Hepatic senescence, the good and the bad

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Abstract
Gradual alterations of cell’s physiology and functions due to age or exposure to various stresses lead to the conversion of normal cells to senescent cells. Once becoming senescent, the cell stops dividing permanently but remains metabolically active. Cellular senescence does not have a single marker but is characterized mainly by a combination of multiple markers, such as, morphological changes, expression of cell cycle inhibitors, senescence associated β-galactosidase activity, and changes in nuclear membrane. When cells in an organ become senescent, the entire organism can be affected. This may occur through the senescence-associated secretory phenotype (SASP). SASP may exert beneficial or harmful effects on the microenvironment of tissues. Research on senescence has become a very exciting field in cell biology since the link between age-related diseases, including cancer, and senescence has been established. The loss of regenerative and homeostatic capacity of the liver over the age is somehow connected to cellular senescence. The major contributors of senescence properties in the liver are hepatocytes and cholangiocytes. Senescent cells in the liver have been implicated in the etiology of chronic liver diseases including cirrhosis and hepatocellular carcinoma and in the interference of liver regeneration. This review summarizes recently reported findings in the understanding of the molecular mechanisms of senescence and its relationship with liver diseases.

Key words: Senescence; Senescence associated secretory phenotype; Hepatocyte; Cholangiocyte; Hepatic stellate cell; Cell cycle arrest; DNA damage

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INTRODUCTION

Definition of senescence
In response to endogenous and exogenous stress, cells in various tissues may enter into a permanent cell cycle arrest. These cells cannot proliferate but remain metabolically active for an extended period of time and are thus termed as senescent cells. Conversion of normal cells into senescent cells occurs throughout the life-time of an organism and plays important roles in tissue homeostasis[1]. In 1961, Leonard Hayflick observed that a human fibroblast cell ceased replication after forty to sixty divisions. As a result of Hayflick’s experiments the limited capacity for cellular division in cell culture was termed as the “Hayflick Limit”. Later it was found that the cause of the Hayflick Limit was the shortening of telomeres, portions of DNA at the ends of chromosomes, which slowly erode as cells replicate.

Hayflick and Moorhead first introduced the term senescence to describe the limited proliferation ability of human fibroblasts in cell culture. However, cells with senescent properties have been discovered in vivo in many types of tissues and the number of senescent cells increases with age[1-6]. Initially senescence was thought to be an artifact in tissue culture with no relevance to the physiology and pathology of an organism. But subsequent studies have proved the importance of senescence in the biological processes, such as embryonic development, tissue repair, tumor suppression and aging[7]. Senescence is now considered as a multistep, dynamic cellular process. When cells are stimulated by senescence-inducing signals, such as oncogene activation, DNA damage, or other stress-mediated signals, they undergo cell cycle arrest or senescence initiation. In the next step, cells with cell cycle arrest undergo chromatin remodeling, present senescence-associated secretory phenotype (SASP), change morphology and gain other characteristics of a full-fledged senescence phenotype. Senescent cells can persist for months[8]. When senescence occurs due to oncogene activation, cell cycle arrest first happens in an autocrine manner, but SASP factors can induce paracrine senescence in other cells at a late stage after the immune system is activated[9,10], thus spreading senescence to neighboring cells across an organ.

There are two hypotheses to explain whether cellular senescence is beneficial or detrimental to an organism. We can consider senescence as a tumor suppressive or anti-cancer process as the senescent cells cannot divide. Therefore, senescence may be good for an organism. On the other hand, cellular senescence may cause loss of regenerative capability of an organ such as the liver. In this respect, it is considered a deleterious process for an organism as it may affect the function and tissue renewal[11]. In either case, more in vivo studies on the mechanisms are needed.

Characteristics of senescence
The senescence phenotype is very stable, unresponsive to mitogenic stimuli and resistant to apoptosis[11]. The telomere dysfunction in normal cells can lead to the conversion to replicative senescent cells. Important stimuli like oncogene over-activation, global DNA damage, and oxidative stress may act individually or synergistically to trigger senescence in normal cells[12]. When cells undergo senescence, they are characterized by alterations in morphology, lysosomal activity and gene-expression. These include expression of cell cycle inhibitors, such as p15INK4B, p16INK4A, and p21Cip1, activation of DNA damage response, alterations of chromatin structures and induction of SASP. Usually senescent cells exhibit enlarged, flat morphology and are frequently multi-nucleated[13]. For example, the report from Aravinthan et al[14] showed that senescent hepatocytes, which overexpress p21Cip1, can be characterized with larger nuclei, compared to non-senescent hepatocytes.

Senescent cells express a higher level of lysosomal β-galactosidase gene (GLB1) and show an increased β-galactosidase activity at a suboptimal pH of 6.0 (optimal pH
level is 4.4-5), compared to normal cells\textsuperscript{15,19}. Of note, as a single cell based assay, the β-galactosidase activity assay is the most commonly used analysis for the senescence property of cells in vitro or in vivo\textsuperscript{20}. The cumulative effect of various stresses on the small population of affected cells may lead to conversion of a homogenous population of senescent cells\textsuperscript{21}.

