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# Is Senescence Important in Hepatic Diseases?

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## Abstract

Aging is a condition in which a person gradually loses the ability to maintain homeostasis, due to structural alteration or dysfunction. Aging changes biological processes in many organs and tissues. The loss of regenerative capacity is the most dramatic age-associated alteration in the liver. Cellular damage, if not repaired, leads to apoptosis or senescence. The presence of permanent cell cycle arrest, the acquisition of major morphological change, and expression of senescence-associated  $\beta$ -galactosidase (SA- $\beta$ -gal) are the characteristics of cellular senescence (CS). Interestingly, CS plays a crucial role in aging of both individual organs and the entire organism; consequently, senescent cells accumulate in organs and decline in organ function. Senescent cells have considerable influence on their microenvironment and exert both beneficial and detrimental effects through secretory associated senescent phenotype (SASP) factors. CS has attracted considerable recent interest with recognition of pathways linking aging, malignancy, and insulin resistance and the current focus on therapeutic interventions to extend healthspan. There are major implications for hepatology in the field of fibrosis and cancer, where cellular senescence of hepatocytes, cholangiocytes, stellate cells, and immune cells has been implicated in chronic liver disease progression.

**Keywords:** senescence, aging, chronic liver diseases

## 1. Introduction

Aging is a biological process that consists of a series of structural and functional changes that appear over time and are not a consequence of diseases or accidents. In general, tissues are comprised of specific cells that determine the tissue microenvironment. Various factors affect the cellular environment, such as oxygen pressure, oxidative stress, temperature, and several

growth factors secreted by cells. Sometimes the cell's microenvironment alters the tissue homeostasis, causing a permissive environment that favors tumor promotion and development. This progressive aging is caused by multiple factors. The following nine characteristics are generally considered to contribute to the aging process: altered intracellular communication, genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, stem cells exhaustion, and cellular senescence [1].

## 2. Cellular senescence

Cellular senescence (CS) is a stable form of cell cycle arrest that limits the proliferative potential of cells [2]. Senescence is the result of various degenerative changes characterized by shortening of the telomeres, chromosomal instability, DNA damage, oxidative stress producing reactive oxygen species (ROS), and oncogene overexpression [3–10]. Some researchers consider CS the natural solution to prevent cell damage, which may lead to various diseases including cancer. In contrast, with age, CS is considered to promote several chronic diseases. The pathological effects of CS may affect the health of elderly people due to the accumulation of senescent cells in several organs and tissues because CS can impair tissue regeneration. Furthermore, senescent cells can release several molecules, such as growth factors, cytokines, and chemokines that alter cellular homeostasis [11].

Up to date, two basic mechanisms have been identified to induce senescence: replicative senescence and premature senescence. Replicative senescence, or the Hayflick limit, is associated with telomere attrition; telomere exhaustion causes the limited proliferative capacity of certain types of *in vitro* cultured cells [12]. Cells react to the loss of telomeres as if it is DNA damage, which therefore triggers the DNA damage response (DDR); the cellular response is similar to that initiated by external agents, for example, ionizing radiation and drugs.

In contrast, premature senescence is related to chromosomal instability, DNA damage, oxidative stress, oncogene overexpression, and epigenetic changes. Senescent cells have a typical morphology in culture: cells are enlarged, vacuolated, and flattened and in some cases, they are multinucleated. However, *in vivo* senescent cells retain the normal morphology according to the tissue to which they belong. It has been found that these cells are metabolically active, and they can secrete growth factors, chemokines, and metalloproteases; this is known as the senescence associated secretor phenotype (SASP) [13].

### 2.1. Senescence markers

Senescent cells have several typical characteristics. First, they are positive for  $\beta$ -galactosidase activity at a suboptimal pH (pH 6), and this activity is based on the increment of lysosomal activity. One limitation of the  $\beta$ -gal assay is the need to use fresh or frozen samples [14]. Another characteristic is that senescent cells are in cellular arrest. Cellular arrest is an essential condition for senescence to occur, as senescent cells are positive for Ki67 protein or negative for the incorporation of 5-bromodeoxyuridine (BrdU) [15]. Other senescent markers are related to tumor suppressors such as p53, p16, p21, and the hypophosphorylated Rb protein. Heterochromatin bodies are known as heterochromatin foci in the nucleolus. In cells with DNA damage, it is common to find

$\gamma$ -H2AX histone phosphorylation which carries the DDR. Another finding is the decrease in lamin B1 levels as a common feature in many types of senescence [16, 17]. On the other hand, senescent cells have been found in biological processes other than aging as in systems such as humans, mice, and chicken embryos, suggesting that these cells are important to embryogenesis [18].

