Hepatic stellate cell senescence in liver fibrosis: Characteristics, mechanisms and perspectives

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Abstract

Myofibroblasts play an important role in fibrogenesis. Hepatic stellate cells are the main precursors of myofibroblasts. Cellular senescence is the terminal cell fate in which proliferating cells undergo irreversible cell cycle arrest. Senescent hepatic stellate cells were identified in liver fibrosis. Senescent hepatic stellate cells display decreased collagen production and proliferation. Therefore, induction of senescence could be a protective mechanism against progression of liver fibrosis and the concept of therapy-induced senescence has been proposed to treat liver fibrosis. In this review, characteristics of senescent hepatic stellate cells and the essential signaling pathways involved in senescence are reviewed. Furthermore, the potential impact of senescent hepatic stellate cells on other liver cell types are discussed. Senescent cells are cleared by the immune system. The persistence of senescent cells can remodel the microenvironment and interact with inflammatory cells to induce aging-related dysfunction. Therefore, senolytics, a class of compounds that selectively induce death of senescent cells, were introduced as treatment to remove senescent cells and consequently decrease the disadvantaged effects of persisting senescent cells. The effects of senescent hepatic stellate cells in liver fibrosis need further investigation.

Keywords:
Liver fibrosis
Hepatic stellate cells
Senescence
Oxidative stress
1. Introduction

Chronic liver diseases of diverse etiology, including toxic injury, viral infection, autoimmune disorders, metabolic and genetic disorders, can evolve into liver fibrosis. Liver fibrosis is characterized by excessive accumulation of extracellular matrix (ECM) and is considered an intermediate stage which can either be reversed or advance to cirrhosis and end-stage liver disease (Zoubeik et al., 2017). Epidemiological data demonstrate that cirrhosis leads to more than 1 million deaths per year worldwide (Tschochatzis et al., 2014). According to recent epidemiological data from the year of 2017 in the USA, age-adjusted mortality associated with cirrhosis was estimated about 9.2 per 100,000 (Moon et al., 2020). The burden of liver fibrosis not only increases the incidence of end-stage liver diseases and mortality but also increases risk of extra-hepatic diseases. Non-alcoholic fatty liver disease (NAFLD), one of the etiological causes of liver fibrosis, increases cardiovascular mortality in children and young adults (Simon et al., 2021). The pandemic of COVID-19 is currently a major health concern worldwide. Liver fibrosis is an independent risk factor for a detrimental outcome of patients with COVID-19 (Campos-Murguia et al., 2021). Chronic viral hepatitis induced liver fibrosis is associated with increased risk of extra-hepatic cancers including prostate cancer, head and neck cancer, squamous cell cancer, lung cancer and hematologic malignancies (Abutaleb et al., 2021). However, no effective medication is approved for clinical application to prevent the progression of liver fibrosis so far.

The main characteristic of liver fibrosis is an excessive production of ECM produced by fibrogenic myofibroblasts (Kisseleva, 2017). Myofibroblasts are rare in normal tissue and only transiently activated during wound healing to produce ECM and scar tissue (Tschochida and Friedman, 2017). Myofibroblasts originate from epithelial cells, mesenchymal stromal cells (MSCs), fibrocytes, mesothelial cells, hepatic stellate cells (HSCs) and portal fibroblasts (PFs) (Kisseleva, 2017). During experimental fibrosis, HSCs are the major precursors of myofibroblasts (Kisseleva, 2017; Lue et al., 2016; Iwaisako et al., 2014).

HSCs represent about 10% of the resident cells in the liver (Tschochida and Friedman, 2017). They originate from mesothelial cells during embryonic development and reside in the subendothelial space of Disse (Rinkevich et al., 2012). In normal healthy liver, HSCs are quiescent (qHSCs) and are characterized by the presence of lipid droplets containing vitamin A and the expression of platelet-derived growth factor receptor-β (PDGFRβ), lecithin retinol acyltransferase (LRAT), desmin and glial fibrillary acidic protein (GFAP) (Tschochida and Friedman, 2017). In response to profibrotic stimuli, qHSCs activate and transdifferentiate into myofibroblast-like cells to actively regulate tissue repair. Compared to qHSCs, activated HSC (aHSC) acquire novel characteristics such as proliferation, contractility, enhanced ECM synthesis, chemotaxis and generation of inflammatory signals (Tschochida and Friedman, 2017). Activated HSCs lose their cytoplasmic vitamin A droplets, acquire a stretched morphology and contractile properties and have different transcriptional characteristics, including increased expression of alpha-smooth muscle actin 2 (ACTA2) and collagen type 1 alpha 1 (COL1A1) and reduced expression of peroxisome proliferator-activated receptor gamma (PPARG) and GFAP (Kisseleva, 2017). ECM accumulation is the outcome of deposition (fibrogenesis) and degradation of ECM components. Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that are the major enzymes responsible for the degradation of ECM components. The MMPs and their endogenous inhibitors, Tissue Inhibitors of Metallo-Proteinases (TIMPs), are therefore key players in the remodeling of ECM and hence the progression and regression of liver fibrosis (Naim et al., 2017). An imbalance in the expression and/or activity of MMPs and TIMPs can promote the progression of liver fibrosis (Robert et al., 2016).

During experimental hepatic fibrosis, the liver can return to a normal state upon removal of pro-fibrotic stimuli - a process known as fibrosis resolution (Troeger et al., 2012; Kisseele et al., 2012) - in which aHSCs spontaneously initiate apoptosis or revert to an inactive phenotype. Apoptosis is involved in the early stages of resolution, whereas inactivation is predominant in mid to late stages (Troeger et al., 2012; Kisseele et al., 2012; Issa et al., 2001). Inactivated HSCs (iHSCs) display reduced expression of activation markers including COL1A1, ACTA2 and TIMP1, re-express quiescence markers like PPARG and restore vitamin A droplets. However, iHSCs are not identical to qHSCs since the characteristic transcription factors of iHSCs are different from that of qHSCs (Liu et al., 2020), and remain more responsive to repeated fibrogenic stimuli compared to qHSCs (Troeger et al., 2012; Kisseele et al., 2012).

Cellular senescence is a physiological process in which proliferating cells enter a state of stable cell cycle arrest in which cells cannot re-enter the cell cycle in response to mitogens (Gorgoulis et al., 2019). Besides apoptosis, senescence is another fate of aHSCs in the reversal of fibrosis (Krizhanovsky et al., 2008). Senescent HSCs are observed during reversal of fibrosis where they contribute to limit the progression of fibrosis (Krizhanovsky et al., 2008; Schnabl et al., 2009). Hence, induction of senescence in HSCs may serve as a promising anti-fibrotic strategy. In the following paragraphs, we review the characteristics, mechanisms and possible effects of cellular senescence on liver fibrosis.

