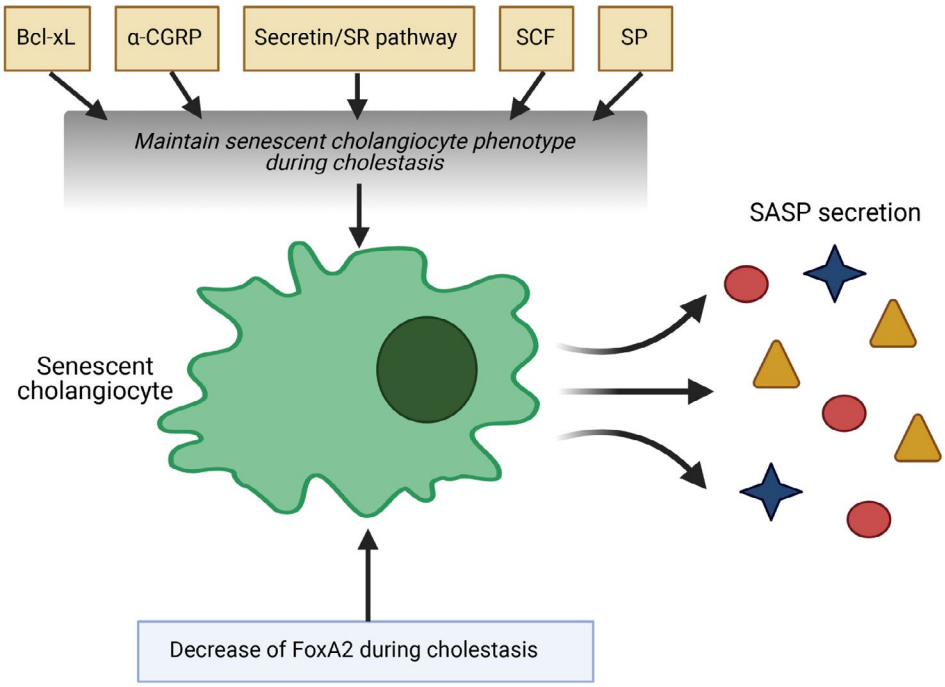

FIG. 2. The molecular determinants of cholangiocyte senescence, identified in experimental models of cholestasis. (This figure was made with BioRender.com under a purchased license agreement.)

biliary tract diseases, and that senescent phenotypes would rise as a standard response of these cells to damage. Other correlative studies matching rodent and human samples supported this view. In a previously mentioned study, FoxA2 activity was nearly undetectable in human PSC or PBC liver specimens, whereas SASP levels (TGF-β1) were increased.[41] The possible link between SP and cellular senescence in PSC was suggested by the finding of increased blood levels of this neuropeptide and its messenger RNA in liver of subjects affected by an advanced stage of this disease.[42] Other correlative assessments regarding α-CGRP, SCF, or vimentin levels were carried out in PSC human tissue to confirm the preclinical data obtained in rodents, as previously described.[49-51] Finally, increased levels of twinfilin-1 were detected in cholangiocytes of patients with PBC or PSC. This protein, in experimental cholestasis, appeared to respond to several microRNA signals and to play a major role in supporting the proliferative/senescent evolution of bile duct cells in this setting.[61]

With regard to other biliary diseases, cellular senesce was also examined in biliary atresia, which is a pediatric progressive cholestatic disease characterized by the preferential obliteration of the extrahepatic biliary duct.[62] In a study, liver evaluation of cell cycle regulators p16 and p21 was carried out in 80 patients with biliary atresia. The results evidenced a constant occurrence of cellular senescence in this disease, similar to other forms of injury of the biliary tract. Moreover, a possible relationship with the progression of injury was suggested.[63] In conclusion, preclinical animal and human data strongly support the role of aging, and in particular of cellular senescence, in the molecular machinery at the base of chronic cholestatic human diseases. Two aspects are particular intriguing at present: (1) how cellular senescence contributes in an integrated process with inflammation, ductular reaction, EMT, and fibrosis in the maintenance of the disease; and (2) how to attain a different regulation of cholangiocyte and HSC senescence, to reduce injury in the course of the diseases of the biliary tract. Further studies will hopefully answer these questions.