## 2.2. Senescence pathways

In the senescence process, there are two key tumor suppressors, the INK4/ARF locus and p53. The INK4/ARF locus allows the expression of three important proteins, p16<sup>Ink4a</sup>, p14<sup>Arf</sup> (p19<sup>Arf</sup> in mice), and p15<sup>Ink4b</sup>. Serrano et al. discovered the protein p16<sup>Ink4a</sup>. The p16<sup>Ink4a</sup> binds to CDK4/6 cyclins, preventing the phosphorylation of the Rb protein family; therefore, the cell cycle is inhibited at the G1 phase and cells enter in a cellular arrest, and this pathway is known as p16<sup>Ink4a</sup>-Rb [19]. In contrast, p53 is a transcription factor, which transcribes target genes to regulate various biological cell processes in response to stress stimuli; the biological cell processes include apoptosis, senescence, energy metabolism, and antioxidant defense. p53 is stabilized and phosphorylated by upstream kinases including ataxia telangiectasia (ATM) and Chk2 [10, 20, 21]. Phosphorylated p53 upregulates the transcription of its target gene, p21, which activates the Rb protein through the inhibition of cyclin E/Cdk2. The hypophosphorylated Rb protein inhibits the transcription of E2F genes, including cyclin A and proliferating cell nuclear antigen (PCNA), which are required for cell cycle progression [22]. On the other hand, p14<sup>Arf</sup> is directly related to p53 and is linked to the murine double minute 2 (Mdm2) protein; therefore, p53 is stabilized, and p21 is expressed and inhibits cyclin E/Cdk2 [23]. This senescent pathway is called the p53-p21 pathway. Another senescence-signaling pathway is through the p38 protein, which is a mitogen activated protein kinase (MAPK) protein that transforms a variety of stress stimuli into a common senescence signal [24]. The inactivation of p38 delays the onset of various forms of cellular senescence including replicative, oxidative-stress-induced, and oncogene induced senescence. In contrast, enforced activation of p38 by a constitutive active form MAPK kinase (MKK6EE) of upstream kinase, MKK6, acutely induces cellular senescence [25, 26].

In recent years, other elements have been incorporated into the senescence pathway regulation. Studies on genetic regulation in human fibroblasts and mice embryonic fibroblasts (MEFs) showed the presence of long noncoding RNAs (lncRNAs), which are derived from noncoding RNAs (ncRNAs). Tumor suppressors have specific lncRNAs; for example, several lncRNAs, such as focally amplified lncRNA on chromosome 1 (FAL 1), BRAF-activated non-protein coding RNA (BANCR), and long intervening noncoding RNAs (LINCRNA) p21, have been found in p21 [27–30]. In the case of p53, lncRNA metastasis associated lung adenocarcinoma transcript 1 (MALAT1) as well as 7SL have been related to p53 expression [31, 32]. The INK4a-ARF locus and p16<sup>Ink4a</sup>mRNA are linked with lncRNAs VAD, antisense non-coding RNA in the INK4 locus (ANRIL), MIR31 host gene (MIR31), and Urothelial cancer associated 1 (UCA 1) [33–38]. Another lncRNA associated with senescence is senescence-associated long non-coding RNA (SALNR), which is related to NF- $\kappa$ B [39]. telomeric repeat containing RNA (TERRA) is a lncRNA associated with telomere length [40]. Researchers have shown that lncRNAs are tightly regulated and tissue specific. Moreover, the dysregulation of lncRNAs has been associated with human diseases. Further studies must be performed to accurately determine the functional role of these lncRNAs in senescence and aging [41].