2. Characteristics of senescent cells

Cellular senescence is a stable cell cycle arrest program, first described as a state of replicative exhaustion of primary cells in vitro (Hayflick and Moorhead, 1961; Hayflick, 1965). Due to the ‘end-replication problem’ of DNA polymerase, telomeres, the end-caps of chromosomes, become progressively shorter with every cell division (Olovnikov, 1973; Watson, 1972), eventually reaching a critical size triggering activation of the DNA Damage Response (DDR) machinery leading to p53-mediated senescence (d’Adda di Fagagna et al., 2003; Kuilman et al., 2010). It is now established that stressors additional to telomere shortening can induce states of premature senescence (Kuilman et al., 2010; Serrano and Blasco, 2001). Senescence-associated phenotypes include loss of DNA synthesis, enlarged and flattened cell morphology, enhanced β-galactosidase (SA-β-gal) activity, accumulation of heterochromatin foci (SAHFs) and a hyper-secretory phenotype (Fig. 1). However, some of these phenotypic characteristics are variable and heterogeneous and dependent on the context, stimulus and cell type (Gorgoulis et al., 2019; Hernandez-Segura et al., 2017). The phosphorylation of the C-terminal of the core histone protein H2AX (termed γH2AX when phosphorylated) is known to recruit DDR proteins and ensure the retention of those proteins in the DSB sites. Therefore, γH2AX has been used as a signature of DDA damage associated senescence (Siddiqui et al., 2015). Endogenous β-galactosidase is a lysosomal enzyme, with maximum activity at pH 4.0–4.5 (Lee et al., 2006). SA-β-gal activity is usually measured at pH 6.0 with the artificial substrate X-Gal and this assay is most commonly used to determine senescence. Determination of β-galactosidase activity at the suboptimal pH 6.0 demonstrates a high level of expression in senescent cells (Debacq–Chainiaux et al., 2009). However, this activity can also be detected in cells with increased lysosomal number and size, e.g. during autophagy. Contact inhibition in long-term cell cultures also induces β-galactosidase activity in quiescent cells (Sharpless and Sherr, 2015). Therefore, a high β-galactosidase activity is not specific for senescent cells and a combination of markers for the identification of senescent cells should be preferred. Several studies have identified senescence-associated gene expression signatures which revealed changes in cell-cycle regulators (Hernandez-Segura et al., 2017; Mason et al., 2004; Trougakos et al., 2006; Seshadri and Campisi, 1990). Two cell-cycle inhibitors that are often expressed by senescent cells are the cyclin-dependent kinase inhibitors (CDKIs) CDKN1a (also termed Waf1, encoding p21Cip1) and INK4a (also termed CDKN2a, encoding p16INK4a). On the other hand, senescent cells repress genes that encode proteins involved in cell-cycle progression; such as E2F targets (e.g. replication-dependent histones, c-FOS, cyclin A, cyclin B and PCNA) (Seshadri and Campisi, 1990; Pang et al., 2006).
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Fig. 1. Characteristics of senescent cells. The triggers, morphology, phenotype and biomarkers of cellular senescence are illustrated. ROS, reactive oxygen species; SASP, senescence associated secretory phenotype; SAHF, senescence associated heterochromatin foci; SA-β-Gal, senescence associated β galactosidase.

and Chen, 1994). Interestingly, the quest for senescence-specific gene expression signatures also revealed that besides changes in cell-cycle regulators, senescent cells exhibited changes in genes that appeared to be unrelated to growth arrest (Hernandez-Segura et al., 2017; Mason et al., 2004; Shelton et al., 1999). A classic example of senescence heterogeneity is the senescence-associated secretory phenotype (SASP), a collection of secreted factors that negatively or positively modulates the functionality of the microenvironment and of surrounding cells. Recent findings established the SASP as a highly heterogeneous plethora of circulating factors, including cell-derived small extracellular vesicles or sEVs (Basisty et al., 2020). Factors secreted by senescent fibroblasts can promote cancer progression (Krtolica et al., 2001; Parrinello et al., 2005; Demaria et al., 2017), but also reinforce growth arrest and promote tumor clearance by the immune system (Xue et al., 2007). SASP-derived IL-1 promotes cancer progression (Lau et al., 2019; Laberge et al., 2015). SASP derived from senescent colon fibroblasts contributes to proliferation and invasion of colon cancer cells (Guo et al., 2019). Conversely, a tumor-suppressive role of SASP has also been demonstrated. NF-κB driven SASP increases chemosensitivity of cancer cells (Chien et al., 2011). P53-dependent SASP derived from hepatic stellate cells promotes tumor-suppressive M1 polarization of macrophages and inhibits epithelial tumorigenesis in liver (Lujambio et al., 2013). However, some reports suggested that the senescence secretome derived from HSCs has tumour-promoting effect (Yoshimoto et al., 2013; Takahashi et al., 2018). Plasminogen activator inhibitor (PAI)-1 is necessary and sufficient for the induction of senescence (Kortlever et al., 2006). Insulin-like growth factor-binding protein 7 (IGFBP7) was shown to mediate senescence induced by oncogenic BRAF (Wajapeyee et al., 2008). Likewise, pro-inflammatory cytokines and chemokines secreted by senescent cells, such as IL-8, CXCL1, IL-6 and their receptors have been shown to be upregulated during senescence and their depletion partially bypasses replicative and oncogene-induced senescence (Acosta et al., 2008; Kulman et al., 2008).

Facultative heterochromatins are condensed, transcriptionally silent chromatin regions that can reverse DNA condensation to allow gene transcription (Trojer and Reines, 2007). SAHF was first described in nuclei of senescent cells containing 30–50 bright, punctate DNA-dense foci that can be distinguished from chromatin of normal cells (Narita et al., 2003). SAHF are specialized domains of facultative heterochromatin that are characterized by heterochromatic histone modifications and the presence of heterochromatic proteins, histone variant macro H2A, high-mobility group A (HMG) proteins and late replicating regions in the genome (Nacarelli et al., 2017). Histone methylation, specifically histone 3 lysine 4 trimethylation (H3K4me3) (activating) and H3K27me3 (repressing) are epigenetic modifications that are highly associated with gene transcription and linked to lifespan regulation in many organisms (Sen et al., 2016). More than 30 % of chromatin is reorganized in senescent cells. This kind of reorganization includes the formation of large-scale domains (mesas) of H3K4me3 and H3K27me3 on lamin-associated domains (LADs), as well as depletion (canyons) of H3K27me3 outside of LADs, indicating profound changes in the transcriptional profile of senescent cells (Shah et al., 2013).

