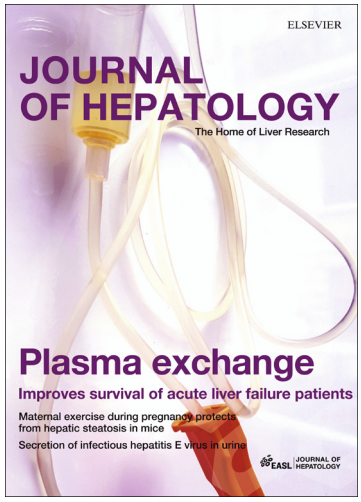

Review

Senescence in chronic liver disease: is the future in ageing?

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TITLE PAGE

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Abbreviations: mTORC1 – mammalian target of rapamycin complex 1
SADF – senescence-associated DNA-damage foci
SAHF – senescence-associated heterochromatic foci
SASP – senescence-associated secretory phenotype
SA-β-GAL – senescence-associated β-galactosidase

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ABSTRACT

Cellular senescence is a fundamental, complex mechanism with an important protective role present from embryogenesis to late life across all species. It limits the proliferative potential of damaged cells thus protecting against malignant change, but at the expense of substantial alterations to the microenvironment and tissue homeostasis, driving inflammation, fibrosis and paradoxically, malignant disease if the process is sustained. Cellular senescence has attracted considerable recent interest with recognition of pathways linking aging, malignancy and insulin resistance and the current focus on therapeutic interventions to extend health-span. 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. This review focuses on cellular senescence in chronic liver disease and explores therapeutic opportunities.

KEY POINTS BOX

1. Senescence of hepatocytes, cholangiocytes, stellate cells and immune cells is a key feature of chronic liver disease independent of aetiology.
2. Cellular senescence plays an important role in the progression of chronic liver disease.
3. Understanding cellular senescence may extend therapeutic options in preventing or even reversing chronic liver disease progression.

POSTCRIPT

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CELLULAR SENESCENCE

What is cellular senescence?

Cellular damage, if not repaired, leads to apoptosis or senescence, the fundamental cellular mechanisms of cancer prevention with a long evolutionary history. Apoptosis, a process of programmed cell death, eliminates damaged cells in a rapid but regulated manner with minimal interference to the microenvironment. Senescence on the other hand, which limits the proliferative potential of damaged cells through the induction of stable cell cycle arrest [1-4], results in substantial alterations to the microenvironment and tissue homeostasis. It is unclear why an injured cell that is incapable of repair should trigger a pathway to either apoptosis or instead to cellular senescence (and is beyond the scope of this review); however, one suggestion is that the greater the severity of DNA damage the more likely it is that a cell will undergo apoptosis [5]. Nevertheless, both pathways should be considered as normal, healthy responses to injury that protect both the organ and the organism.

Cellular senescence was first described over half-a-century ago as a consequence of replicative exhaustion (replicative senescence), the concept that each individual cell has a finite potential for cell division [6]. Cellular senescence also occurs in a much more rapid manner as part of an immediate response to cellular stresses such as oxidative stress, DNA damage, oncogene activation and disruptions to the epigenome (stress-induced premature senescence), approaches which have been exploited in laboratory studies [1, 4] (figure 1).

Although cellular senescence plays a crucial role in ageing of both individual organs as well as the entire organism, as might be imagined from the nomenclature, it is important to appreciate that cellular senescence is also a normal part of wound healing and plays a critical part in normal embryonic development in animals and humans [7-9]. Cellular senescence under these circumstances can be considered an acute, time-limited event, which is part of the normal

physiological response protecting the organ and in which senescent cells are removed subsequently, thus indicating that the process of cellular senescence can be terminated or reversed. If, however senescent cells persist i.e. when the senescence process is disordered or chronic, this represents a breach of the normal healing process and the organ is then susceptible to injury mediated by the senescent cell *per se* as well as the initial cause of the DNA damage – the yin and yang of cellular senescence.

Once cellular senescence is established, cells become resistant to apoptosis [10, 11]. As a consequence, senescent cells then accumulate in organs and the decline in organ function with ageing is thought to be related, at least in part, to the accumulation of senescent cells with altered physiology [12-14]. Evidence from skin naevi suggests that senescent cells may persist in tissue for years [15].

Markers of cellular senescence

Cells undergo significant morphological changes during senescence. Senescent cells become enlarged and flattened and contain enlarged nuclei. They express senescence-associated β-galactosidase (SA-β-GAL) detectable at pH 6, which differentiates senescent cells from pre-senescent and terminally differentiated cells [16].

Senescent cells are classically cell cycle arrested at G1/S transition, thus typically display a DNA content characteristic of the G1 phase and express cell cycle inhibitors such as p21, p16 and p53 [17]. Nuclear accumulation of senescence-associated heterochromatic foci (SAHF) and senescence-associated DNA-damage foci (SADF) further discriminate senescent cells. Proteins involved in the formation of SAHFs induce senescence when over-expressed and are required for stable suppression of proliferation-associated genes [18, 19], while SADFs contain proteins crucial for DNA-damage response and senescence [20].