**SASP and senescence**

Senescent cells can secrete a large number of proinflammatory cytokines, chemokines, growth factors and proteases that may have a variety of effects on neighboring cells. This phenotype of senescent cells is known as senescence-associated secretory phenotype (SASP). Senescent cells are highly secretory and they execute a diverse set of functions mediated by SASP\textsuperscript{8,9}. As senescent cells are viable and actively secreting cells, they have a profound influence on the surrounding cells. SASP affects senescent cells and their microenvironments in both autocrine and paracrine fashions. SASP factors are highly dynamic in expression and the composition changes over the period of senescence. This change in the composition of SASP in senescent cells may determine whether the effect is beneficial or harmful in the respective organs\textsuperscript{22}. The mechanisms of the initiation and regulation of SASP and its influence in inflammation and disease processes have not been fully elucidated yet.

**SIGNALING PATHWAYS IN SENESCENCE**

The reactive oxygen species (ROS) is considered as one of the main activators of senescence cells\textsuperscript{15,19}. The imbalanced ROS may cause oxidative stress and DNA damage, leading to the activation of DNA damage response in the affected cells.

**Signaling pathways that control cell cycle arrest**

The p53 and Rb signaling pathways may become activated in response to extrinsic and intrinsic stressors, including DNA damage. p53 and Rb can affect transcriptionally both upstream regulators and downstream effectors in the senescence pathway\textsuperscript{20}. The inactivation of either p53 or Rb prevents or significantly delays senescence\textsuperscript{20} (Figure 1). ATM and ATR, as well as their downstream targets, Chk1 and Chk2, become activated during DNA damage, which in turn activate p53. Activated p53 is considered as one of the main players in converting normal cells to senescent cells. The phosphorylated form of p53 is the essential contributor of replicative senescence in human fibroblast\textsuperscript{8,9}. Acetylation of p53 at Lys161/Lys162 is also important for cell cycle arrest and senescence\textsuperscript{16}. Once p53 is activated by posttranslational modification, it in turn activates its transcriptional target, p21\textsuperscript{cip1} (CDKN1A). p21\textsuperscript{cip1} is the inhibitor of Cyclin E/CDK2 complex and it promotes cell cycle arrest at G1/S phase of cell cycle (Figure 1). Of note, p21\textsuperscript{cip1} can be activated by both p53-dependent and p53-independent mechanisms\textsuperscript{20}.

Retinoblastoma protein (Rb) is a tumor suppressor and a well-established cell cycle regulator. Rb is expressed in all tissues and controls cell cycle progression through interactions with the E2F family of transcription factors\textsuperscript{20,26}. Other Rb family members, namely, p107 and p130, also play active and important role in driving senescence independently of Rb\textsuperscript{20,22}. p21\textsuperscript{cip1} has been found to be both a positive and negative regulator of Rb by regulating either Rb phosphorylation or degradation, respectively\textsuperscript{20}. p16\textsuperscript{ink4a} (CDKN2A) inactivates Cyclin-dependent kinases, which phosphorylate Rb, and Rb phosphorylation status in turn has an impact on the expression of p16\textsuperscript{ink4a}. In normal cells, Cyclin D and Cyclin E bind to CDK2 and CDK4/6, respectively, since p21\textsuperscript{cip1} and p16\textsuperscript{ink4a} level remain at the basal level. Hyperphosphorylation of Rb permits the E2F factor to participate in the production of replication proteins and hence cell cycle progress. But a higher level of either p16\textsuperscript{ink4a} and/or p21\textsuperscript{cip1} can keep Rb in the hypophosphorylated state so that E2F remains bound with it. Therefore, cell cycle is arrested, which ultimately lead to cellular senescence (Figure 1).

Another cell cycle inhibitor, CDKN3 is a phosphatase and dephosphorylates Rb thus ensuring that cell cycle progression is blocked. Thus, CDKN3 is considered as an important contributor to cellular senescence\textsuperscript{27,29,30}. CDKN3 can also interact with Mdm2 and form a complex with p53 and Mdm2. Once the complex is formed, p53 losses its ability to induce p21\textsuperscript{cip1}\textsuperscript{30,31}.

G1-phase cell cycle arrest can also be induced by transforming growth factor-beta (TGFβ)\textsuperscript{31,32}. TGFβ can keep Rb in hypophosphorylated state, suggesting that it may suppress Rb phosphorylation and thereby interfering with cell cycle progression\textsuperscript{32}. p15\textsuperscript{ink4b} (CDKN2B), a homologue of p16\textsuperscript{ink4a}, is activated by TGFβ, acts on CyclinD-CDK4/CDK6 complex and contributes to cell cycle arrest, and hence to senescence\textsuperscript{15} (Figure 1). Since p15 \textsuperscript{ink4b} and p16 \textsuperscript{ink4a} have a common mode of action, in a tissue...
Both external and internal stresses can induce DNA damage and the activation of p16\(^{INK4A}\) and/or p15\(^{INK4B}\). DNA damage can activate p53 ataxia telangiectasia mutated (ATM) and ATM and RAD3-related (ATR) pathway. Activated p53 induces p21\(^{Cip1}\) expression. Expression of p21\(^{Cip1}\) can also be regulated by p53-independent mechanisms. The Cyclin dependent kinases activate Rb but are inhibited by p15\(^{INK4B}\)/p16\(^{INK4A}\) and p21\(^{Cip1}\) which leads to cell cycle arrest and senescence. The senescent cells express senescence-associated secretory phenotype (SASP). The SASP factors may induce senescence in neighboring cells in a paracrine fashion. ATM: Ataxia telangiectasia mutated; SASP: Senescence-associated secretory phenotype.

where either one does not express, the other can possibly compensate the functionality.