A heterogeneous phenotype of senescent cells has been described before. Therefore, a combination of senescence signatures were recommended to identify senescent HSCs in vitro and in vivo (Sharpless and Sherr, 2015). Senescent human HSCs, induced by repetitive passaging, have been reported to express enhanced p21 and γH2AX and secrete increased IL-8 protein in vitro (Odagiri et al., 2019). Because of the lack of a single, specific and reliable marker to identify senescent cells in vivo, a combination of SA-β-Gal staining with staining for P21, P16 and/or γH2AX and the HSC marker αSMA on serial sections may help to identify senescent human HSCs (Yoshimoto et al., 2013). More recently, single-cell RNA sequencing (scRNA-seq) technique has been introduced for cell-tracing studies. The identification of senescent cells in various organs by scRNA-seq, can contribute to the discovery of novel in vivo signatures of senescent cells (Tabula Muris, 2020). On the other hand, in some specific conditions, senescent cells are very rare, which may decrease the accuracy and/or feasibility of identifying senescent cells by scRNA-seq (Kim and Kim, 2021).

3. Mechanisms that can be involved in regulation of hepatic stellate cell senescence

3.1. DNA damage response

Isolated primary HSCs can only be cultured for a limited number of passages as they rapidly undergo replicative senescence (Knittl et al., 1999; Saile et al., 2002). In addition, HSCs senescence can be triggered
by a variety of intrinsic stressors, e.g. lysosomal stress, the unfolded protein response (UPR), oncogene activation and reactive oxygen species (ROS) generation leading to unresolved DNA damage (Kuilmann et al., 2008; Gonzalez et al., 2016; Aravinthan and Alexander, 2016; Tilstra et al., 2012; Yang et al., 2013; Liu et al., 2014).

In a typical mammalian cell, the incidence of spontaneous DNA damage is estimated to be less than 2 × 10⁻²⁷ (Campos-Murguia et al., 2021) lesions per day (Ciccia and Elledge, 2010). The majority of damaged DNA can be repaired and is unlikely to initiate the senescence process. However, a persistent DDR can drive cell senescence (White and Vijg, 2016). Single-stranded and/or double-stranded DNA breaks (SSBs or DSBs, respectively) are activators of the DDR (Bielak-Zmijewska et al., 2018). The Mre11-Rad50-Nbs1 (MRN) complex is the sensor of double-stranded DNA damage (Lamarche et al., 2010). Upon DSB, MRN complexes recruit and activate Ataxia-Telangiectasia Mutated (ATM) or ATM- and Rad3-related (ATR) kinases that activate CHK1 and CHK2 (check point kinase 1 and 2, respectively) (d’Adda di Fagagna et al., 2003; Blackford and Jackson, 2017). Activated CHK1 promotes the degradation of CDC25A, a phosphatase that removes inhibitory modifications from cyclin dependent kinases (CDKs). ATM can phosphorylate p53 on multiple sites, including S15, which has been demonstrated to inhibit its interaction with the ubiquitin ligase MDM2, resulting in p53 stabilization and initiation of senescence (Blackford and Jackson, 2017). Upon SSB, poly-(ADP)ribose polymerase (PARP), predominantly PARP1, which senses the breaks, initiates DDR and probably induces p16-dependent cellular senescence (Nassour and Abbadie, 2016).

Senescent cells display a heterogeneous phenotype and induction of senescence is driven by multiple stressors. It is a highly coordinated and regulated process (Fig. 2). An overview of the main regulators and pathways of senescence is presented in the subsequent paragraphs.

### 3.2. Senescence associated secretory phenotype and the cGAS-STING pathway

Cytoplasmic chromatin fragments (CCFs) are fragments of nuclear chromatin generated in the process of autophagosomal-lysosomal degradation of the nuclear envelope in senescent cells (Ivanov et al., 2013; Dou et al., 2015). CCFs are γH2Ax and H3K27me3-positive, which suggests that the formation of CCFs is associated with the DDR response (Ivanov et al., 2013). Cytoplasmic DNA from CCFs activates cyclic GMP-AMP (cGAMP) synthase (cGAS) and its downstream effector STimulator of Interferon Genes protein (STING) to maintain the SASP secretome (Dou et al., 2017). Interleukin-8 (IL-8) and TGF-β are SASP components in senescent HSCs (Nguyen et al., 2020). C-X-C motif chemokine receptor 2 (CXR2), the receptor for IL-8, is essential for SASP-driven paracrine senescence (Acosta et al., 2008). TGF-β has been shown to trigger hepatocyte senescence in a model of acute liver injury (Ivanov et al., 2013). Cytoplasmic DNA from CCFs activates cyclic GMP-AMP (cGAMP) synthase- STimulator of Interferon Genes protein (cGAS-STING) to maintain the SASP (Hari et al., 2019).

The gut microbiome is known to modulate chronic liver diseases and carcinogenesis (Moreno-Gonzalez and Beraza, 2021). The intrahepatic immune microenvironment in the fibrotic liver is modulated by the gut microbiome by shaping the T-cell receptor immune repertoire (Liang et al., 2020). Deoxycholic acid, a secondary bile acid produced by gut microbiota, increases the number of senescent HSCs and augments SASP-induced hepatocellular carcinoma development in high fat diet-fed mice (Yoshimoto et al., 2013).

#### 3.3. NF-κB signaling

The hierarchy of the networks inducing the secretory phenotype is still unclear. Yet, the transcription of several SASP genes primarily depends on two transcription factors (TFs): Nuclear Factor-kappa B (NF-κB) and CCAAT/enhancer binding protein beta (C/EBPβ) (Chien et al., 2011; Acosta et al., 2008; Kuilmann et al., 2008).