Further, senescent cells secrete a wide variety of factors (table 1), a phenomenon known as the senescence-associated secretory phenotype (SASP) [21-24]. Autophagy, which allows cells under conditions of stress to digest internal constituents to generate energy and metabolic precursors, is closely associated with senescence [25]. This catabolic pathway is spatially and temporally coupled to the mammalian target of rapamycin (mTOR)-associated anabolic pathway, allowing protein degradation to feed raw materials directly into protein synthesis for the SASP [26].

It is important to stress that there is no single marker of cellular senescence. The presence of permanent cell cycle arrest, the acquisition of major morphological change, expression of SA-β-GAL, accumulation of SAHFs and SADFs, acquisition of SASPs, increased ROS production and autophagy are known collectively as the 'cellular senescence signature' and is used to define cellular senescence [27-29] (figure 2).

Senescent-associated secretary phenotype

Senescent cells have considerable influence on their microenvironment and exert both beneficial and detrimental effects through SASP factors. These induce senescence in neighbouring cells in a paracrine fashion [30, 31], and promote inflammation, which aids clearance of senescent cells and cell debris [32], encouraging tissue repair and remodelling. In addition, SASP helps removal of both pre-malignant and malignant cells, thus representing an important aspect of tumour surveillance [33, 34]. SASPs are also involved in refining embryogenesis. On the other hand, the SASP may also promote tumourigenesis [23, 24, 35], which is occasionally referred to as the tumour-associated secretary phenotype. Such deleterious effects of the SASP are seen characteristically when senescent cells accumulate and become 'chronic' as seen with aging and in chronic diseases (figure 3).

CELLULAR SENESCENCE IN CHRONIC LIVER DISEASE

Fibrosis progression, decompensation and the development of liver cancer are common to all chronic liver disorders, independent of aetiology. Hepatocyte injury *per se*, as opposed to aetiology, is therefore likely to play a crucial role in chronic liver disease progression. Cellular senescence of hepatocytes, cholangiocytes, stellate cells and immune cells has been demonstrated in a wide spectrum of chronic liver disorders using *in-vitro* and *in-vivo* experiments in cell lines, animal models and humans over many years. The involvement of senescence in liver disease has attracted increased attention recently. With clinical hepatologists and hepatobiologists in mind, this review examines the evidence and clinical implications of cellular senescence in chronic liver disease.

Cellular senescence in epithelial cells (hepatocytes and cholangiocytes)

Senescence of hepatocytes has been demonstrated indisputably in a range of chronic parenchymal liver disorders, including chronic viral hepatitis B and C [36-39], alcohol-related liver disease [40], non-alcohol-related fatty liver disease [41-43] and genetic haemochromatosis [44]. Hepatocyte senescence appears an almost universal phenomenon in chronic liver disease, irrespective of aetiology [45]. Evidence of hepatocyte senescence has also emerged from tolerant human liver allografts with normal hepatic function and histology [46]. Hepatocyte senescence has also been demonstrated in animal models of liver disease [47].

As with hepatocytes in chronic parenchymal liver disease, senescence of cholangiocytes was detected readily within damaged small bile ducts and bile ductules in chronic biliary liver disorders such as primary biliary cholangitis (previously primary biliary cirrhosis) [48, 49] and primary sclerosing cholangitis [50]. Cholangiocyte senescence has also been demonstrated in advanced stages of chronic parenchymal liver diseases such as chronic viral hepatitis and non-alcohol-related fatty liver disease [51]. The mechanism for involvement of neighbouring cells has not yet been dissected, but is likely to reflect paracrine effects driven by senescent hepatocytes; these

observations may have clinical significance if resting bile duct cells retain stem cell function [52, 53], although this is contentious.

In contrast to other organs/tissues, ageing per se does not seem to induce senescence of hepatocytes and cholangiocytes in normal liver [54]. This finding may be of great value in the field of liver transplantation, as more and more liver grafts are being used from older donors who are otherwise healthy. It is conceivable that senescence markers might in the future be used to select suitable livers from older donors.

Cellular senescence in non-epithelial cells (hepatic stellate cells and immune cells)

Despite a potential and important role in fibrosis regression (see below), senescence of hepatic stellate cells has been studied only rarely in chronic liver disease. A study comparing hepatocytes with stellate cells in chronic liver disease demonstrated significantly longer telomeres in hepatic stellate cells [45]. On the other hand, senescence of hepatic stellate cells has been demonstrated in animal models of obesity [35], which has not yet been shown in humans.

Immune senescence has also been examined in patients with chronic liver disease. Accelerated telomere shortening in peripheral lymphocytes and has been demonstrated in chronic viral hepatitis [55], cirrhosis [56], and liver transplant recipients with established grafts, independent of the aetiology of liver disease [57, 58]. Despite emerging evidence, the cause of immune senescence in chronic liver disease remains unknown. Peripheral monocyte telomere shortening has been demonstrated in type-2 diabetes mellitus, a disease which has strong associations with non-alcohol-related fatty liver disease [59]. Immune senescence in circulating mononuclear blood cells has been demonstrated in patients with non-alcohol-related fatty liver disease (Shah M & Alexander GJ, personal communication). No studies have yet examined senescence in intrahepatic immune cells.