**Signaling pathways that regulate SASP**

The connection between senescent cells and the immune system is mediated by SASP. The inflammation signature of SASP include chemokines, cytokines and other immune modulators. SASP is regulated at multiple levels such as, chromatin modification, transcription, translation, mRNA stability and secretion\(^{[34]}\). The autocrine and paracrine positive feedback loops also regulate the signaling of SASP. Most senescent cells express multiple cytokines such as IL-8, CCL2 (MCP-1), CCL7 (MCP-3), CCL8 (MCP-2), CCL13 (MCP-4), CCL3, CCL16, CXCL1, CXCL2, CXCL3 \(ef^{[35,36]}\). The most characterized SASP components include multiple pro-inflammatory cytokines, such as interleukin-1 (IL-1), and interleukin-6 (IL-6). IL-1 and IL-6 produced in senescent epithelial and fibroblast cells are capable of inducing cellular senescence in adjacent cells. SASP helps spreading senescence phenotype among nonsenescent cells by creating an inflammatory environment in tissues. The knock-down of IL-6 and IL-8 receptors IL-6R and CXCR2, respectively, prevents senescence\(^{[34]}\).

SASP is induced and regulated by several signaling pathways leading to the activation of the nuclear factor-xB (NF-xB) and/or CCAAT/enhancer-binding protein-\(\beta\) (C/EBP\(\beta\)). In senescent cells, activated NF-xB and C/EBP\(\beta\) regulate the expression of SASP factors by controlling, mainly at the transcriptional level, the inflammatory SASP molecules, IL-1\(\alpha\), IL-6 and IL-8. These inflammatory SASP factors can enhance SASP signaling through activating NF-xB\(\alpha\), and C/EBP\(\beta\) in an autocrine feed-forward fashion\(^{[37,38]}\) (Figure 2).

**NOTCH signaling pathway:** This pathway has been implicated as an important regulator of SASP. Works from Hoare et al\(^{[39]}\) suggested a global upregulation of
Figure 2  Senescence-associated secretory phenotype signaling pathways. Nuclear factor kappa light chain enhancer of activated B cells can be activated via multiple signaling pathways such as the GATA binding protein 4, cyclic GMP-AMP synthase-stimulator of interferon genes, and nicotinamide adenine dinucleotide -nicotinamide phosphoribosyltransferase NAD+-NAMT pathways, which lead to the expression of senescence-associated secretory phenotype (SASP) proteins. SASP can be positively regulated through C-X-C motif chemokine receptor 2, or negatively by NOTCH via CCAAT-enhancer-binding proteins. SASP can induce senescence in both autocrine and paracrine manners. SASP can be anti-tumorigenic in the early phase of senescence, but can be pro-tumorigenic in the late phase of senescence. NF-κB: Nuclear factor kappa light chain enhancer of activated B cells; GATA4: GATA binding protein 4; cGAS-STING: Cyclic GMP-AMP synthase-stimulator of interferon genes; SASP: Senescence-associated secretory phenotype; CXCR2: C-X-C motif chemokine receptor 2; C/EBP: CCAAT-enhancer-binding protein.

NOTCH1 accompanied by dynamic alterations of its downstream activity in senescence. They also proposed NOTCH1 as a master regulator of SASP composition via a temporal and functional switch between two different secretomes, TGFβ and pro-inflammatory cytokines through down-regulation of C/EBPβ.

The NOTCH1 was shown to be upregulated in cells undergoing oncogene-induced senescence (OIS), which caused upregulation of TGFβ in the first phase of senescence. The inhibition of NOTCH signaling substantially reduced TGFβ induction in OIS cells. The inhibition of TGFβ in NOTCH1-driven senescent cells prevented the upregulation of the TGFβ-targeted cell cycle inhibitor, p15INK4b. In addition, Hoare et al[39] found that NOTCH1 negatively regulated C/EBPβ but had no effect on NF-κB pathway in the later phase of senescence. The overexpression of activated Notch1 receptors (N1ICD - NOTCH1 intracellular domain) inhibited the ability of C/EBPβ to induce IL-1, IL-6 and IL-8[40]. Moreover, Hoare et al[39] found that inhibiting Notch signaling accelerated clearance of senescent cells in the liver. Therefore, NOTCH signaling pathway may be associated with a complicated mechanism of SASP regulation. But the key questions that remain to be addressed are: (1) Which factor(s) involved in the switching from NOTCH1-TGFβ to NOTCH1-C/EBPβ; (2) What the cross-talk is between NOTCH1-mediated C/EBPβ and NF-κB regulation in senescent cells.