### 2.3. Chromatin alterations

Alterations in the structure of chromatin are believed to contribute to the irreversible nature of the senescent state. Narita et al. found that in DNA cells stained with 4',6-diamidino-2-phenylindole (DAPI), chromatin bodies called foci can be observed at the nucleus [42]. It is likely that DAPI-stained foci correspond to highly compacted heterochromatin, as senescent cell chromatin is resistant to micrococcal nuclease digestion; moreover, DAPI foci contain numerous proteins characteristic of transcriptionally inactive heterochromatin, including hypoacetylated histones, methylated histone H3 on Lys 9 (H3K9 me), and heterochromatin protein 1 (HP1). The formation of heterochromatin structures known as heterochromatin foci is associated with senescence-associated heterochromatin foci (SAHF). Various forms of stress induce SAHF formation as well as other senescence phenotypes, suggesting that SAHFs can be used to identify senescent cells *in vivo* and that SAHF formation somehow contributes to the mechanisms of cellular senescence; although, not all senescent cells exhibit SAHFs [43].

### 2.4. Oxidative stress and liver senescence

Under normal physiological conditions, cells are protected from oxidative stress by an array of endogenous antioxidants that maintain a balance between pro-oxidant production and antioxidant capacity. An imbalance occurs when intracellular antioxidants are unable to neutralize the pro-oxidants such as ROS, which are a number of highly reactive molecules derived from molecular oxygen [44]. ROS are mainly produced by the mitochondrial respiratory chain, cytochrome P450, and autooxidation of endogenous substrates such as heme proteins, catecholamines, quinones, among others [45]. ROS exert a broad array of biological effects, ranging from physiological regulatory functions to several oxidative modifications, which can contribute to the pathogenesis of various diseases. ROS can also impact the cell by altering physiological and biochemical processes such as gene expression, cell adhesion, cell metabolism, cell cycle, cell disease, and cell death [46, 47]. Thus, oxidative stress has been confirmed to be a common participant in several diseases including liver diseases [48]. It has been proposed that the damage induced by oxidative stress may represent a common link between different chronic liver diseases. This proposal is supported by the fact that free radicals are key players not only in normal liver function but also in the genesis and progression of liver steatosis, fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [49, 50]. Therefore, a strong association is expected between oxidative stress and the pathogenesis of liver diseases. Oxidative stress is an inherent component in the liver as the activity of many enzymes involved in lipogenesis, fatty acid oxidation, cholesterol, and phospholipid metabolism is regulated by the redox status of liver parenchyma, which can influence the redox balance of the cell microenvironment [51]. Thus, following liver injury by different etiological factors [52], endogenously produced ROS may have an accumulative impact (mainly evidenced as an increase in lipid peroxidation), which plays a critical role in the progression of chronic liver diseases [53].

Several studies have proposed that ROS production is one of the main activators and stabilizers of CS. Although this is a preliminary proposal, there is evidence demonstrating



a direct contribution of ROS to both the DDR and the redox-activated pathways [54, 55] and induces telomere shortening [56], which results in a DDR and senescence activation. Up to date, both experimental and clinical studies have strongly supported the association of CS with chronic liver diseases. In addition to specific parenchymal or nonparenchymal liver structures, studies have highlighted the appearance of CS in two paradoxical settings, as a tumor suppressor and as a tumor promoter, and in two physiological processes, aging and tissue repair [57, 58]. Cellular senescence is involved in multiple physiological processes; the complexity of CS may be elucidated by deciphering the mechanisms that induce its activation, such as oxidative stress. In this regard, evidence has shown that increased ROS production by mitochondrial dysfunction is triggered after a DDR and subsequent CS activation, which in turn is a consequence of p21 activation. The sequence of events maintains a dynamic feedback loop that is necessary for the stability of growth arrest during the establishment of the senescent phenotype [59]. A similar phenomenon was observed during the activation of the p16/RB pathway, which induces high intracellular levels of ROS and thereby activates protein kinase C- $\Delta$  (PKC $\Delta$ ); consequently, further ROS are generated to establish a positive feedback loop to sustain ROS-PKC $\Delta$  signaling [60]. Other studies have reported that activation of the RAS, p21, and p53 pathways are also closely related to elevated ROS production [61, 62]. In these reports, N-acetylcysteine treatment blocked cell cycle arrest and thereby CS, demonstrating that ROS accumulation is an important mediator of CS; furthermore, researchers proposed that ROS are a causal agent of CS. The evidence confirms that ROS play a key role in the CS process; however, it is still unclear whether ROS are a consequence or cause of CS.