Liver Sinusoid

The hepatic vascular compartment has a unique structure that allows maintenance of liver homeostasis. Liver blood flow enters into the liver through two vessels, the portal vein and the hepatic artery, altogether supplying the hepatic tissue with oxygen, nutrients, hormones, and other substances including inflammatory factors and toxins. The segment of the hepatic microcirculation where the exchange of substances occurs is the hepatic sinusoid, a unique vascular bed consisting of a layer of liver sinusoidal endothelial cells (LSECs) surrounded by vascular perisinusoidal cells (HSCs), and flanked by plates of nonparenchymal cells or hepatocytes. The thin area where HSCs reside is called the space of Disse. In addition, the hepatic sinusoid houses an important part of the phagocytic system, Kupffer cells (KCs), the resident macrophages, and other immune cells like T lymphocytes and pit cells (also known as hepatic natural killer cells), altogether making the liver a key organ for innate immune system.[64] Immune cells are anchored to the luminal side of the sinusoidal endothelium, and therefore are exposed to the bloodstream. The complex functions of the liver in molecule biosynthesis, metabolism, inflammation, and clearance of bloodstream are tightly dependent on an adequate microcirculation, guaranteed by a healthy phenotype and proper paracrine signaling within the liver sinusoid.[65]

Aging of Liver Sinusoidal Cells

LIVER SINUSOIDAL ENDOTHELIAL CELLS

LSECs are specialized endothelial cells that form the vascular wall in the liver sinusoids. In normal conditions, LSECs form a permeable barrier due to fenestrae presence in their membrane and due to absence of basal membrane. These properties make the liver endothelium discontinuous, increasing crosstalk between hepatocytes and liver sinusoidal cells. LSECs also play a role in regulating hepatic vascular tone through the production of vasoactive molecules.[66]

Data from experimental models demonstrated that LSECs de-differentiate during healthy aging, a process initially defined by partial loss in the number and diameter of fenestrae, also termed as pseudocapillarization.[67] Subsequent studies improved the

description of LSEC de-differentiation in aging, clearly defining that such distinctiveness was not limited to morphologic changes but accompanied by significant deregulations in the LSEC phenotype.[68,69] It has been described that alterations in key vasodilatory pathways, including the nitric oxide one, may contribute to increment the hepatic vascular resistance. The reduction in vasodilators is of relevance considering the roles of nitric oxide regulating the hepatic vascular tone,[70,71] exerting anti-inflammatory effects and maintaining other nonparenchymal cell phenotypes through paracrine interactions.[72,73] Aged LSECs exhibit decreased expression of diverse angiocrine receptors (e.g., SE-1, stabilin-2 [Stab2], kdr) and angiocrine factors (including wingless-type [wnt2] and hepatocyte growth factor [hgf]), which may affect hepatocyte regeneration.[69,74] LSECs are in a moderate pro-inflammatory state, which, together with down-regulation of sirtuin 1 and up-regulation of p16, suggest senescence of this cell type in aging.[75] Finally, LSEC scavenger functions are also compromised in aging, as demonstrated by the reduction in endocytic capacity.[76] Interestingly, a recent study suggested that scavenger capacity dynamics during a lifespan would be a key mechanism to promote LSEC senescence.[75] Middle-aged mice exhibit a significant increase in scavenger receptors expression, which may fuel the intake of toxic substances, ultimately leading to mitochondrial oxidative stress and senescence. Following senescence progression, heterochromatic silences, thus suppressing, the expression of several scavenger receptors and endocytosis genes to ultimately reduce LSEC endocytic capacity in aging.[77] The mentioned deregulations due to aging have a clear effect on LSEC response in front of an injury, either acute or chronic. In fact, preclinical studies that aimed to analyze the effects of aging on the liver microcirculation demonstrated that LSECs exhibit profound changes in response to an injury, including further reduction in fenestrae, loss of angiocrine mediators, and decline in vasodilators synthesis.[78,79] These alterations have an effect on vascular functionality, maintenance of other liver cells, and development of clinical complications of liver injury including portal hypertension.