The NF-κB transcription factor family consists of five proteins: p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1) and p100/52 (NF-κB2) that associate with each other to form distinct transcriptionally active homo- and heterodimeric protein complexes. In most cells, NF-κB dimers are sequestered in an inactive form in the cytosol through their interaction with IκB proteins. Degradation of these inhibitors upon their phosphorylation by the IκB kinase (IKK) complex leads to nuclear translocation of NF-κB dimers and induces transcription of their target genes (Ockenghaus and Ghosh, 2009). NF-κB activation includes two major signaling pathways: the canonical and the non-canonical NF-κB signaling pathways. The canonical pathway is induced by inflammatory stimuli including TNFα, IL-1 or LPS and uses a large variety of signaling adaptors to engage IKK activity. The non-canonical pathway depends on NIK (NF-κB-inducing kinase)-induced activation of IKKα. The canonical pathway mediates the activation of NF-κB1/p50, RelA/p65 and c-Rel, whereas the non-canonical NF-κB pathway selectively activates p100-sequestered NF-κB1, predominantly NF-κB2/p52 and RelB (Sun, 2017). Among all the dimers, the p50/65 heterodimer is the most abundant. The proportion of NF-κB dimers varies depending on the cell type. In addition, not all combinations of NF-κB dimers are transcriptionally active. Only p65, RelB and c-Rel contain carboxy-terminal transactivation domains that induce NF-κB dependent gene transcription. Homo- and heterodimers of p50 and p52 inhibit NF-κB dependent gene transcription via competition with other dimers to bind to the κB sites of genes (Ockenghaus and Ghosh, 2009).

As mentioned before, the DNA damage response is essential for the induction of senescence. NF-κB is a master regulator of the genotoxic stress-induced cell response and both of the canonical and the non-canonical pathway can be activated by the DNA damage response (McCool and Miyamoto, 2012). E.g., NF-κB-regulated gene expression increases in fibroblasts in response to UVA or UVB irradiation-induced DNA damage (Vile et al., 1995). Following DNA damage, ATM is activated and causes sustained NF-κB activation, independent of p53 signaling (Piret et al., 1999). In addition, the DNA damage response kinases ATM and ATR activate GATA4 which in turn activates NF-κB to promote cell senescence. PI3K, Phosphoinositide 3-kinase. GSK3β, Glycogen synthase kinase 3 beta. mTOR, The mammalian target of rapamycin. ROS, Reactive oxygen species. NF-κB, Nuclear factor kappa B. H₂S, Hydrogen sulfide. IL-22, Interleukin-22. STAT3, Signal transducer and activator of transcription 3. Alpha-1 AR, Alpha-1 adrenergic receptor. cGAS-STING, cyclic GMP-AMP synthase- STimulator of Interferon Genes protein.

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**Fig. 2.** The major pathways that regulate cellular senescence. PI3K, Phosphoinositide 3-kinase. GSK3β, Glycogen synthase kinase 3 beta. mTOR, The mammalian target of rapamycin. ROS, Reactive oxygen species. NF-κB, Nuclear factor kappa B. H₂S, Hydrogen sulfide. IL-22, Interleukin-22. STAT3, Signal transducer and activator of transcription 3. Alpha-1 AR, Alpha-1 adrenergic receptor. cGAS-STING, cyclic GMP-AMP synthase- STimulator of Interferon Genes protein.
facilitate senescence induction thus constituting a positive feedback loop (Kang et al., 2015). NF-κB and C/EBPβ are activated and enriched in the chromatin fraction in oncogene-induced senescent cells (Chien et al., 2011; Kuilman et al., 2008). NF-κB also showed increased DNA-binding activity in replicative-, irradiation- and chemotherapy-induced senescent cells (Chien et al., 2011; Freund et al., 2011; Jing and Lee, 2014; Jing et al., 2011). Depletion of the NF-κB subunit p65 reduced expression of many pro-inflammatory SASP factors in oncogene-induced senescence and irradiation-induced senescence (Chien et al., 2011; Freund et al., 2011). NF-κB directly controls transcription by binding to promoters of SASP components including IL-8, IL-6 and GM-CSF (Freund et al., 2011). C/EBPβ on the other hand is recruited specifically to the IL-6 promoter and C/EBPβ knockdown caused a collapse of the whole inflammatory network mainly due to disruption of IL-6 autocrine signaling (Kuilman et al., 2008). NF-κB and C/EBPβ are critical regulators of the SASP. In fact it seems that DDR and p38 signaling converge to NF-κB activation (Freund et al., 2011).

Sustained activation of NF-κB is required for the progression of senescence. In a mouse model of aging driven by Ercc1 deficiency and presenting as the failure to repair stochastic endogenous DNA damage, additional genetic depletion of p65 attenuates aging symptoms (Tilstra et al., 2016). In contrast, knockdown or pharmacological inhibition of NF-κB leads to increased fibroblast resistance to apoptosis, indicating that NF-κB activation contributes to the anti-apoptotic phenotype of senescent cells (Liberatore et al., 2009). In line with this, loss of Nfkβ1, one of the canonical NF-κB subunits, promotes nuclear translocation of the p65/p65 homodimer, leading to reduced apoptosis and increased accumulation of senescent cells with DNA damage (Bernal et al., 2014). In addition, NF-κB signaling is known to control the senescence-associated secretory phenotype and may play a key role in SASP-induced paracrine senescence (Lopes-Paciencia et al., 2019; Malaquin et al., 2016). In contrast, knockdown or pharmacological inhibition of NF-κB reduces the number of senescent cells (Tian et al., 2019; Xu et al., 2018).

3.4. PI3K-Akt pathway

The phosphatidylinositol-3-kinase (PI3K) pathway regulates a wide range of target proteins and Akt is one of the main downstream targets of PI3K. The PI3K-Akt axis modulates a variety of cellular process, including survival and proliferation and is also involved in cellular senescence. During induction of senescence, Akt phospho-Ser473 (pS473) and phospho-Thr308 (pT308) are increased and accompanied by increased P53 expression (Kim et al., 2017; Boichuck et al., 2019). Hyperactivation of the PI3K-Akt pathway via PTEN deletion or PIK3CA mutant expression induces cellular senescence in the absence of DNA damage (Aistle et al., 2012). Pharmacological inhibition of Akt by the PI3K inhibitor LY294002 reduces P21G1P1 expression and SA-β-gal positivity in fibroblasts (Kim et al., 2017).

Akt has a variety of substrates, including Glycogen Synthase Kinase 3 (GSK3), Forkhead box a (FOXO) family transcription factors, Tuberosis Sclerosis Complex 2 (TSC2) and mTORC1 (Manning and Toker, 2017). These downstream targets of Akt also influence cellular senescence in various ways. GSK3 is one of the main targets of Akt and is phosphorylated by active Akt at its two subunits GSK3α (Ser21) and GSK3β (Ser9) (Manning and Toker, 2017). Ser9 phosphorylated GSK3β increases stability of P21G1P1 post-translationally. Conversely, overexpression of a mutant GSK3β unable to phosphorylate Ser9 decreases P21G1P1 protein expression (Rossig et al., 2002). Inability to phosphorylate FOXO3a by Akt inhibits transcription of the antioxidative enzyme SOD2 and consequently promotes ROS production, leading to DNA damage and cellular senescence (Jinai et al., 2014). PI3K-Akt activation triggers mTOR and P53 activation (Aistle et al., 2012). Significant activation of Akt-mTOR is also observed in replicative senescence (Tan et al., 2016). Furthermore, SASP components are regulated by the Akt-mTOR pathway in premature senescent cells (Bent et al., 2016).