## 2.5. Reactive oxygen species and hepatic diseases

A variety of free radicals are found in the liver and can be classified as mitochondrial, principally from complexes I and III, and extra mitochondrial, such as cytochrome P450, xanthine oxidase, nitric oxide synthase, and nicotinamide adenine dinucleotide phosphate reduced (NADPH) oxidase. Where neutrophils and Kupffer cells primarily produce free radicals, the cytochrome P450 system and mitochondria are the major sites of ROS release in hepatocytes. An important mechanistic source of ROS is lipid metabolism, which is dependent on the redox balance of the liver, and its alteration is closely linked to both alcoholic and nonalcoholic steatohepatitis [51, 63]. The above-mentioned sources of ROS are strictly controlled in normal hepatocytes; however, in hepatic diseases, an overproduction of free radicals can overcome the antioxidant defenses and induce liver injury [51]. Consequently, the liver is a continuous target of oxidative stress and redox balance. It is not surprising that an increasing number of findings have shown a close association between liver diseases and ROS production [63]. Clinical and experimental reports have suggested that CS onset may be an intrinsic phenomenon from the beginning of liver injury, such as the appearance of neoplastic nodules [64, 65]. This proposal is supported by the presence of replicative and premature senescence in both parenchymal and nonparenchymal liver cells [58, 66, 67]. Using a liver injury model to mimic sequential HCC progression (from fibrosis to cirrhosis and HCC) [68], we recently confirmed that CS appearance is intrinsic to the initiation of liver injury, as a chronological increase of CS alongside HCC progression was observed [69]. We did not determine ROS levels in this investigation; however, HCC was induced with continuous diethylnitrosamine administration, a hepatotoxic and potent

ROS producer [70], suggesting that the chronological appearance of CS was accompanied by persistent ROS induction. In a recent study, the role of hepatocyte senescence in the development of insulin resistance was investigated [71]. Using HepG2 cells, they demonstrated that H<sub>2</sub>O<sub>2</sub> treatment induced CS despite insulin stimulation. This phenomenon was accompanied by a persistent nuclear localization of FoxO1, a transcription factor that, among others, promotes the expression of genes involved in cell cycle arrest and detoxification of ROS. Moreover, an increase and decrease in the expression of glucose transporters 2 and 4 (GLUT2 and GLUT4), respectively, were proposed as senescence markers, as this pattern was found in senescent HepG2, in human cirrhotic liver tissue, and in publicly available liver disease datasets. In a different study by the same research group, gene expression profiles of HepG2 cells subjected to oxidative stress were compared with public microarray datasets obtained from human cirrhotic samples. The results showed that the gene upregulation profile of senescent HepG2 cells induced by H<sub>2</sub>O<sub>2</sub> treatment was similar to that in human cirrhotic livers. Interestingly, gene expression profile patterns of senescent hepatocytes were markedly linked to alterations in cell cycle regulation, morphology, metabolism, and stellate cell activation, which occur alongside impaired synthetic function in senescence [72]. Although these results suggest a close relationship between hepatocyte senescence and ROS action, the use of an external stressor agent to induce CS may be a key factor to work in an asynchronous manner in the sequence of molecular events involved in intrinsic ROS production. More direct evidence has demonstrated a strong interconnection between senescence, ROS, and TGF- $\beta$  signaling in chronic liver diseases. Borkham-Kamphorst et al. showed that the overexpression of cysteine-rich protein 61 (CCN1/CYR61) significantly inhibited the production of collagen type I and induced ROS, leading to CS and apoptosis *in vivo* and *in vitro*. This effect was associated with an attenuation of TGF- $\beta$  signaling; as a result, CCN1/CYR61 overexpression mitigated liver fibrogenesis in a bile duct ligation model. This analysis is a clear example of ROS and CS induction as a potential molecular mechanism for tissue repair and remodeling promoted by the manipulation of CCN1/CYR61 during hepatic injury [73]. In a similar study with contrasting reports, dioscin, a saponin plant steroid, promoted CS but decreased the oxidative stress induced by CCl<sub>4</sub> in activated hepatic stellated cells (HSC). Among others, this antifibrotic effect was associated with the attenuation of TGF- $\beta$ /Smad, Wnt/ $\beta$ -catenin, MAPK, and mitochondrial signaling pathways [74]. Recent investigations have shown that Sir2 enzyme members, collectively called sirtuins, are involved in CS associated with liver diseases. Sirtuin 6 (SIRT6), an enzyme that promotes resistance to DNA damage and oxidative stress, plays a central role in the CS induced by ROS and TGF- $\beta$  signaling in HCC cells [75]. In this report, ROS and TGF- $\beta$  upregulated SIRT6 expression by inducing sustained activation of extracellular signal-regulated kinase (ERK) and Smad pathways. Upregulated SIRT6 not only abrogated the inducing effect of TGF- $\beta$  and ROS on CS but was also required for the ERK pathway to suppress p16 and p21 expression. Additionally, upregulation of SIRT6 promoted HCC cell tumorigenicity and contributed to the inhibitory effect of the ERK pathway on cellular senescence. This feedback loop activation integrated by SIRT6 and TGF- $\beta$  has the potential to modulate both tumorigenicity and senescence and represents a clear example of the molecular complexity around senescence activation. Another investigation group demonstrated that SIRT1, an enzyme that deacetylates nonhistone proteins and allows mammalian cell survival under oxidative stress, is downregulated in aged and middle-aged