cGAS-STING pathway: cGAS (cGMP-AMP synthase) is a cytosolic DNA sensor and it is activated in response to cytosolic DNA. This activated cGAS binds and activates Stimulator of Interferon Gene (STING). During cellular senescence, the organization of chromatin changes and undergoes degeneration. The integrity of the nuclear envelope is disrupted due to the loss of the nuclear lamina protein Lamin B1. Eventually, small chromatin fragments migrate from nucleus to cytoplasm to become cytoplasmic chromatin fragments (CCF) in senescent cells[40]. cGAS can be activated by any double-stranded DNA irrespective of the sequence[41]. Therefore, CCF from the senescent cells can activate cGAS-STING pathway and upregulate SASP expression via inducing type-1 interferons (IFN-1). The cGAS-STING signaling pathway drives the production of inflammatory SASP cytokines through type I interferon via IRF3 and proinflammatory responses via NF-κB[42], thereby facilitating senescence. Gluck et al[43] demonstrated that cGAS deficiency in the liver abrogated the induction of
senescence in hepatocytes, which suggests the importance of cGAS in the process of senescence in vivo.

Cecco et al.\textsuperscript{[44]} showed that in oncogene-induced senescence and stress-induced premature senescence, activation of retrotransposable element, L1, and IFN-1 occur in the late phase. They found three factors that caused L1 activation, RBl, FOXA1 and TREX1. RBl expression was declined in senescent cells but was enhanced in proliferating cells. Uprogulation of FOXA1 and downregulation of RBl and TREX1 were found to be associated with L1 activation and stabilization. The activation of L1 ensured a strong activation of INF-1 response in senescent cells. In addition, when the cGAS-STING pathway was disrupted, the INF-1 response was inhibited and SASP response such as the induction of CCL2, IL-6, and MMP3 were downregulated in the late phase of senescent cells.

**NAD'-NAMT pathway:** NAD\textsuperscript{+} and the rate-limiting enzyme of its synthesis, nicotinamide phosphoribosyltransferase (NAMPT), have a critical role in ageing and cancer\textsuperscript{[45-47]} Recently, Nacarelli et al\textsuperscript{[45]} demonstrated a mechanism of SASP regulation that involves these factors and HMGA proteins HMGA1 and HMGA2\textsuperscript{[47]}. HMGA proteins are known to regulate the chromatin structure and to promote senescence\textsuperscript{[48]}. Now, these proteins are found to regulate NAMPT gene expression by binding at the enhancer element during oncogene-induced senescence of fibroblasts\textsuperscript{[47]}. NAMPT upregulates the expression of proinflammatory SASP fators such as IL-1β, IL-6 and IL-8, which are dependent on the enzymatic activity of NAMPT. This proinflammatory activity are regulated by increased NAD\textsuperscript{+}/NADH ratio. Nacarelli et al\textsuperscript{[47]} have also demonstrated that increased NAD\textsuperscript{+}/NADH ratio causes suppression of AMPK, which interferes with p53-mediated inhibition of p38 MAPK. p38 MAPK controls the transcriptional activity of NF-κB by regulating acetylation of p65. The NF-κB mediated proinflammatory activity is therefore enhanced.

In addition to HMGA proteins, another high mobility group protein, HMGB1, has been implicated in the senescence and SASP. Davalos et al\textsuperscript{[49]} reported the existence of a relationship between HMGB1 and senescence. They found that altered expression of HMGB1 induced senescence in a p53-dependent manner in fibroblasts. Exogenous HMGB1 treatment activated NF-κB activity and IL-6 secretion whereas depletion of HMGB1 attenuated the senescence phenotype. The disruption of normal HMGB1 levels can induce a p53-dependent cell cycle arrest. Interestingly, secreted HMGB1 is essential for optimal secretion of IL-6 and MMP-3, both are important SASP components.

**Other pathways:** The other known regulators of SASP are bromodomain containing protein 4 (BRD4), lysine methyltransferase MLL1 and G9A\textsuperscript{[50]}. The recruitment of BRD4 to senescence-activated enhancers located adjacent to SASP genes is probably required to induce SASP\textsuperscript{[51]}. But the upstream signaling that regulate these transcriptional activators are not completely known. SASP helps spreading senescence phenotype among nonsenescent cells by creating an inflammatory environment in tissues.

For SASP regulation, mTOR plays an important role as well. The 4E-BP1 is phosphorylated by mTOR. It is a repressor of translation of mRNA. In order to regulate SASP, 4E-BP1 represses IL-1α and MAP kinase-activated protein kinase 2\textsuperscript{[52]}. The degradation of mRNA of proinflammatory SASP factors is associated with a RNA binding protein, zinc finger protein 36L1 (ZFP36L1); and MAP kinase-activated protein kinase 2 inhibits ZFP36L1\textsuperscript{[53]}. By regulating the stability of SASP mRNA, mTOR regulates SASP in senescent cells.