HEPATIC STELLATE CELLS

HSCs are the main collagen-synthesis cells of the liver, and by producing extracellular matrix (ECM) and interacting with neighboring cells, they play a key role in liver architecture modification and function. HSCs' first described function was to store fat in the capillary wall of the human liver (also known as fat-storing cells or Ito cells). This property has led their identification in the liver tissue and their isolation using density gradient methods. However, during liver damage, these quiescent HSCs acquire an "activated" phenotype, losing their fat-storing cell phenotype and becoming pro-contractile, proliferative, and matrix-secreting myofibroblast-like cells.[80]

In healthy aging, HSCs exhibit a slight, but remarkable, activation state, as evidenced by increased proliferation, significant increments in the expression of specific markers including α-smooth muscle actin, collagen I and collagen IV, together with increase in inflammatory, pro-oxidant and senescence markers, with evidence of matrix deposition within the parenchyma or sinusoids.[69,81] Interestingly, and opposite to what is observed in chronic liver disease, aged HSCs present increased intercellular accumulation of lipids, presumably due to alterations in retinoid metabolism and lipid breakdown like down-regulation in cellular retinol binding protein 1 and overexpression of patatin-like phospholipase domain containing 3.

In response to an injury, HSCs become overactivated and therefore actively contribute to the aggravation and perpetuation of liver diseases. Indeed, preclinical models of acute liver injury (due to ischemia and reperfusion injury) and chronic liver disease (due to chronic carbon tetrachloride [CCl_4] administration) in aging demonstrated hyperresponse of HSCs, leading to hypercontraction, elevated production, and secretion of ECM components, which ultimately led to exaggerated architectural distortion and elevation in the hepatic vascular tone.[78]

HEPATIC MACROPHAGES

Liver resident macrophages, also known as KCs, are situated in the sinusoidal lumen. KCs are exposed to blood, as well as to antigens and bacterial endotoxins, therefore representing the first line of defense to maintain immune system in the liver. Current evidence indicates that macrophages perform a wide repertoire of functions in inflammation and repair. The factors determining the behavior of

macrophages at sites of inflammation are complex, and dependent on several variables of the organ and model of tissue injury.[82]

During aging, and in addition to LSECs, KCs become pro-inflammatory, displaying elevated levels of IL-6, a cytokine with key roles in liver regeneration, infection defense, and metabolism regulation, together with alterations in other polarization makers.[69,83] The pro-inflammatory state of KCs, and LSECs may contribute to the recruitment of circulating inflammatory cells including neutrophils and monocyte-derived macrophages, observed in aged livers. In situations of chronic liver disease, aged animals exhibit further deterioration in the hepatic inflammatory phenotype, exhibiting increased recruitment of inflammatory macrophages together with down-regulation in anti-inflammatory and pro-resolutive cytokines.[78] The polarization of resident macrophages and the increased myeloid content in the aged cirrhotic liver may indeed derive from the exaggerated activation of the hepatic endothelium (as described previously) and from increased gut-derived bacterial products, ultimately aggravating and perpetuating CLD in aged individuals. In Table 1, we summarize the findings on the aging process of LSECs, HSCs, and macrophages.

HUMAN DATA

Little is known about the effect of aging in the human liver sinusoid. Epidemiological data evidenced higher prevalence of chronic liver diseases coursing with microcirculatory dysfunction in the elderly, together with faster progression and worse prognosis,[57,84,85] altogether suggesting that the hepatic vascular bed might be unprotected in front of an injury also in the human being. In accordance with this hypothesis, morphological analysis of human livers suggested pseudo-capillarization of LSECs and slight activation of HSCs,[86] and recent data supported the concept of sinusoidal unprotection due to aging. In the context of healthy aging, characterization of the liver sinusoid in young and aged human liver tissues validated most of phenotypic deregulations observed in preclinical models.[69] Human aged livers exhibit features of LSEC de-differentiation, as suggested by reduced angiocrine and vasodilatory genes, including endothelial