mice. This condition increased mice susceptibility to the effect produced by chronic alcohol consumption and exacerbated alcoholic liver injury and fibrosis in hepatocytes and hepatic stellate cells [76]. This evidence clearly reveals that the senescence and ROS pair in the liver of elderly patients boosts alcohol liver disease, likely by the modification of liver metabolic functions. This proposal is in accordance with a recent report of Cyp2e1 as a central player in aging-dependent hepatic steatosis, apoptosis, and fibrosis. The investigation demonstrated that Cyp2e1 increased the production of reactive nitrogen species and ROS in aged mice; as a result, high levels of lipid peroxidation, oxidative protein modifications, oxidative DNA damage, and inflammation were found. These changes were absent or decreased in aged Cyp2e1-null mice [77]. In the serum of patients with nonalcoholic steatohepatitis (NASH) and NASH-related HCC, oxidative stress correlated with the levels of NASH activity markers, while the antioxidative function was preserved in younger patients as well as in patients with a well-preserved liver function; in contrast, patients with NASH-related HCC tended to be older and exhibited a diminished antioxidative function [78]. In addition to the complexities involved with senescence and ROS in liver diseases, there is evidence of SIRT1 and Cyp2e1 as possible targets for preventing aging-related liver disease.

Senescence induction has also been another means for researchers to determine the efficiency of chemotherapeutic agents against cancer. Most of these agents are strong oxidative stress inducers such as cisplatin. In a recent study, this compound promoted a senescent phenotype in HCC cell lines dependent on p53 and p21 activation and intracellular ROS generation [79]. Although senescence activation appears to be a natural consequence of oxidative stress induction, ROS generation may dictate different cellular consequences depending on the overall concentration at steady-state levels and site of generation [80]. In support of this, an advanced investigation has conferred an important role for ROS production and its participation as a decisive factor in the progression and aggressiveness of cancer [81], and has also raised the complexity of the senescence and ROS pair. Investigators reported that the normally high potential for melanoma cells to form subcutaneous tumors is limited after intravenous or intrasplenic transplantation; this occurs because metastatic cells experience a charge of oxidative stress in the blood and visceral organs, which is not present in established subcutaneous tumors. To overcome that barrier, cells undergo reversible metabolic changes during metastasis that increase their capability to withstand oxidative stress. It was found that mitochondrial membrane potential, mass, and ROS levels declined more in circulating metastatic cells compared to subcutaneous tumors, raising the possibility that mitochondrial function is reduced in circulating melanoma cells in an effort to reduce ROS generation [81]. During the normal aging process, mitochondrial metabolic changes are implicated in the induction and maintenance of CS [82]. While the investigation did not show whether oxidative stress also limits the initiation and early growth of primary cutaneous cancer cells [81], it is reasonable to hypothesize that senescence activation and/or ROS generation are determinant factors of the appearance and progression of different multifocal HCC types. This intriguing hypothesis stems from a proposal that multifocal HCCs are either from an HCC of multicentric origin or HCC of intrahepatic metastasis [83]. Thus, in this scenario, ROS may modulate CS activation in the HCC microenvironment. As a result, senescence and ROS may be the decisive molecular pair to establish the outcome of multifocal HCC. This hypothetical proposal also raises the question of whether the appearance of