Mechanisms of cellular senescence in chronic liver disease

Despite the convincing evidence for senescence of hepatocytes and cholangiocytes, the mechanisms that drive the evolution of senescence in chronic liver disease remain debatable. Telomere attrition from repeated cell proliferation causing replicative exhaustion (replicative senescence) has been considered the basis for hepatocyte senescence in chronic liver disease [37, 38, 45]. However, this hypothesis is not supported by more recent studies, which place greater emphasis on stress-induced hepatocyte senescence [41, 44, 60]. In particular the absence of hepatocyte proliferation in liver biopsies from patients with chronic liver disease, even in the early stages and the demonstration of a failure of cycle progression of hepatocytes in chronic liver disease make it unlikely that increased cell turnover underpins hepatocyte senescence [36, 40, 41]. The disparity in many studies in the literature regarding hepatocyte proliferation may be explained by a common misunderstanding of markers used to measure cell proliferation. Mcm, PCNA and Ki67, proteins commonly studied as markers of cell proliferation in these studies, are detectable throughout all or most phases of the cell cycle, at varying concentrations but not in the resting phase. Therefore, their presence cannot be interpreted exclusively as an indication of cell proliferation since cells that have entered the cell cycle but are in stable cell cycle arrest also express the same proteins.

It has been argued that oxidative stress, a common cellular stress, leads to both telomeric and non-telomeric DNA damage and thereby causes telomere-dependent and telomere-independent cellular senescence [27]. Similarly, stress-induced senescence has also been proposed for cholangiocyte senescence in chronic biliary liver disease [48-50]. Nevertheless, it is unlikely that a single mechanism is solely responsible; both stress-induced and replicative senescence is likely to contribute to cellular senescence in chronic liver disease.

Moreover, genetic mutations of components of the telomerase enzyme complex (telomerase reverse transcriptase and telomerase RNA component), which maintain telomere length, have been demonstrated in cirrhosis with a wide range of aetiologies [56, 61]. These studies highlight the genetic involvement of hepatocyte senescence in liver disease progression and show telomerase mutations as a risk factor for the development of cirrhosis [62].

Accumulation of senescent cells in chronic liver disease

Senescent hepatocytes have been shown to accumulate with progression of chronic liver disease [36, 37, 40-42]. An on-going liver insult, whether in the form of continuous alcohol ingestion, fat accumulation, viral or immune-related damage is likely to generate senescent hepatocytes continuously, leading eventually to accumulation. Lack of immune-mediated clearance of senescent hepatocytes due to immune senescence in patients with chronic liver disease is also likely to contribute towards further accumulation of senescent hepatocytes. Induction of senescence in hepatocytes in the immediate vicinity of an already senescent hepatocyte, a phenomenon known as senescence-induced senescence, could also contribute to the accumulation and explain the clustering of senescent hepatocytes [30]. Although the lifespan of senescent hepatocytes in humans is not yet known, given the anti-apoptotic nature of senescent cells and immune senescence in chronic liver disease, senescent hepatocytes are likely to persist. In mice, senescent hepatocytes have been shown to persist up to 7 weeks after a single brief exposure to a DNA-damaging agent [63]. In this study, the use of DNA-damaging agent may also have impaired immune responses, thus allowing senescent cells to persist longer (chronic senescence), mimicking the clinical scenario in chronic liver disease, as opposed to the prompt clearance of senescent hepatocytes reported by Kang *et al* (acute senescence) [34], in which immune responses remained healthy, since senescence was induced specifically and exclusively in murine hepatocytes via oncogene activation.

Senescent cholangiocytes have also shown to increase in number with progression of chronic liver disease [51]; evidence for a similar increase in number is lacking for other intrahepatic cell lineages.

CONSEQUENCES OF CELLUALR SENESCENCE IN CHRONIC LIVER DISEASE – PHYSIOLOGY TURNED PATHOLOGY

Cellular senescence and liver fibrosis

Accumulation of senescent cells is likely to influence disease progression through an impact on the microenvironment and tissue homeostasis. The diseased liver generates an enormous burden of senescence since as many as 80% of hepatocytes are senescent in advanced disease [40, 41] and the evidence indicates that these have a marked effect on disease progression. A number of studies in chronic parenchymal liver disease demonstrated an increase in the proportion of senescent hepatocytes with inflammatory activity and fibrosis stage [36, 37, 40-42]. Similar associations were also demonstrated between hepatocyte nuclear area and fibrosis in alcohol-related liver disease, a finding that predates the concept of cellular senescence in the field of hepatology [64, 65]. A rise or fall in the proportion of senescent hepatocytes with progression or regression of fibrosis respectively, has also been shown in chronic liver disease [41]. Moreover, a close geographical association is evident between hepatocyte senescence and activation of hepatic stellate cells in chronic liver disease [40]. Furthermore, the rate of hepatocyte telomere shortening has been shown to correlate with the rate of fibrosis progression [37]. These data indicate a likely role for hepatocyte senescence in stellate cell activation and fibrosis progression. This is further corroborated in a study of p53-decifient mice with nutrition-directed steatohepatitis, which demonstrated reduced hepatocyte p21 expression and reduced hepatic stellate cell activation (reduced α-SMA and collagen expression) compared to the wild type counterpart [66]. However, experimental evidence for such direct causal link between hepatocyte senescence and stellate cell activation (and therefore fibrosis) is lacking in man.