**Autophagy and senescence**

Autophagy is a highly regulated cellular program responsible for recycling intracellular proteins and damaged/nonfunctional organelles. Autophagy was found to be activated in senescence in human fibroblasts due to the negative feedback of mTOR pathway\textsuperscript{[54]}. A number of genes (e.g., Ulk3 and LC3) associated with autophagy are upregulated in senescent cells and inhibition of autophagy essential genes like Atg5 or Atg7 caused interference of senescence phenotype including SASP\textsuperscript{[55]}. Reciprocally, Kang et al\textsuperscript{[54]} showed that knockdown of autophagy essential genes such as Atg7, Atg12 or lysosomal associated membrane protein 2 (LAMP2) caused senescence in human fibroblasts. This senescence pathway was found to be ROS and p53-dependent. In contrast, Garcia-Prat et al\textsuperscript{[55]} demonstrated that muscle stem cells maintained a reversible quiescence state and do not enter to senescence when the autophagy mechanism is active. This group showed that the muscle stem cells underwent senescence if autophagy was defective and restoration of autophagy prevented senescence in satellite cells. Therefore, autophagy may both induce and prevent senescence, which may be dependent on cell type or the type of experimental
model. However, it is still an open question as to whether autophagy is necessary for senescence to occur or it inhibits the senescence process. Kang et al\(^\text{[48]}\) reported that a transcription factor, GATA4, played an important role in the cellular senescence mechanism. This group suggests that GATA4 is a senescence and SASP regulator. In normal cells, GATA4 binds to an autophagy adaptor, p62, which is eliminated by the selective autophagy process. This autophagy process is suppressed when senescence is induced. DNA damage activates ATM and ATR kinases, which inhibit p62-mediated GATA4 degradation. The stabilized GATA4 then activates NF-κB and IL-1α. NF-κB, one of the master regulators of senescence, initiates and maintains SASP, and facilitates senescence. The GATA4 activation is found to be independent of p53 and p16\(^\text{INK4A}\), but is dependent on ATM and ATR kinases. This finding suggests that GATA4 may represent a separate branch of DNA damage repair response that induces SASP and hence favors senescence. Kang et al\(^\text{[48]}\) has suggested a clarification on the argument whether autophagy induces or inhibits senescence. Selective autophagy prevents cells from undergoing senescence by limiting GATA4 level when senescence-inducing stimuli, such as irradiation are given but later general autophagy lead to cellular senescence.\(^\text{15}\)

At least, one more member of the GATA family, GATA6, has been implicated in the senescence mechanism. Perlman et al\(^\text{[14]}\) reported that GATA6 overexpression could upregulate the expression of p21\(^{\text{Cip1}}\) so that the proliferation of vascular smooth muscle cells and fibroblasts were inhibited. Zhang et al\(^\text{[14]}\) suggested the existence of a direct relationship between autophagy and GATA6. Autophagy decreased accumulation of GATA6 and p62 was found to be a negative regulator of GATA6 accumulation. Zhang et al\(^\text{[14]}\) also determined the relationship between an antimalarial drug, dihydroartemisinin (DHA) and GATA6, and their contribution to the hepatic senescence. DHA treatment enhanced the expression of p53, p21\(^{\text{Cip1}}\) and p16\(^{\text{INK4A}}\) and induced senescence in hepatic stellate cells (HSCs) in rat livers. GATA6 accumulation further accelerated the expression of p53 and p16\(^{\text{INK4A}}\) in DHA-treated livers and promoted senescence. The knock-down of GATA6 by siRNA significantly reduced DHA-induced upregulation of p53 and p16\(^{\text{INK4A}}\), and thereby the senescence.

**SENESCENCE IN THE LIVER**

The detailed mechanism and biological function of cellular senescence in liver diseases have not been fully elucidated. But meaningful progresses have been made in this field. Senescence can adversely affect liver functions, cellular viability and tissue regeneration under pathological conditions.\(^\text{24}\). Senescence in hepatocytes is well documented in different liver diseases such as cirrhosis.\(^\text{23,43}\). Senescence in cholangiocytes is also known to occur in such biliary diseases as primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC). Senescence in HSCs has been reported in fibrosis.\(^\text{12}\).

**Mouse models to study liver cell senescence**

There are various mouse models developed to study senescence-associated liver diseases. Demaria et al\(^\text{[10]}\) developed a mouse model named p16\(^{\text{INK4A}}\)-MSC to visualize and to eliminate senescent cells \textit{in vivo}. The promoter of p16\(^{\text{INK4A}}\) was used in this model to express a fusion protein, composed of luciferase, red fluorescence protein (RFP) and herpes simplex virus 1 thymidine kinase. This viral thymidine kinase converts ganciclovir (GCV) into an apoptosis inducer. GCV treatment induces p53 accumulation andsurges cell surface expression of CD95 and tumor necrosis factor receptor. Utilizing the p16\(^{\text{INK4A}}\)-MSC mice and imaging, researchers can investigate a vigorous, but transient, upsurge in senescent cells \textit{in vivo}.