senescence/ROS is a transitory, sporadic, or specific event of a determined HCC development stage. We have recently shown that, concomitant to the HCC development induced by diethylnitrosamine, there is also a chronological appearance of CS [69]; however, its association with ROS generation and effect on the outcome of multifocal HCC type has not been investigated. This is an interesting and comprehensive proposal worth investigating.

### **3. Is cellular senescence important in the development of hepatic diseases?**

Aging changes the biological processes in many organs and tissues of mammals, leading to the development of age-associated diseases and aberrant body homeostasis. Nearly 13% of the population in the United States is over the age of 65 years; this percentage and the number of elderly people will increase substantially over the next 50 years [84]. Although the aging process does not cause death, elderly people appear to be predisposed to a variety of diseases; therefore, aging facilitates the onset and/or progression of various pathologies including diseases of the liver [84]. The ability to withstand hepatic insult decreases each decade, including those associated with high triglyceride levels, nonalcoholic fatty liver diseases, and HCC [85–87]. The percentage of deaths caused by liver diseases increases dramatically in humans over the age of 45 years. Accordingly, age is a critical determinant in the outcome of hepatitis C infection [88] and liver transplantation [89]. However, the most dramatic effect of aging on the liver is its delayed and/or reduced proliferation after a loss of mass from a surgical or chemical injury [90–92]. Based on these correlations, it has been suggested that elderly people are predisposed to liver diseases. The livers of young individuals regenerate after these treatments and maintain homeostasis; conversely, there is a dramatically reduced regeneration of the livers of older people. The clinical outcome of human acute liver injury is also partially dependent on the potential for hepatic regeneration. In older people, there is a higher incidence of acute liver failure and a higher mortality with acute hepatitis A [93, 94]. In a cohort of patients with acute liver failure secondary to viral hepatitis, age was an independent predictor of a poor outcome, with the worst outcomes in people over the age of 50 years [95]. Wong et al. and Poynard et al. were among the first to demonstrate that age at infection was a major risk factor for subsequent fibrosis progression and that the rate of fibrosis progression accelerated with increasing age [96, 97]. Studies of other liver diseases have demonstrated similar findings. Age is an independent risk factor for poor outcomes in primary biliary cirrhosis (PBC) in addition to the presence of portal hypertension and impaired liver function [98]. Similarly, age, independent of bilirubin, prothrombin time, and renal function, predicted the outcome in patients with alcoholic hepatitis [99]. There is some evidence that age may influence disease progression in nonalcoholic fatty liver disease (NAFLD) [100, 101].

The role of CS in diverse chronic liver diseases has come into recent research. The structure of hepatocytes changes with age. The volume of hepatocytes increases with development and maturation but declines with senescence [84]. The relative volumes of hepatocyte organelles also change during aging. The universal change in diagnostic liver biopsy specimens is the

age-related cytoplasmic accumulation of highly oxidized insoluble protein lipofuscin or age pigment, which in turn reflects a concomitant increase in the volume of the dense body compartment [102]. Accelerated hepatocyte aging and the accumulation of senescent hepatocytes have been found in diverse chronic liver disorders [103–108]. Furthermore, the studies have shown that the increased proportion of senescent hepatocytes is associated independently with increased fibrosis stage, impaired hepatic function, and an adverse liver-related outcome including liver-related death [103–109]. However, it is still unknown whether hepatocyte senescence contributes causally to fibrosis progression, declining hepatic function, or the increased risk of an adverse liver-related outcome.