3.5. mTOR complexes

mTOR is a serine/threonine protein kinase belonging to the PI3K-related kinase (PIKK) family. It is composed of two different protein complexes mTORC1 and mTORC2. mTORC1 consists of three core components: mTOR, Raptor (regulatory protein associated with mTOR) and mLST8 (mammalian lethal with Sec13 protein 8, also known as G0B). mTORC2 also contains mTOR and mLST8 but contains Rictor (rapamycin insensitive companion of mTOR) instead of Raptor (Saxton and Sabatini, 2017). The two mTOR complexes regulate different cellular processes because they target different downstream kinases. mTORC1 promotes protein synthesis largely through the phosphorylation of two downstream kinases: p70S6 Kinase 1 (S6K1) and elf4E Binding Protein (4EBP) (Lipton et al., 2015; Morita et al., 2015). mTORC1 activates the sterol responsive element binding protein (SREBP) in response to low sterol levels to promote de novo lipogenesis (Porstmann et al., 2006). PGC1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha), a key regulator of mitochondria biogenesis is also regulated by mTORC1 (Summer et al., 2019). The mTORC2 complex mainly controls proliferation and survival, primarily by phosphorylating several members of the AGC (PKA/PKG/PKC) family of protein kinases (Saxton and Sabatini, 2017). The most well-documented role of mTORC2 is to activate Akt via phosphorylation of Ser473 (Sarbassov et al., 2005). Recent research also suggested a positive feedback loop between Akt and mTORC2 (Yang et al., 2015). Furthermore, Akt-dependent phosphorylation of specific kinases including FoxO1/3a and TSC2 is also required for mTORC2 activation (Saxton and Sabatini, 2017; Jacinto et al., 2006).

In senescent cells, mTORC1 signaling is dysregulated (Summer et al., 2019; Carroll et al., 2017). In normal cells, the removal of mitogenic cues such as growth factors and amino acids inactivates mTORC1. However, in senescent cells, mTORC1 acquires resistance to inactivation by nutrient starvation and growth factor removal (Carroll et al., 2017; Zhang et al., 2000). Sensitivity of mTORC1 to amino acids is increased in replicative senescent cells (Chen et al., 2010). This phenomenon is in line with evidence that the Ras-related small GTP-binding protein Rag is activated by amino acids and in turn activates mTORC1 (Saxton and Sabatini, 2017). Palmitate-induced lipotoxicity and accumulation of lipids is accompanied by increased expression of senescence markers, suggesting a link between lipogenesis and senescence (Inoue et al., 2017; Chang et al., 2018). It has also been demonstrated that high glucose-induced cellular senescence promotes intracellular lipid accumulation via the PI3K-Akt-mTOR pathway (Chen et al., 2019). In addition, mTORC1 directly regulates phosphorylation of p53 in PTEN-loss induced senescence (Jung et al., 2019). In contrast to the clear evidence of dysregulation of mTOR signaling in senescence, the results of mTORC1 inhibition to decrease senescence are inconsistent. Rapamycin has been reported to reduce oxidative stress-induced senescence by inhibiting SASP (Wang et al., 2017). However, although rapamycin and Torins decrease the number of SA-β-gal positive cells they fail to restore the proliferation ability of senescent fibroblasts (Leontieva and Blagosklonny, 2017). Knockdown of Raptor decreases phosphorylation of S6K and expression of senescence markers including P16INK4a and SA-β-gal (Ito et al., 2017). However, S6K and 4EBP1 activities seem to be dispensable in PTEN-loss induced senescence (Jung et al., 2019). The interpretation of the heterogeneous effects of mTORC2 on senescence may be hampered by the lack of detailed knowledge on the role of mTORC2 and senescence (Saxton and Sabatini, 2017; Schreiber et al., 2015; Sarbassov et al., 2006). Another layer of complexity in understanding the role of mTOR in senescence is the role of mTOR in regulating autophagy (see next section).

3.6. Autophagy

Autophagy is an evolutionary conserved, dynamic process that involves the scavenging of intracellular components and facilitates the
Hydrogen sulfide is one of various gasotransmitters that regulate a variety of cellular processes. The antioxidant effect of H_{2}S has been extensively investigated. The desulfhydration of cysteine is the major source of H_{2}S in mammals. The process is catalyzed by two pyridoxal-5'-phosphate- (PLP-) dependent enzymes, cystathionine β-synthase (CBS) and cystathionine γ-lyase (CSE) (Xie et al., 2016). H_{2}S improves redox homeostasis in various ways. In physiological conditions, the concentration of H_{2}S is in the submicromolar range and not sufficient to serve as a direct reductant (Xie et al., 2016). It is speculated that H_{2}S can be a direct scavenger of ROS at much higher concentrations, e.g., by exogenous administration of H_{2}S or chemical H_{2}S donors (Lan et al., 2011). H_{2}S producing enzymes CSE and CBS can be upregulated by long-term supplementation of H_{2}S donors in vivo (Wu et al., 2017). H_{2}S increases glutathione biosynthetic pathways, increases the cellular glutathione pool and decreases homocysteine levels (Parsanathan and Jain, 2018). Furthermore, increased H_{2}S levels promote post-translational sulhydrylation of KEAP1 to facilitate the release of NRF2, which is a transcription factor that increases transcription of various antioxidant genes (Corsello et al., 2018).

It has been demonstrated that H_{2}S production is increased during activation of hepatic stellate cells and increases the bioenergetics of mitochondria (Damba et al., 2019). Genetic ablation of CSE in mouse embryonic fibroblasts stimulates cellular senescence by increasing oxidative stress. In these fibroblasts, H_{2}S production is significantly reduced (Yang et al., 2013). Decreased levels of NAD^{+} cause senescence and aging (Nacarelli et al., 2019). Supplementation of exogenous H_{2}S restores intracellular NAD^{+} level and induces SIRT1 expression to reduce senescence (Das et al., 2018). SIRT1 is a member of the mammalian class III histone deacetylases and regulates PGC-1α activity in mitochondrial biogenesis (Tang, 2016). Autophagic degradation of SIRT1 is associated with cellular senescence (Xu et al., 2020). In addition, H_{2}S has been shown to modulate various kinases involved in senescence (Lee et al., 2018). This evidence suggests that H_{2}S regulates cellular senescence via various mechanisms.