AhCre\(^{-}\)Mdm2\(^{2f/f}\) mice\(^\text{[11]}\): Murine Double Minute 2 (MDM2) interferes the transcriptional activity of p53 and guides degradation of p53 through ubiquitination. The disruption of the p53–MDM2 interaction by knocking out MDM2 genetically leads to p53-mediated cell-cycle arrest through inducing the activation of cell cycle inhibitor, p21\(^{\text{Cip1}}\). Deletion of Mdm2 causes upregulation of p53 and thus p21\(^{\text{Cip1}}\). The AhCre system allows expression of Cre recombinase in hepatocytes in response to β-naphthoflavrin, which in turn deletes the floxed Mdm2. This AhCre Mdm2\(^{2f/f}\) mouse model induces p21\(^{\text{Cip1}}\)-dependent hepatocellular senescence conditionally.\(^\text{11}\). The rescue of growth arrest may be achieved by crossing the AhCre Mdm2\(^{2f/f}\) mouse strain to p21\(^{\text{Cip1}}\)-deficient mouse strain.

K19-Mdm2\(^{2f/f}\)tdTomLSL.\(^\text{[8]}\): In this mouse model, Mdm2 can be conditionally deleted in cholangiocytes as the Cre recombinase expression is under the control of Krt19 promoter. This model exhibits features of biliary diseases upon induction of senescence in cholangiocytes. The Cre activity in senescent cholangiocytes can be
monitored following the expression of tdTomato[60].

Mdr2(Abcc4)(-/-) mouse model is useful for the study of chronic biliary liver disease. The mdr2 gene encodes a membrane protein that transports phosphatidylcholine. mdr2-deficient mice are unable to secrete phosphatidylcholine into the bile by hepatocytes which leads to cholestatic liver disease. Researchers have used this model to study senescence in cholangiocytes and fibrosis in the liver[68-70].

Senescence-accelerated mouse prone8 (SAMP8) is widely studied for aging mechanism, but is also useful for the study of liver diseases because of its phenotype of liver dysfunction[9]. The SAMP8 mouse strain exhibits an oxidative stress-induced age-related phenotype and severe mitochondrial dysfunction related liver pathology[3].

**Senescence in hepatocytes**

The function and the regenerative capacity of the liver deteriorate with age[61]. The accumulation of reactive oxygen species (ROS) during aging could lead to a significant level of oxidative stress. A number of studies have suggested a causative connection between cellular injury and oxidative stress in aged individuals[62-64]. Intano et al[65] reported that DNA damage could be caused by ROS and the repair response was slowed down in aged mouse hepatocytes. ROS and DNA damage trigger cells to overexpress cell cycle inhibitors and halt proliferation of the damaged cells by inducing senescence. The liver can repair and regenerate normally if the damage is mild. But when the degree of damage to the liver is severe, hepatocytes lose regenerative capacity, and undergo necrosis, apoptosis or senescence[66-70].

Hepatocellular senescence can cause remarkable changes in tissue homeostasis, and microenvironment through SASP, which may lead to fibrosis and hepatocellular carcinoma. In the early stage of liver diseases, hepatocellular senescence may serve as a protective role by blocking proliferation of affected or injured liver cells. Therefore, the risk for these affected cells to be transformed to cancer cells would be reduced. But at the later stage of the diseases, senescent cells secrete SASP factors with an abnormal level of proinflammatory cytokine/chemokines, such as CCL2, and TGFβ, which can promote the conversion of non-senescent cells to senescent cells in a paracrine manner. Rudolph and DePinto have hypothesized that chronic liver injury causes progressive and repetitive liver damage and regeneration, which leads to accumulation of shorter telomeres in hepatocytes. This in turn may lead to hepatocellular senescence and ultimately cirrhosis[67]. In addition, Wiemann et al[68] showed that telomere shortening and hepatocellular senescence were positively correlated with progression of fibrosis in human cirrhosis samples, which denoted hepatic senescence as a general marker of cirrhosis.

The accumulation of fat in hepatocytes can promote telomere shortening and DNA damage, which may be mediated by oxidative stress. The critically shorter telomere and damaged DNA may induce senescence in hepatocytes[71,72]. Aravinthan et al[73] demonstrated that hepatocytes in non-alcohol-related fatty liver disease (NAFLD) could enter the senescent state after they overexpressed p21Cip1, and presented a larger nuclear area and γ-H2AX positive foci. The presence of hepatocyte senescence positively correlated not only with the fibrotic stage of the liver but also with the diabetic state associated with NAFLD.

Any repetitive wave of insult that can cause damage to hepatocytes such as alcohol intake, hepatitis viral infection, immune disorder or autophagy deficiency, may compromise liver regeneration and promote senescence in hepatocytes and other types of cells[74-76]. The normal liver contains a basal level of senescent hepatocytes (3%-7%), but in chronic hepatitis and cirrhosis the liver may exhibit 50%-100% of hepatocytes in senescence[61,69]. The generation of fibrotic scars in cirrhosis is thought to be the consequence of hepatocyte senescence, which may be used as a marker for cirrhosis[2].