### 3.1. Hepatic fibrosis

Hepatic fibrosis is a common pathological consequence of chronic liver diseases and results from the progressive accumulation of an excess quantity of extracellular matrix (ECM) tissue. In many patients, continuous liver parenchyma insult ultimately leads to cirrhosis [110]. The ECM accumulation during chronic liver injury is caused by a heterogeneous population of myofibroblasts derived mainly from hepatic stellate cells and from resident fibroblasts. Myofibroblasts are highly proliferative cells with enhanced survival that migrate and accumulate at sites of liver damage in response to a liver insult that caused a wide variety of growth factors, cytokines, and lipid mediators. Recently, many studies have observed that the presence of an increased proportion of senescent hepatocytes is associated with an increased fibrosis stage. This results in impaired hepatic function and an adverse liver-related outcome, including liver-related death [103, 104, 106–108].

### 3.2. Cirrhosis and HCC

In humans, cirrhosis is induced by a variety of liver diseases. Regardless of its etiology, cirrhosis evolves slowly over many years, and chronic hepatocyte death and renewal are major predisposing factors [111]. Cirrhosis is associated with life-threatening complications due to a decreased functional parenchymal reserve and altered hepatic blood flow. A recent report by WHO indicates that cirrhosis accounts for 170,000 deaths in Europe per year [110]. The cellular growth arrest and CS appear to be in a pro-fibrotic state. There is existing research on the relation between cirrhosis and telomere shortening, showing that telomere shortening is a marker of cirrhosis and correlates with senescence-associated  $\beta$ -galactosidase in 84% of cirrhosis samples, specifically in hepatocytes [110]. CS contributes to age-related tissue dysfunction, macroH2A1, and a variant of histone H2A. CS is also a marker of senescence-associated heterochromatic foci that synergizes with DNA methylation to silence tumor-suppressor genes in human fibroblasts. MacroH2A1, a variant of histone H2A, is a marker of senescence-associated heterochromatic foci that synergizes with DNA methylation to silence tumor-suppressor genes in human fibroblasts. In a recent study, Borghesan et al. investigated the relationship between macroH2A1 splice variants, macroH2A1.1 and macroH2A1.2, and liver carcinogenesis. It was found that protein levels of both macroH2A1 isoforms were increased in the livers of very elderly rodents and humans, and were strong immunohistochemical markers of human cirrhosis and HCC [112].

### 3.3. Chronic hepatitis B

Chronic hepatitis B virus (HBV) infection can lead to the development of chronic hepatitis, cirrhosis, and HCC. HBV infection is associated with age, cell cycle arrest, and CS. In a recent study, the authors assessed the HBV antigen production in relation to cell cycle arrest and CS *in vitro* using hepG2 and hepG2.2.15 cell lines [113]. The authors found that cell cycle arrest induced *in vitro* by the addition of H<sub>2</sub>O<sub>2</sub> caused increased levels of supernatant HBsAg and HBV DNA and increased expression of HBcAg. In contrast, there was no observed effect on HBsAg or HBV DNA production in senescent cells, with only a minor increase in cytoplasmic HBcAg staining [113]. Widespread telomere shortening is consistent with accelerated aging in chronic HBV [114].

### 3.4. Chronic hepatitis C

Chronic infection with hepatitis C virus (HCV) affects approximately 170 million people around the world. Moreover, 20% of these affected people develop cirrhosis and are at great risk to develop HCC. An increased risk of HCV-related cirrhosis is associated with hepatic steatosis, older age, and higher alcohol consumption [88, 115]. It has been reported that during chronic HCV infection, telomere shortening is present in liver tissue [116], likely due to increased hepatocyte turnover. Critically short telomeres trigger replicative senescence. Marshall et al. found a strong correlation between hepatocyte G1 arrest, dysfunctional hepatic regeneration, and increased cirrhosis in patients with chronic HCV. They showed increased hepatocyte cell cycle arrest measured by the expression of the mini-chromosome maintenance protein 2 (Mcm-2) in liver biopsy samples. The researchers also found that p21 expressed predominantly in hepatocytes and it was correlated with the stage of fibrosis [107].