Cellular senescence plays a crucial role in wound healing. Induction of fibrosis by senescent hepatocytes is a way of limiting tissue injury as part of the normal wound healing process. Recruitment of immune cells for the clearance of cell debris and senescent cells also contributes

towards wound healing. However, the continuing nature of insult seen in chronic liver disease allows continued senescence of hepatocytes, tipping the balance towards accumulation. Immune senescence of chronic liver disease may also contribute to such accumulation through impaired clearance of senescent cells. Simultaneously, accumulation of senescent hepatocytes leads to continuous activation of stellate cells and thereby fibrosis progression, making a physiological phenomenon pathological.

An increase in the proportion of senescent cholangiocytes with fibrosis progression has also been demonstrated in both chronic parenchymal and biliary liver disease, although this was more marked in the latter [50, 51]. Whether senescent cholangiocytes induce stellate cell activation or whether both cholangiocyte senescence and stellate cell activation are parallel consequences of biliary injury is not yet known.

Whilst senescence of hepatocytes and cholangiocytes is associated with fibrosis progression, senescence of hepatic stellate cells caused fibrosis regression in a murine model [32]. Stellate cell senescence, which has been attributed to replicative exhaustion, has been shown to limit fibrosis progression and lead to resolution of fibrosis upon withdrawal of the damaging agent [32]. In these circumstances, senescence in the hepatic stellate cell can be regarded as a normal part of the repair process, in which the senescent stellate cells would be cleared at a later date.

Unfortunately none of the above studies have looked at senescence of hepatocytes, cholangiocytes and stellate cells contemporaneously in order to understand the influence of contrasting populations upon liver fibrosis in chronic liver disease. Such studies are essential.

Cellular senescence and impaired hepatic function

Hepatocyte senescence has strong links with liver dysfunction in chronic liver disease. The strongest evidence of such an association comes from a time when cellular senescence was not known to be associated with chronic liver disease. A study in 1988 demonstrated very elegantly a strong correlation between hepatocyte nuclear area and the development of jaundice, ascites, and encephalopathy in alcohol-related liver disease [65]. These findings were corroborated in a more recent study, which showed an association between an increased proportion of senescent hepatocytes and a decline in liver function in alcohol-related liver disease [40]. The decline in hepatocellular function is likely to be due to an alteration in the metabolic function of senescent hepatocytes. Differential expression of genes involved in the major metabolic pathways has been demonstrated in hepatocyte senescence [67]. Such changes in cellular function become more pronounced and detrimental when the balance is skewed towards senescent hepatocyte accumulation, leading to a decline in hepatic function and decompensation.

In vivo and *in vitro* experiments demonstrated an alteration in the expression of bilirubin transporters (MRP2 and MRP_3) in senescent hepatocytes [68]. MRP2, which is restricted to the canalicular (apical) membrane was down-regulated whilst MRP_3, which is found only in the sinusoidal (basolateral) membrane of hepatocytes, was up-regulated in senescent hepatocytes [68]. Thus, accumulation of senescent hepatocytes in advanced chronic liver disease leads to increased transport of conjugated bilirubin into the hepatic sinusoids rather than the bile canaliculi and therefore explains the development of jaundice (conjugated bilirubinaemia) in advanced liver disease [68]. Similar *in vitro* experiments also show reduced synthetic function of senescent hepatocytes [67], which at least in part explains the decline in liver synthetic function in advanced liver disease.

The metabolic alterations of senescent hepatocytes in conjunction with the deterioration of hepatic function in advanced liver disease is corroborated by the independent association demonstrated between the proportion of senescent hepatocytes and a subsequent adverse outcome in patients with chronic liver disease [40, 41]. Progressive accumulation of senescent hepatocytes in chronic liver disease, which do not function like normal hepatocytes, is likely to disturb the metabolic function of the liver when sufficient cells are affected and thereby lead to decompensation and then an adverse outcome. Consistent with this is that the proportion of senescent hepatocytes was a stronger predictor of an adverse outcome than the clinical parameters used currently [40].

A number of studies have demonstrated an association between hepatocyte senescence and insulin resistance or type-2 diabetes mellitus [41, 42, 60]. Induction of senescence in hepatocytes has been shown cause selective insulin resistance through dissociation of Akt downstream pathways [69]. This *in vitro* finding, which has not been confirmed *in vivo*, might perhaps explain the development of insulin resistance in advanced liver disease, a phenomenon known as 'hepatogenous diabetes'. Furthermore, altered expression of glucose transporters in hepatocyte senescence has been demonstrated in humans and is also related to clinical outcome in patients with chronic liver disease and could be used in both stratification and prognostication [69].