3.8. STAT3 pathway

STAT3 is a member of the STAT (Signal Transducers and Activators of Transcription) family that has DNA binding activity and stimulates expression of innate immune mediators in the liver in response to interferon (Hillmer et al., 2016). It has been demonstrated that numerous cytokines and growth factors including IL-6, IL-10 family members, G-CSF (Granulocyte colony-stimulating factor) and growth factors that act through protein tyrosine kinase receptors (e.g. epidermal growth factor) are capable to activate STAT3 (Hutchins et al., 2013; Nguyen-Jackson et al., 2012; Lo et al., 2005). In addition to its transcriptional activities, non-transcriptional functions of STAT3 have been described as well. Mitochondrial STAT3 associates with mitochondria and regulates electron transport chain function as well as glycolysis and oxidative phosphorylation independent of its transcriptional action (Wegrzyn et al., 2009). Furthermore, STAT3 directly interacts with EIF2AK2 (Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2) to inhibit autophagy (Niso-Santano et al., 2013).

STAT3 is activated by extracellular inflammatory signals derived from SASP and regulates senescence. IL-6 is one of the well-established SASP components that regulates cellular senescence via activation of STAT3 (Kojima et al., 2013). IL-22, a member of the IL-10 family, induces hepatic stellate cell senescence via phosphorylation of STAT3 and upregulation of SOCS3 (suppressor of cytokine signaling 3) (Kong et al., 2012). IL-10 is also able to promote hepatic stellate cell senescence via STAT3 (Huang et al., 2018, 2020). Oxidative stress-induced senescence is accompanied by hyperactivity of the STAT3 pathway and disruption of the IL-6–STAT3 axis reduces cellular senescence (Waters et al., 2019). In addition, STAT3 interacts with mTOR to negatively regulate autophagy during cellular senescence (Yang et al., 2019a).
Adrenergic receptors (AR) are a family of transmembrane receptors coupled to G-proteins. Three types have been identified: alpha-1, alpha-2 and beta, each one characterized by its structure and function (Strosberg, 1993). Alpha-1 AR is coupled to the Gq protein and regulates PKC activation via diacylglycerol synthesis as well as calcium fluxes via PLC signaling and IP3 generation (Suzuki et al., 1990; van Bremen and Saida, 1989; Burch et al., 1986; Han et al., 1990; Wilson and Minneman, 1990). Previous studies demonstrated an important relationship between intracellular calcium levels and senescence, which could be related to the alpha-1 AR response. It has been demonstrated that calcium levels increase in response to different inducers of senescence inducers like telomere shortening and oxidative stress. This dysregulation of intracellular calcium homeostasis was linked to the PLC/IP3/IP3R pathway in human mammary epithelial cells during the induction of senescence (Wiel et al., 2014). In addition, the use of BAPTA-AM, a calcium chelator, reduces the number of cells with a senescent phenotype related to the alpha-1 AR response. It has been demonstrated that calcium-dependent enzyme, coupled to G-proteins. Three types have been identified: alpha-1, alpha-2 and beta, each one characterized by its structure and function (Borodkina et al., 2016). Calmodulin (CaM), a calcium-dependent protein, is required for G1 transit by regulating the activity of calcineurin a Ca2+/-CaM-dependent protein, which increases the synthesis of cyclin D1 in fibroblasts (Kahla and Means, 2004). Thus, Ca2+ appears to be important for both cell proliferation and senescence and its precise effects depend on the intracellular concentration and location which is associated with the activation and regulation of alpha 1 AR. Protein kinase C is part of the family of serine/threonine protein kinases and can modulate cell cycle progression (Nishizuka, 1984). PKCα has been related to cell cycle arrest during G1 phase, because it induces the phosphorylation of p21 at Ser146 and the dephosphorylation of Thr160 of Cdk2 in keratinocytes (Kashivagi et al., 2000). PKCα also showed effect on the cell cycle during the transition from G1 to S phase, by decreasing the expression of cyclin D1 (Detjen et al., 2000; Hizli et al., 2006) and increasing the expression of p21 (Detjen et al., 2000; Tibudan et al., 2002; Clark et al., 2004). PKCα is also able to promote cellular senescence in TIG-1 cells, increasing the activity of β-galactosidase (Katakura et al., 2009). However, in addition to the role of some PKC isoforms in senescence, PKC isoforms are also important for cell growth. PKCα has growth-stimulatory effects which have been observed in different cell types including hepatocytes (Liu et al., 1997; Alisi et al., 2004). Apparently, the effect of protein kinase C depends mainly on the isoform as well as the cell type in which it is present. Beta ARs are coupled to the Gs protein and regulate cAMP production and protein kinase A activation. It has been demonstrated that the β-AR agonist isoproterenol promotes proliferation of U251 cells, and this effect was inhibited by propranolol, a selective antagonist of β-AR. However, the mechanism of the antagonism of beta AR has not been fully elucidated yet (He et al., 2017). Moreover, it has also been demonstrated that propranolol decreases the proliferation of breast cancer cells by increasing expression of p53, reducing expression of bcl-2 and increasing apoptosis (Montoya et al., 2019). In hepatic stellate cells, activation of ARs is mainly related to proliferation (Oben et al., 2003). This proliferative effect is mainly linked to the activation of alpha-1 AR and the downstream response involves PI3K and the Erk family of mitogen activated protein kinases. In addition, hepatic stellate cells are able to produce and release catecholamines, including norepinephrine, which is important for autocrine stimulation of their proliferation (Oben et al., 2003). Doxazosin, a selective alpha1-AR antagonist, has antifibrotic properties in the liver by reducing the deposition of the extracellular matrix and reduction of the stellate cell marker α-smooth muscle actin (Serna-Salas et al., 2018). However, whether doxazosin has a direct effect on HSCs via alpha 1 AR remains to be elucidated. We hypothesize that the antagonism of adrenergic receptors may be linked to the resolution of fibrosis via the induction of senescence of HSCs since decreased collagen production and decreased proliferation have been observed in senescent HSCs.