Teoh et al[77] reported the induction of p53, p21Cip1 and p16(Ink4a) in senescent hepatocytes during chronic liver diseases but the detailed mechanism was not studied. Recently Bird et al[78] published a seminal article showing that acute liver injury could also induce hepatocyte senescence. Using a hepatocyte specific senescence induction model (AhCre-Mdm2[60/60]) they found that hepatocytes undergoing cell-autonomous senescence can transfer senescence properties to normal hepatocytes via a SASP factor, CCL2, which stimulates the production of TGFβ from hepatic macrophages.

The TGFβ pathway was found to be activated in senescent hepatocytes in human fulminant liver failure. The targets of TGFβ pathway, SMAD7 and pSMAD2/3, were upregulated in hepatocytes near the necrotic area during acute liver injury. The interference of monocyte recruitment with an anti-CCL2 antibody in this model led to reduction of senescence spreading from senescent hepatocytes to normal hepatocytes, and enhanced hepatocyte proliferation. This finding suggests that TGFβ and CCL2
can work together in a non-cell-autonomous senescence pathway. The inhibition of TGFβ-TGFβ-R1 interaction by specific inhibitors or the depletion of macrophages by Liposomal Clodronate reduced senescence progress and enhanced liver regeneration[9]. This study thus suggests that the senescence process in acute liver injury may be therapeutically manageable.

Senescent hepatocytes can recruit and activate immune cells using their SASP property. The activated immune cells can remove senescent hepatocytes, which is termed as “senescence surveillance”, hence preventing malignant transformation[8]. Hepatic senescence harboring additional mutations like, p53 mutation, can cause aggressive hepatocellular carcinoma (HCC)[9]. Restoration of p53 in liver tumors can activate immune cell-mediated clearance of senescent tumor cells[68]. Kang et al[82] found that CD4+ T cells clear senescent hepatocytes with the help of activated monocytes/macrophages, which also suggested to the importance of senescence surveillance as an antitumor barrier for the HCC.

**Senescence in cholangiocytes**

The existence of senescent cholangiocytes and their potential deleterious effects in biliary diseases (PBC and PSC) have been described[65,66]. To have a better understanding of the detailed mechanism of cellular senescence in cholangiopathies, an in vitro model (K19-Cre-Mdm2[60]) was recently created to study the mechanism of cholangiocyte mediated biliary senescence in the liver by conditionally activating senescence in cholangiocytes. The cell-autonomous senescence, where the induction of p21[Cip1] and p16[INK4A] are dependent on p53 induction in cholangiocytes, results in the conversion of normal hepatocytes of close proximity into non-cell-autonomous senescence, where the induction of p21[Cip1] and p16[INK4A] are independent on p53[9]. As in the AhCre-Mdm2[60]/[61] model this paracrine senescence was found to be TGFβ-dependent as the blocking of TGFβ signaling pathway partially interfered with the paracrine senescence effect. The involvement of other SASP factors in addition to TGFβ may need to be explored in additional details.

Both studies by Ferreira-Gonzalez et al[60] and Bird et al[61] have indicated the importance of TGFβ in the transmission of senescence properties from senescent cells to otherwise normal cells. TGFβ has been shown to be involved in the liver disease progression from injury to hepatocellular carcinoma[60]. Therefore, TGFβ via small molecule inhibitors could be a valuable therapeutic target to treat cholangiopathies and hepatocellular injury and preventing senescence-associated tumorigenesis.

In cholestatic liver diseases and cholangiopathies, the bile acid (BA) secretion can be defective and bile acids may not reach to intestine, which may cause accumulation of bile acids in the liver, and cholestatic liver injury, which in turn can induce senescence in liver cells[83,84]. Whether a specific BA species can directly cause senescence in liver cells is not known. In vitro treatment of bile duct epithelial cells with deoxycholic acid (DCA) and lithocholic acid (LCA) could not induce SASP. BA may induce senescence in other cells, such as HSCs.

**Senescence in HSC**

HSCs are in quiescent state in normal healthy livers but become activated following liver injury. Activated HSC play an important role in liver fibrosis. There are three possible fates for the activated and fibrogenic HSC: (1) Apoptosis, to limit the HSC involvement in extracellular matrix (ECM) deposition; (2) Reversal from the activated status to quiescence; and (3) Senescence, to limit the degree of fibrosis in the liver. Multiple inducers of senescence in HSC have been reported, including IL-22, CCN1 (CYR61), retinoic acid, and Substance P (SP)[60].

Hepatocellular fibrosis often results from the excessive deposition of ECM by activated stellate cells. The general concept is that senescent hepatocytes are associated with fibrosis, impaired liver function and increased mortality. On the other hand, Krizhanovsky et al[60] reported that in response to liver injury, activated HSC initially proliferated and contributed to ECM deposition. However, senescent HSC could limit the extent of fibrosis. Moreover, they found that if HSCs were deficient in key senescent genes in the p53 or Rb pathways, these cells continued to proliferate and secreted excessive ECM. These finding suggest that senescent HSCs are probably beneficial for the injured liver but the senescent hepatocytes are not.

A secondary bile acid, DCA can induce SASP in HSC[60]. The enterohepatic circulation of DCA seemed important for the senescence of HSC. DCA can induce DNA damage by upregulating ROS, which may lead to senescence in HSC but the underlying mechanism is unclear. It is not known if the bile acids can induce senescence in other liver cells.