Cellular senescence and liver cancer

Although the primary role of cellular senescence is cancer prevention, senescence is also involved in tumourigenesis [27], and hepatocyte senescence is no exception to this. A study using a murine model illustrated elegantly the development of hepatocellular carcinoma from senescent hepatocytes in the absence of immune clearance of senescent cells [34]. This model is highly relevant to the development of hepatocellular carcinoma in advanced chronic liver disease in humans where both accumulation of senescent hepatocytes and immune senescence (thus

impaired immune clearance of senescent hepatocytes) coexist. This may also explain the rarity of hepatocellular carcinoma outside the context of cirrhosis.

Escape of hepatocytes from the senescent state is considered to be the primary mechanism involved in the development of hepatocellular carcinoma. Whilst non-malignant hepatocytes of chronic liver disease demonstrated DNA-damage foci, shorter telomeres and cell cycle inhibitors indicating hepatocyte senescence, hepatocellular carcinoma from the same liver only demonstrated DNA-damage foci and shorter telomere but not cell cycle inhibitors [70], indicating escape from senescent state. Gene expression studies of cirrhosis and hepatocellular carcinoma also demonstrated involvement of hepatocyte escape form senescence in the development of hepatocellular carcinoma [71]. Whether on-going insult leads to further DNA damage in already senescent hepatocytes, which help these cells overcome senescence barriers is not yet known.

Tumours may also develop from neighbouring non-senescent hepatocytes, which are in the vicinity of senescent hepatocytes. Senescent cells, through the SASP, are involved in angiogenesis and recruitment/proliferation of newer cells to replenish damaged cells, an essential role of senescent cells towards tissue integrity. This physiological phenomenon, however, is suspected to be involved in tumourigenesis [72, 73]. Accumulation of senescent hepatocytes in large proportions, as in advanced chronic liver disease, results in a SASP-rich microenvironment, which in turn may promote tumourigenesis in neighbouring DNA-damaged, pre-senescent hepatocytes.

Although, based on the above association between hepatocyte senescence and development of hepatocellular carcinoma, it is tempting to postulate an association between cholangiocyte senescence and cholangiocarcinoma in chronic biliary disease, no studies have yet investigated this possible link.

Cellular senescence and viral kinetics

Finally, in contrast to chronic hepatitis C virus infection, the replication of hepatitis B virus seems to decline with disease progression. This association appears to be related to hepatocyte senescence. In a recent study, the markers of hepatitis B viral replication were confined to biologically younger hepatocytes, as opposed to older or senescent hepatocytes [39]. This may explain the low levels of hepatitis B viral DNA and HBsAg usually seen in cirrhotic patients who have high proportions of senescent hepatocytes.

THE FUTURE – PROGNOSTIC & THERAPEUTIC POTENTIAL OF CELLULAR SENESCENCE

The crucial role of cellular senescence in fibrosis and liver decompensation, both critical landmarks in the course of progressive liver disease, make it an ideal potential biomarker for disease stratification and prognostication. In fact, specific markers of hepatocyte senescence have already been demonstrated to have a role in predicting the outcome of chronic liver disease [40, 41]. However, the requirement for liver tissue (in the form of liver biopsy) for the analysis of hepatocyte senescence is a clear disadvantage. Liver biopsy is invasive, associated, on occasions, with serious complications and as ever, the biopsy sample may not represent the state of hepatocyte senescence of the entire liver. It would be advantageous to identify and validate serum markers of hepatocyte senescence, which could improve the landscape for the evaluation of chronic liver disease, with the added value in some cases to anticipate fibrosis as an alternative to measuring established fibrosis in serum or within the liver.

Secondly, accumulation of senescent hepatocytes is considered as a driver of chronic liver disease progression. Thus, treatment targeted towards eliminating senescent hepatocytes should, in theory, help alleviate senescence-related pathology. Consistent with this approach, clearance of senescent cells was shown to retard age-associated organ dysfunction, delay tumorigenesis and extend lifespan in murine models [74, 75]. A new class of drugs known as "senolytics", which selectively target senescent cells, are under investigation in animal models, but so far with little effect on general applicability [76]. An exciting, recent study, using a specific inhibitor of the anti-apoptotic proteins BCL-2 and BCL-xL, demonstrated selective elimination of senescent cells in culture in a cell type- and species-independent manner [77]. The same agent mitigated premature aging of the haemopoietic system through removing senescent bone marrow hematopoietic stem cells in a murine model [77]. This study indicates that pharmacological elimination of senescent cells with restoration of tissue function is now a real possibility.

In an alternative approach in an experimental animal model, restoration of telomere function through telomerase gene delivery has been shown to alleviate cirrhosis and improve liver function [78], thus corroborating the concept that progressive chronic liver disease could be halted by elimination or prevention of cellular senescence.