4. Consequences of cellular senescence in liver fibrosis

In the CCl4 (carbon tetrachloride) model of liver fibrosis, senescent cells mainly derive from activated hepatic stellate cells (Krizhanovsky et al., 2008). Activated hepatic stellate cells that undergo replicative senescence exhibit a less fibrogenic phenotype and are prone to spontaneous apoptosis (Schnabl et al., 2003). Induced premature senescence of aHSCs decreases fibrogenesis in vivo (Krizhanovsky et al., 2008; Kong et al., 2012; Jin et al., 2016). Senescent HSCs express cell surface ligands for receptors on NK cells that promote the elimination of senescent HSCs (Jin et al., 2017). It has been suggested that the SASP of senescent HSCs initiates cellular senescence of surrounding non-senescent HSCs (Lujambio et al., 2013; Sugihara et al., 2018). Interleukin-22 has been demonstrated to induce hepatic stellate cell senescence and alleviate liver fibrosis (Kong et al., 2012). It has also been verified that the bioactive compound curcumin attenuates liver fibrosis via inducing hepatic stellate cell senescence, in a PPAR-γ/p53-dependent manner (Jin et al., 2016, 2017). These data underscore the biological rationale of therapy-initiated HSC senescence as a strategy to treat liver fibrosis. Excessive numbers of senescent cells have a significant impact on the microenvironment and tissue homeostasis. The chronic secretion of SASP factors by persistent senescent cells may promote paracrine senescence, disrupt tissue homeostasis and cause infiltration of inflammatory cells, exacerbating local inflammation (Aravintan and Alexander, 2016). It should be also noted that persistent existed senescent HSCs may promote tumorigenesis, strategies that eliminate senescent HSCs should be considered following therapy-induced senescence.

Senescence of hepatocytes has been observed in various liver diseases including chronic viral hepatitis, alcohol-related liver disease, non-alcoholic fatty liver disease (NAFLD) and genetic haemochromatosis (Aravintan and Alexander, 2016). Replicative senescence of hepatocytes has been described in both normal and fibrotic liver. In fibrotic liver, the number of senescent cells is higher than in non-fibrotic liver (Paradis et al., 2001). In biopsies of NAFLD patients, P21GIP1-positive hepatocytes are present and correlate with the grade of fibrosis (Richardson et al., 2007). It has been reported that lipid accumulation in hepatocytes induces senescence and that senescent hepatocytes promote the activation of HSCs via an Nrf2-dependent mechanism (Yu et al., 2021). Therefore, measurement of senescence markers in total liver may not be very informative since the consequences of senescence for fibrosis is dependent on the cell type undergoing senescence. Apart from direct injury or aging, cytokine-induced senescence of hepatocytes appears to be the most important cause of senescence in hepatocytes (Kandhaya-Pillai et al., 2017; Wan et al., 2014). Hepatocyte senescence is involved in the pathogenesis of chronic liver diseases. TGF-β receptor 1 inhibitor reduces P21-dependent hepatocyte senescence and restores liver regenerative ability in a mouse model of acute liver injury (Bird et al., 2018). Hepatocyte-specific senescence promotes intracellular fat accumulation in hepatocytes in experimental NAFLD models and positively correlates with the grade of steatosis (Ogrodnik et al., 2017). The mechanism of increased lipid accumulation in senescent hepatocytes is associated with mitochondrial dysfunction that occurs during aging (Ogrodnik et al., 2017; Ogrodnik and Jurk, 2017). Likewise, extrahepatic adipoocyte senescence correlates with adipocyte hypertrophy of visceral adipose tissue and obesity-induced metabolic dysfunction (Palmer et al., 2019). These results demonstrate that accumulation of senescent cells correlates with metabolic dysfunction. However, it should be considered that the phenotype of senescent hepatocytes may vary depending on the inducers of senescence. In contrast to diet-induced senescence, senescent hepatocytes in chronic alcohol-induced injury appears to be more resistant to apoptosis and steatosis (Wan et al., 2014). Hence, in view of the long-term disadvantages of senescence, the induction of senescence in non-HSC liver cell types should be considered and, if possible, avoided.

Since the accumulation of senescent cells seems to favor progression of aging-related disorders, the specific removal of senescent cells may
help to attenuate these symptoms and increase life-span. Senolytics represent a novel class of compounds that induce selective death of senescent cells (Pignolo et al., 2020). Dasatinib (D) in combination with quercetin (Q) were the first described senolytic drugs (Zhu et al., 2015; Khosla et al., 2020). The dasatinib and quercetin (D + Q) were shown to reduce p21, PAI-1 and BCL-xl of senescent cells (Zhu et al., 2015). Several studies demonstrated that the combination of dasatinib and quercetin reduce the number of senescent cells with high specificity and efficiency (Ogrodnik et al., 2017; Palmer et al., 2019; Zhu et al., 2015; Sierra-Ramirez et al., 2020). In a mouse model of diet-induced hepatic steatosis, administration of D + Q decreases the number of senescent hepatocytes and ameliorates the severity of NASH (Ogrodnik et al., 2017). Likewise, the BCL-2 family inhibitor ABT-737 reduces P21 expression and the SASP, demonstrating its potential for senotherapy (Hari et al., 2019). In addition, elimination of senescent hepatocytes has been demonstrated to improve liver regeneration and decrease the expression of P21 and p53 H3K27 demethylases may also be a promising strategy to induce HSC senescence and reduce fibrosis (Jiang et al., 2021). In addition, inhibition of p16 and p53 in vitro may not only induce senescence and also do not avoid the generation of senescent cells (Khosla et al., 2020). A potential harmful effect of the removal of senescent cells may be the impairment of wound repair (Kowald et al., 2020). Therefore, intermittent administration of senolytics may be required to improve their advantageous effect and decrease their disadvantageous effect. In addition to senolytics, preventing generation of senescent cells may induce unwanted effects as well. Genetic ablation of P21 in the liver enhances DNA damage, cholestasis and carcinogenesis (Ehedego et al., 2015). The effect of hepaticocyte senescence in carcinogenesis is bidirectional. Hepatocyte senescence is conserved as a tumor-suppressing mechanism because of the accompanying permanent proliferation arrest (Papatheodorid et al., 2020). On the other hand, senescent hepatocytes that escape from the senescent state and re-enter the cell cycle could be pre-malignant (Chan and Narita, 2019). Mitochondrial ROS generation is associated with mitochondrial dysfunction and is a driver of cellular senescence. Therefore, improvement of mitochondrial function could be a strategy to inhibit cellular senescence. Histone deacetylase (HDAC) inhibitors increase the expression of mitochondrial genes, reduce CCF formation and restore mitochondrial function. Therefore, HDAC inhibitors could be potent anti-senescent drugs (Vizioli et al., 2020). Furthermore, it was demonstrated that inhibition of the histone H3K27 methyltransferase Ezh2 induced senescence and apoptosis of HSCs in vitro and reduced collagen accumulation in vivo in a model of fibrosis, indicating that epigenetic modifications via methyltransferases and demethylases may also be a promising strategy to induce HSC senescence and reduce fibrosis (Jiang et al., 2021). In addition, inhibition of TGF-β signaling blocks paracrine senescence and restores liver regenerative ability, revealing another potential target for anti-senescent strategy (Bird et al., 2018).