### 3.5. Nonalcohol-related fatty liver disease (NAFLD)

NAFLD is a leading cause of chronic liver disease worldwide [117], which presents a wide range of liver disorders including simple steatosis, steatohepatitis, cirrhosis, and HCC [117]. In ob/ob mice, NAFLD is associated with an increased expression of the cell cycle inhibitor p21 and impaired liver regeneration [118]. The combination of impaired regeneration and increased incidence of cancer in NAFLD suggests accelerated aging. In the literature, NAFLD has been separately described by the appearance of premature telomere shortening, increased hepatocyte nuclear area, advanced fibrosis, and p21 expression [103, 104]. Recently, Aravinthan et al. performed a study on 105 archived formalin-fixed paraffin-embedded liver needle biopsy specimens from 70 patients within the spectrum of NAFLD, and 43 liver needle biopsies at the time of liver transplantation from age- and sex-matched donor livers served as controls. In this study, the authors found a close correlation between hepatic steatosis and short telomeres; additionally, the proportion of hepatocytes with DNA damage identified by the presence of  $\gamma$ -H2AX increased in parallel with steatosis grade. The authors suggested that the accumulation of fat in hepatocytes causes DNA damage and telomere erosion, possibly mediated by oxidative stress. A critically short telomere and a break in double-stranded DNA lead to CS and permanent cell cycle arrest. The authors also found a pattern of predominant hepatocyte G1/S phase cell cycle arrest in NAFLD, with an increased expression of Mcm-2

compared to a normal liver. Furthermore, there was increased expression of the p21 protein. A striking feature was the association of p21 expression with the fibrosis stage, which suggests an accumulation of senescent hepatocytes with disease progression [105].

### 3.6. Alcohol-related liver disease

Alcohol-related liver disease (ALD) is one of the leading causes of liver-related morbidity and mortality in the world. ALD encompasses a broad spectrum of liver injury from simple steatosis to alcohol-related hepatitis, cirrhosis, and HCC. The pathophysiology of ALD is a complex phenomenon; however, oxidative stress is an important factor in the disease since alcohol consumption increases the production of ROS and diminishes cellular antioxidant levels [119]. The validity of clinical criteria in predicting outcomes in ALD has been studied. In one study, it was shown that the increase of the hepatocyte nuclear area was related to hepatic dysfunction and suggests hepatocyte senescence, since nuclear enlargement is a recognized morphological characteristic of CS [120]. In contrast, irreversible cell arrest that limits the proliferative potential of cell is a hallmark of CS and is mediated by p21 [121]. Thus, p21 has a vital role in the stability of cell cycle arrest and the induction of senescence [121].

### 3.7. Senolytic drugs

The accumulation of senescent cells in tissues and organs contributes to age-related diseases; however, the presence of radiations or genotoxic agents may contribute to the development of senescence phenotype. This accumulation is important because senescent cells contribute to alterations in the microenvironment. This can support conditions for diseases such as cancer to develop, but senescent cell quantity is important for organ function in the case of aging.

Senolytic agents may be used for clearance of senescent cells in the organs. Chang et al. used ABT263, a specific inhibitor of apoptosis genes BCL2 and BCL2L1, to selectively induce apoptosis in senescent cells in culture. They carried out experiments using a mouse model with known p16-3 MR and administered ABT263 to mice and observed that p16-positive senescent cells were depleted in bone marrow, lung, and muscle tissue via apoptosis. The data demonstrate that ABT263 is a senolytic drug that acts in a highly specific manner on various tissues to target senescent cells both in culture and *in vivo*. These findings significantly advance clinical targeting of cell senescence [122].

## 4. Conclusion

Until now, the role of CS is poorly understood and little studied in hepatology. There are major implications for hepatology in the field of fibrosis and cancer, and liver chronic diseases. Further investigation into the molecular basis of senescence in liver diseases is necessary, and the collaboration between basic and clinical researchers is fundamental to arrive to better diagnosis and treatment.

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