Senescent cells affect neighbouring cells and microenvironment through a broad variety of SASP factors, which induce and reinforce senescence, activate immune responses, induce fibrosis and harbour pro-tumourigenic properties [27]. The mammalian target of rapamycin complex 1 (mTORC1) is involved in the SASP production and inhibition of mTORC1 has been to shown to attenuate SASP production [79, 80] and improve organismal longevity [81, 82]. In an alternative approach, reprogramming of the SASP through inhibition of the JAK2/STAT3 pathway enhanced immune surveillance and tumour clearance [83]. Similar such opportunities could, therefore, be optimally exploited to intervene in the process of hepatocyte senescence of chronic liver disease for therapeutic advantages.

Paradoxically, there is a potential role for pro-senescent therapy for certain complications of chronic liver disease. Treatment of hepatocellular carcinoma with TGFβ, a potent hepatocyte cell cycle inhibitor, has been shown to induce senescence in tumour cells and inhibit tumour growth in a mouse model [84]. Reactivation of p53 in a mouse model of hepatocellular carcinoma using RNA interference has also been shown to cause tumour senescence and tumour regression [33]. A targeted pro-senescent therapy approach, therefore, has immense clinical potential. Similarly, senescence-inducing therapy targeting the hepatic stellate cells based on murine work could slow or even prevent fibrosis progression in chronic liver disease. Although such pro-senescent therapies are currently being tried in cancer treatment, cell-specific pro-senescent therapies are not yet available for clinical or research purposes.

CONCLUSION

Senescence is a fundamental cellular process with colossal diagnostic, prognostic and therapeutic potential in liver disease. However, the role of cellular senescence is poorly understood and little studied in hepatology. Strong collaboration between basic and clinical scientists could rejuvenate the liver.

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Table 1: Common senescence-associated secretary phenotype (SASP) factors secreted by senescent cells (Source: adopted with modification from reference [23]). Please note that this table does not include all known SASP factors.

Interleukins (IL)	**Growth regulators/factors**	**Proteases and regulators**
IL6	Amphiregulin	MMP-1, -3, -10, -12, -13, -14
IL7	Epiregulin	TIMP-2
IL1a, IL1b	Heregulin	PAI-1, -2; tPA; uPA
IL13	EGF	Cathepsin B
IL15	βFGF	
Chemokines (CXCL, CCL)	HGF	**Soluble receptors/ligands**
IL8	KGF (FGF7)	ICAM-1, -3
GRO-α, -β, -γ	VEGF	OPG
MCP2	Angiogenin	sTNFRI
MCP4	SCF	TRAIL-R3, Fas, sTNFRII
MIP1a	SDF1	uPAR
MIP3a	PIGH	SGP130
HCC4	IGFBP-2, -3, -4, -6, -7	EGF-R
Eotaxin-3		
Other inflammatory factors	**Nonprotein soluble factors**	**Insoluble factors (ECM)**
GM-CSF	PGE2	Fibronectin
MIF	Nitric oxide	Laminin, Collagen

Abbreviations: IL, interleukin; GRO, Growth-regulated oncogene; MCP, Monocyte chemoattractant protein; MIP, Macrophage inflammatory protein; GM-CSF, Granulocyte-macrophage colony-stimulating factor; MIF, Macrophage migration inhibitory factor; EGF, Epidermal growth factor; FGF, Fibroblast growth factor; HGF, Hepatocyte growth factor; KGF, Keratinocyte growth factor; VEGF, Vascular endothelial growth factor; SDF1, Stromal cell-derived factor 1; IGFBP, Insulin-like growth factor-binding protein; PGE2, Prostaglandin E2; MMP, Matrix metalloproteinase; TIMP, Tissue inhibitor of metalloproteinase; PAI-1, Plasminogen activator inhibitor-1; tPA, Tissue plasminogen activator; ICAM, Intercellular Adhesion Molecule 1; sTNFRI, Soluble tumour necrosis factor receptor I; TRAIL-R, TNF-related apoptosis-inducing ligand receptor.

Figure legend

Figure 1: Consequences of cellular injury

The figure illustrates the potential outcomes of DNA damage and cellular injury. In the setting of 'controlled' injury, the outcome depends on whether repair can be undertaken. The cell will attain pre-injury, normal status if damage is repaired completely. When the damage is irreparable, it leads to either apoptosis or cellular senescence. 'Uncontrolled' (extreme) cellular injury leads to death by lysis or necrosis, as often seen in acute liver failure.