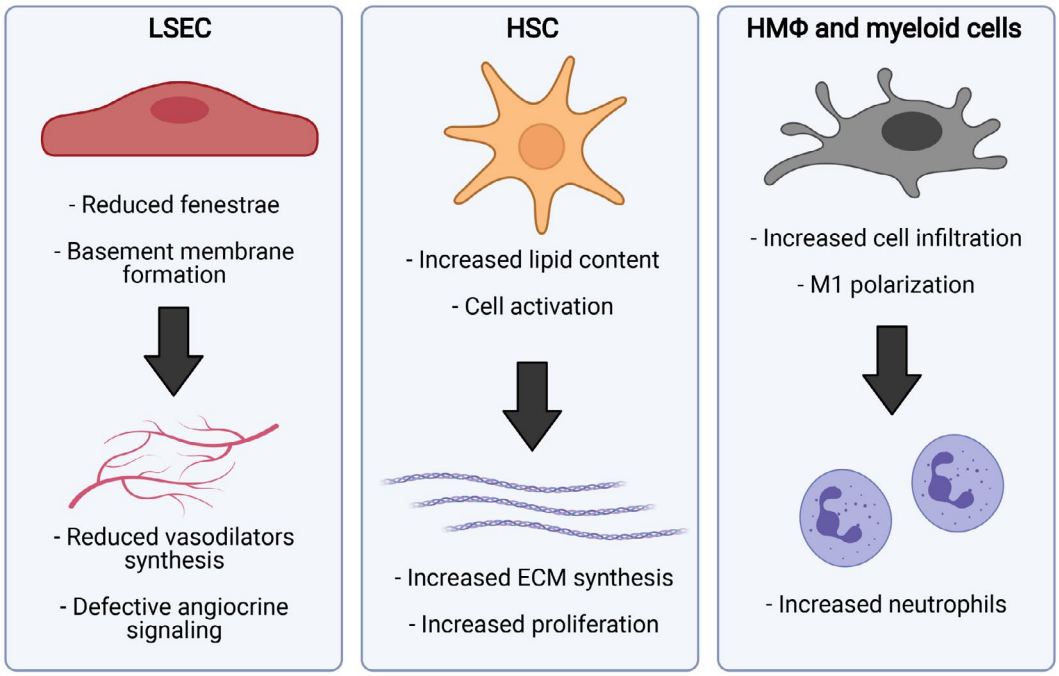

FIG. 3. Major phenotypic modifications due to aging in liver sinusoidal cells. Abbreviation: HMΦ, hepatic macrophage. (This figure was made with BioRender.com under a purchased license agreement.)

TABLE 1. MAIN EFFECTS OF AGING ON THE CELLS OF LIVER VASCULATURE

Cell Type	Pathophysiological Event	Specific Evidence	References
LSEC	Vasoconstriction	⇩NO, ⇩eNOS, ⇩cGMP, ⇩HO-1, ⇩KLF2	(69,78)
	Inflammation	⇧ICAM, ⇧IL-6, ⇧CD6, SASP: ⇧(cytokines IL-1α, IL-1β, IL-15, I-L18), ⇧(chemokines Ccl2, Ccl6, Ccl8, Ccl24, Cxcl9, Cxcl12, Cxcl13, Cxcl16)	(68,69)
	Scavenging dysfunction	⇩SR(FSA), ⇩(Msr1, Marco, ScarB1, ScarB2, CD36r, CD68r, Mrc1, Stab1, Stab2), ⇩(Ox-LDL, Ac-LDL)	(75,91)
	Angiocrine deregulation	⇩Stab2, ⇩CB32b, ⇩VEGF-R2, ⇩HGF, ⇩Wnt2, ⇩Hamp, ⇩Axin2	(69,78,92)
	Capillarization	Pseudo-capillarization (partial loss of fenestrae and basement membrane formation)	(86,93)
HSC	ECM synthesis	⇑α-SMA, ⇑collagen 1α1, ⇑collagen 1α2	(56,69,78)
	Proliferation	⇑PDGF-receptor β, ⇑desmin, ⇩telomere length, ⇑Ki67	(69,94)
	Hypercontraction	⇑Rhok, ⇑ α-SMA, ⇑LPA	(10,95-97)
	Paracrine deregulation	⇑SASP, ⇑SA-β-gal, ⇑p21, ⇑p53, ⇑p16, ⇑IL-22, ⇑CCN1, ⇑retinoic acid, ⇑SP	(42,98,99)
HMΦ	Inflammation	⇧ICAM, ⇧IL-6, ⇩TNFα, ⇩Mrc1, ⇩Arg1, ⇩IL-10	(68,69,78)