As mentioned previously, senescent hepatocytes promote HSC activation (Yu et al., 2021). The effect of senescent HSCs on hepatocytes is unknown and remains an interesting topic to be studied. Senescent HSCs interact with macrophages to create a tumor-suppressing microenvironment (Lujambio et al., 2013). IL-10 and IL-22 have been shown to induce senescence of HSCs. The induction of senescence by IL-10 requires intact p53 and Stat3 signaling (Kong et al., 2012; Huang et al., 2020). Interestingly, induction of HSC senescence via a p53-dependent mechanism by miRNA-145 has also been reported (Yang et al., 2019b). Senescent HSCs promote recruitment of NK cells in the liver (Jin et al., 2017). Whereas infiltrating B-lymphocytes inhibit senescence of HSCs, they exacerbate fibrogenesis and maintain a tumorogenic inflammatory microenvironment (Faggioni et al., 2018). These data suggest that the immune microenvironment of liver cells is to a large extent determined by the interaction of senescent HSCs and (infiltrating) immune cells.

Senescent liver sinusoidal endothelial cells (LSECs) have been demonstrated as the major p16 positive cells in aging mice (Grose et al., 2020). Although the role of senescent LSECs in the pathogenesis of liver fibrosis is not elucidated, elimination of senescent LSECs in 18-month old mice induces deregulation of blood-vessel permeability and subsequent fibrosis because the LSECs lose the capacity of self-renewal. These results indicate a potential side-effect of senolytics in conditions of deficient regenerative capacity. Urokinase-type plasminogen activator receptor (uPAR) is identified as a cell surface marker of senescent cells in fibrotic liver. Chimeric antigen receptor (CAR) T cells that target uPAR efficiently reduce the number of senescent cells and alleviate fibrosis (Amor et al., 2020). Extrahepatic senescent adipocytes accumulate in conditions of obesity and are associated with metabolic dysfunction. Clearance of senescent adipocytes with dasatinib and quercetin improve glucose homeostasis and insulin sensitivity. Interestingly, increased adipogenesis was observed after the administration of D + Q. This effect was not entirely unexpected, since senolytics improve the proliferative and differentiation potential of adipocyte progenitors (Palmer et al., 2019). In view of the varying results obtained with senolytics, the cell-type specificity and regenerative capacity may be the key factors that determine the outcome of senolysis (Table 1).

5. Conclusions and future perspectives

HSC activation is involved in the pathogenesis of liver fibrosis and their induction into senescence seems to reduce the fibrogenic phenotype. Senescent HSCs can acquire genetic changes, e.g. gene repair

| Model | Species | Inducer/inhibitor | Phenotypic changes | Reference |
|-------|---------|-------------------|--------------------|-----------|
| HSCs, in vitro aging | Human | Culture-induced senescence | Inflammation and stress-related genes; apoptosis; ECM proteins; p21; pH2AX; telomere length ↓ | Schnabl et al., 2003; Odagiri et al., 2019 |
| CCL4-induced fibrosis | Mouse | Fibrosis-induced senescence | ECM proteins; ECM-degrading proteins; | Krizhanovsky et al., 2008; Lujambio et al., 2013 |
| HSCs |Mouse| IL10; IL-22| SA-β-Gal; p53| Kong et al., 2012; Huang et al., 2018, 2020 |
| CCL4-induced fibrosis |rat | P35/Stat-3-dependent | SA-β-Gal; p21; p53; Acta2; fibrosis markers | Yang et al., 2019b |
| HSCs, HSC cell lines | Mouse; human; rat | miRNA-145; p53-dependent | SA-β-Gal; cell cycle arrest; | Jiang et al., 2021 |
| HSCs, HSC cell lines; in vitro CCL4 | Human; mouse | Inhibition of H3K27 methyltransferase Ezh2 | SA-β-Gal; cell cycle arrest; | Yang et al., 2019b |
| HSC cell line; rat in vivo CCL4 model | Human; rat | Curcumin; p53/PPAR-γ-dependent | SA-β-Gal; p21; p16; NK cell receptors; ECM proteins | Jin et al., 2016, 2017 |
resulting from the DNA damage response as well as epigenetic changes, e.g. activation of facultative heterochromatin and histone modification, that profoundly alter their cellular signaling pathways and intracellular metabolic processes. E.g., both increased as well as decreased autophagy are involved in senescence induction. mTOR inhibitors failed to reverse all aspects of the senescent phenotype, suggesting a complex regulation of signaling pathways leading to senescence.

Evidence that promoting HSC senescence reduces fibrogenesis is the rationale for exploring induction of senescence as a strategy for the treatment of liver fibrosis. However, accumulation of senescent hepatocytes may lead to hepatic dysfunction. This potential detrimental consequence requires a comprehensive evaluation of the effects of inducing HSC senescence in the liver as a therapeutic strategy. Therefore, targeting of senescence-inducing drugs is essential but remains a challenge. Effective, reproducible and specific targeting of compounds to activated HSCs has not been reported yet, although some progress has been made, e.g. targeting of IL-10 to activated HSCs (Rachmanawati et al., 2007). IL-10 might be a promising candidate to induce senescence of activated HSCs. Other candidate targets to induce senescence may be (downstream) targets of p53 and epigenetic modifications via targeting methyltransferases/demethylases. Furthermore, senescent HSCs need to be removed to exclude unwanted long-term effects of accumulated senescent HSCs. Another issue is the duration of senescence-induced therapy for fibrosis. Partial permanent induction of senescence in activated HSCs may hamper the normal wound-healing response in the liver and/or the maintenance of the normal extracellular matrix which is essential to preserve liver architecture and function. The interaction between senescent HSCs and immune cells, including macrophages, lymphocytes and NK cells, demonstrates that the elimination of senescent HSCs is determined to a large extent by immune cells. This indicates that maintaining the proper micro-environment in the liver is key to the success of immune clearance of senescent HSCs. For this reason, intermittent pro-senescence therapy might be combined with senolitics in order to minimize the disadvantages and detrimental effects of accumulated senescent cells. However, more studies should be conducted to evaluate the potential of such combination of pro- and anti-senescence interventions in liver fibrosis.

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