Figure 2: Common causes and cellular changes in senescence

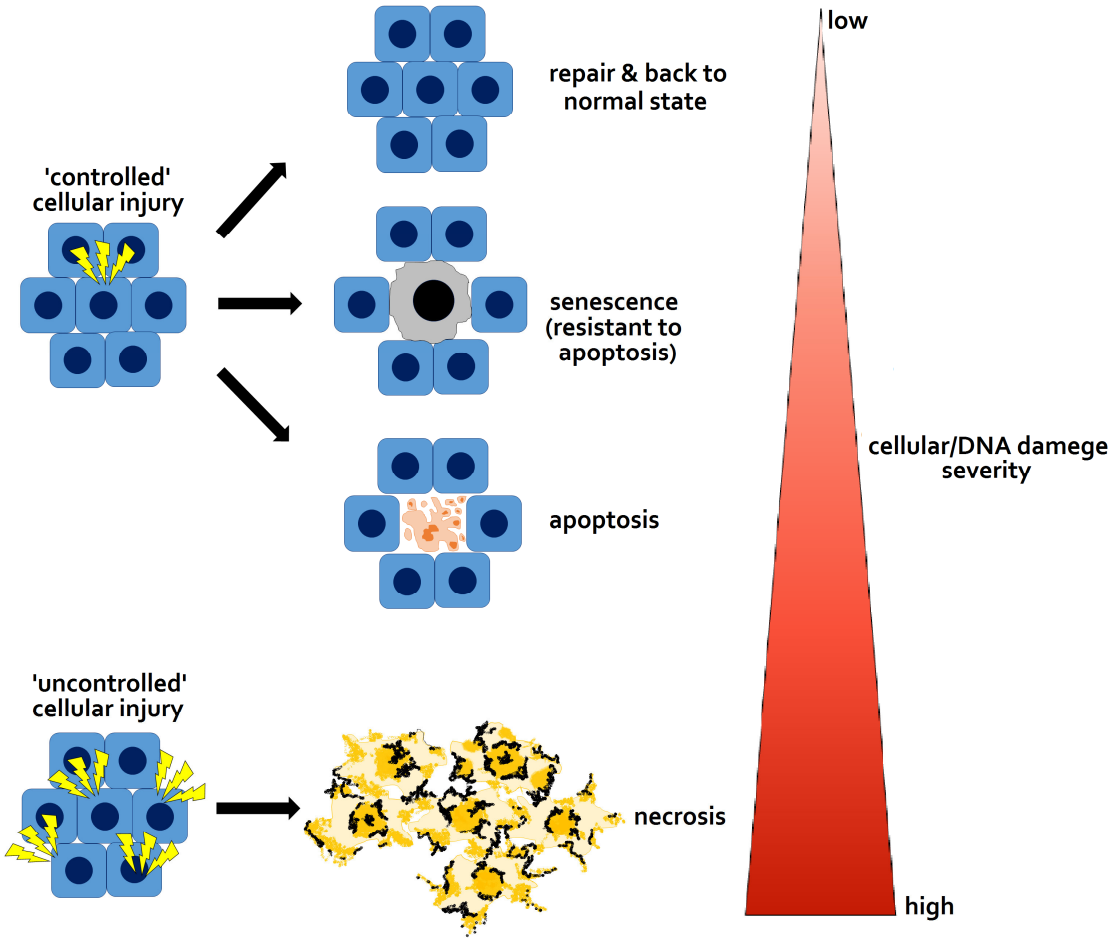
Cellular senescence is activated in response to various forms of cellular stresses, categorised broadly into replicative senescence and stress-induced (genotoxic, oxidative and oncogenic stresses) premature senescence. Cells, once senescent, acquire major morphological changes, become permanently cell cycle arrested, express senescence-associated β-galactosidase (SA-β-GAL), accumulate senescence-associated heterochromatic foci (SAHF) and senescence-associated DNA-damage foci (SADF), attain the senescence-associated secretory phenotype (SASP), increase ROS production and activate autophagy. All play a crucial role in the induction and maintenance of cellular senescence and are known collectively as the 'cellular senescence signature'.

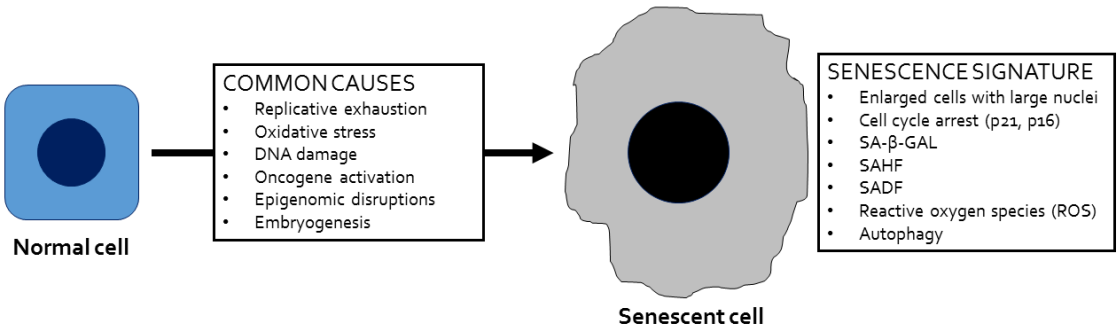
Figure 3: 'Acute' and 'chronic' senescence