Abbreviations: Ac-LDL, acetylated low-density lipoprotein; CCN1, cellular communication network factor 1; cGMP, cyclic guanine monophosphate; Cxcl, chemokine (C-X-C motif) ligand; eNOS, endothelial nitric oxide synthase; FSA, formaldehyde-treated albumin; Hamp, hepcidin antimicrobial peptide; HMΦ, hepatic macrophage; HO-1, heme oxygenase 1; ICAM, intercellular adhesion molecule; KLF2, Krüppel like factor 2; LPA, lysophosphatidic acid; Marco, macrophage receptor with collagenous structure; Mrc1, mannose receptor C-type 1; Msr1, macrophage scavenger receptor 1; Ox-LDL, oxidized low-density lipoprotein; PDGF, platelet-derived growth factor; Rhok, Rho-associated protein kinase; ScarB, scavenger receptor class B; Stab2, stabilin-2; TNF, tumor necrosis factor; VEGF-R2, VEGF receptor 2; Wnt2, wingless-type; α-SMA, α-smooth muscle actin.

nitric oxide synthase, HGF, laminin subunit beta 1 and Stab2, together with moderate activation of HSCs. In the scenario of chronic liver disease, transcriptomic analysis of liver tissue from patients with cirrhosis identified substantial number of genes differentially expressed comparing young (<48 years) versus old (>58 years) individuals. Comprehensive characterization of these data revealed an aged cirrhotic signature that, interestingly, included upregulated pathways related to vascular pathobiology, thus confirming the deterioration of the sinusoidal vascular bed in aged liver disease.[78] These findings, detected in well-characterized patients, may have an important role in the discovery of new therapeutic approaches and treatment of liver diseases in aging. Indeed, these findings suggest that future preclinical studies and clinical trials should consider age in their design (Fig. 3).

Conclusions

In conclusion, while aging represents one of the most important risk factors for several neoplastic and nonneoplastic chronic diseases, important issues remain to be addressed. For example, the identification of appropriate markers of this time-dependent process (as a function of disease onset and course) may possibly improve translational application of preclinical studies in human.[2] Second, inhibition or modulation of cellular senescent phenotypes might be beneficial in pathological conditions. Finally, identification of senescence-related pathways in different tissues would possibly allow us to design new treatment to organ-specific disease. Preclinical animal studies have already shown that ablation of senescent cells (obtained by means of specific drugs or small interfering RNA treatment) was able to improve cardiac reserve in older mice or lifespan in progeroid Ercc1$^{-/\Delta}$ rodents.[87] Several drugs, including dasatinib, quercetin, fisetin and others, have been proposed to lead senescent cells toward deletion/apoptosis.[88] In a pivotal study, intermittent administration of dasatinib and quercetin was conducted for 3 weeks in 14 patients affected by idiopathic pulmonary fibrosis. Physical function was improved by treatment, with an acceptable safety and tolerability.[89] Further findings on senolytic treatment in humans were obtained in a study on patients with diabetes-related renal disease.[90] This research demonstrated, after a 3-day short course of dasatinib + quercetin, a reduced number of adipose tissue senescent cells and decreased circulating levels of SASP. However, large trials on chronic administration of senolytic treatments would require an accurate evaluation of risk and benefit to be undertaken

in the future. Moreover, a ubiquitous depletion of senescent cells might not be beneficial in different pathological settings. For instance, in this review we reported that the improvement in the animal model of cholestasis is not only associated with the ablation of senescent cholangiocytes, but also with the induction of senescence in HSCs. We also reported that different cells are present within the vascular liver compartment that may unevenly react and contribute to the aging/senescent process. This strongly suggests that a specific line of research focusing on aging of biliary and vascular compartments would be necessary to pursue therapeutic options for diseases afflicting these anatomical districts in humans.

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