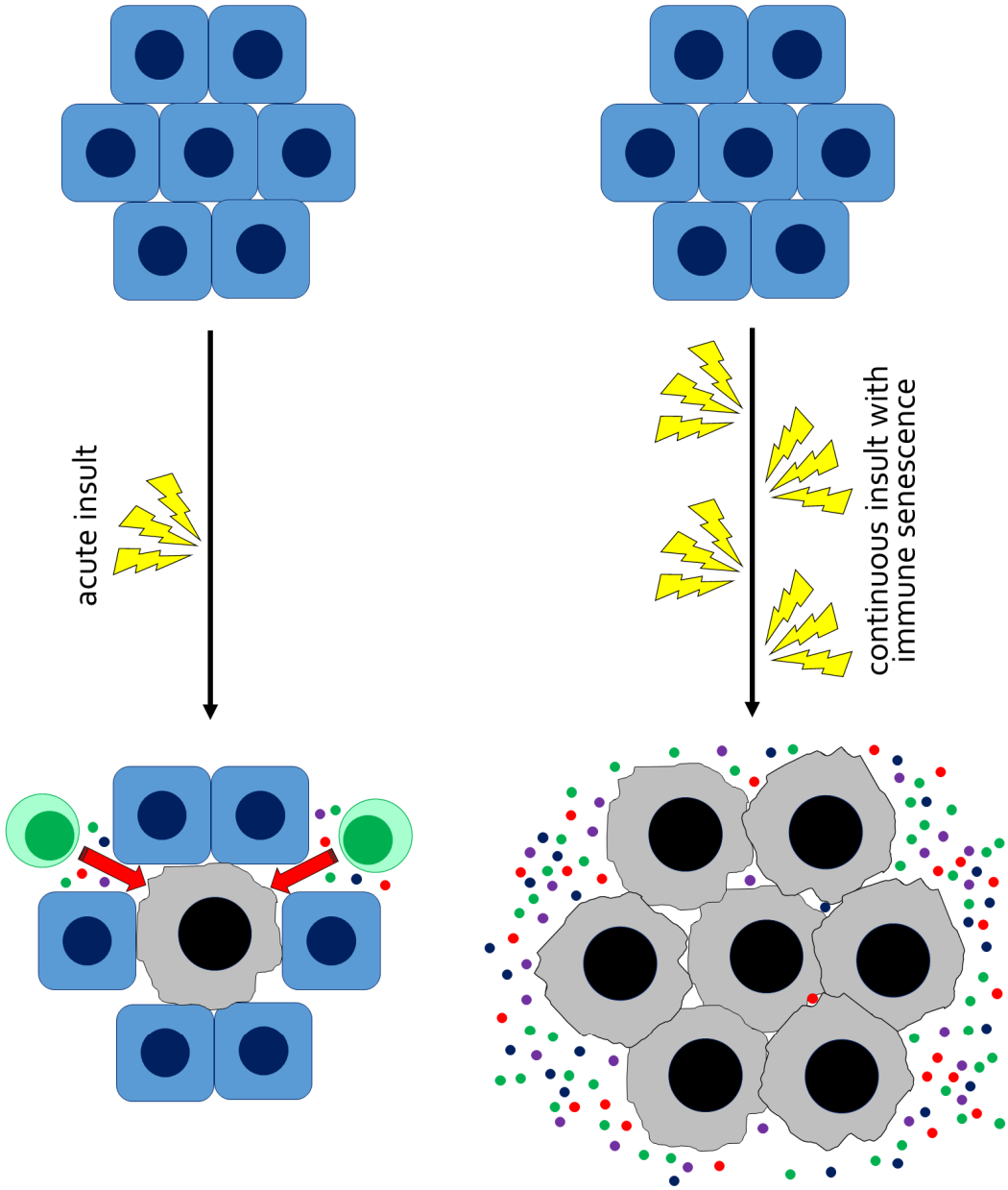
An acute cellular injury leads to cellular senescence, which plays a pivotal role in tumour prevention, would healing and regulation of embryonic development. These senescent cells are removed subsequently by the innate immune system. In contrast, on-going cellular injury, as seen in chronic liver disease, leads to continuous generation of senescent cells, which is accentuated by immune senescence leading to failure of immune-mediated clearance. This leads to a state of 'chronic' senescence, which leads eventually to tissue dysfunction and tumour promotion. Thus cellular senescence is a classic example of antagonistic pleiotropy.

Figure 4: Consequence of hepatocyte senescence and the role of SASP factors in chronic liver disease

Senescent hepatocytes are pro-inflammatory and SASP factors are involved in the activation of innate immune responses. SASP factors reinforce the senescent state in an autocrine manner and induce senescence in neighbouring normal hepatocyte, a phenomenon known as senescence-induced senescence. On the other hand, SASP factors may also promote tumourigenesis in neighbouring cells. Tumours may also develop from senescent hepatocytes if they acquire mutations, which inactivate or help bypass senescence. Senescent hepatocytes, through SASP factors, attract and activate hepatic stellate cells leading to fibrosis. Hepatocytes, once senescent, undergo major metabolic changes such as alteration in handling of bilirubin and acquisition of insulin resistance.







'ACUTE' SENESCENCE
Tumour suppression
Wound healing
Embryogenesis

'CHRONIC' SENESCENCE
Tumour promotion
Tissue dysfunction