When HSC proliferate, they secrete molecules that form ECM such as collagens (e.g., Col1A) and matrix metalloproteins (MMPs). In acute liver injury, senescent HSC may have antifibrotic effects by secreting matrix metalloproteinases that digest MMPs.
and collagens. However, senescent cells of other origins (non-HSC) can express matrix metalloprotenase inhibitors, which would promote fibrosis by antagonizing the effect of senescent HSC. Therefore, detailed studies are required to understand the balance between fibrogenic and non-fibrogenic SASP in senescent HSC and in senescent non-HSC.

CONCLUSION

An increasing number of research on hepatocellular senescence have indicated the importance and significance of the senescence in the liver. The detailed mechanisms of hepatic senescence are yet to be fully determined. Exploring the regulatory mechanisms of SASP discussed above in the liver will provide an important insight to fight against hepatic inflammation, fibrosis or cancer in the liver tissues. Understanding the molecular mechanisms of cellular senescence in liver diseases will help to develop important tools for better diagnosis and treatment of the liver diseases.

REFERENCES

1. He S, Sharpless NE. Senescence in Health and Disease. Cell 2017; 169: 1000-1011 [PMID: 28575665 DOI: 10.1016/j.cell.2017.05.015]
2. Campisi J. Aging, cellular senescence, and cancer. Annu Rev Physiol 2013; 75: 685-705 [PMID: 23140366 DOI: 10.1146/annurev-physiol-030212-183653]
3. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci USA 1995; 92: 9363-9367 [PMID: 7568133]
4. Krishnamurthy J, Torrice C, Ramsey MR, Kovalovs GI, Al-Regaiey K, Su L, Sharpless NE. Inksa/Arf expression is a biomarker of aging. J Clin Invest 2004; 114: 1299-1307 [PMID: 15207862 DOI: 10.1172/JCI22475]
5. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965; 37: 614-636 [PMID: 14315085]
6. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25: 585-621 [PMID: 13905658]
7. van Deursen JM. The role of senescent cells in aging. Nature 2014; 509: 439-446 [PMID: 24848057 DOI: 10.1038/nature13193]
8. Childs BR, Li H, van Deursen JM. Senescent cells: a therapeutic target for cardiovascular disease. J Clin Invest 2018; 128: 1217-1228 [PMID: 29608141 DOI: 10.1172/JCI91546]
9. Bird TG, Müller M, Boulter L, Vincent DF, Ridgway RA, Lopez-Guadamilas E, Lu WY, Jamieson T, Gavare O, Campbell AD, Ferreira-Gonzalez S, Cole AM, Hay T, Simpson KJ, Clark W, Hedley A, Clarke M, Gentaz P, Nixon C, Bryce S, Kiousis C, Sprangaers J, Nikhs RJ, Van Rooijen N, Bartholin L, McGeilal CR, Apte U, Barry ST, Iredale JP, Clarke AR, Serrano M, Roskams TA, Sanoso OJ, Forbes SJ. TGFβ inhibition restores a regenerative response in acute liver injury by suppressing paracrine senescence. Sci Transl Med 2018; 10 [PMID: 30116142 DOI: 10.1126/scitranslmed.aan1230]
10. Acosta JC, Banito A, Wuestefeld T, Georgilis A, Janisch P, Morton JP, Athineos D, Kang TW, Lasitschka F, Androulis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AF, Carroll T, Capper D, Pritchard C, Imman GI, Longerich T, Sansom OJ, Zender L, Gil J. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. Nat Cell Biol 2013; 15: 978-990 [PMID: 23770676 DOI: 10.1038/nclb2784]
11. Campisi J, d’Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 2007; 8: 729-740 [PMID: 17667954 DOI: 10.1038/nrm2223]
12. Ben-Porath I, Weinberg RA. The signals and pathways activating cellular senescence. Int J Biochem Cell Biol 2005; 37: 961-976 [PMID: 15743671 DOI: 10.1016/j.biocell.2004.10.013]
13. Carnero A. Markers of cellular senescence. Methods Mol Biol 2013; 965: 63-81 [PMID: 23296651 DOI: 10.1007/978-1-62703-239-1_4]
14. Arasimhan A, Scarpini C, Tachtatzis P, Verma S, Penryhn-Lowe S, Harvey R, Davies SE, Allison M, Coleman N, Alexander G. Hepatocyte senescence predicts progression in non-alcohol-related fatty liver disease. J Hepatol 2013; 58: 549-556 [PMID: 23142622 DOI: 10.1016/j.jhep.2012.10.031]
15. Lee BY, Han JA, Im JS, Morrone A, Jhung K, Goodwin EC, Kleijer WJ, DiMaio D, Hwang ES. Senescence-associated beta-galactosidase is lysosomal beta-galactosidase. Aging Cell 2006; 5: 187-195 [PMID: 16626397 DOI: 10.1111/j.1474-9726.2006.00199.x]
16. Debacq-Chainiaux F, Ursalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-beta-gal) activity, a biomarker of senescent cells in culture and in vivo. Nat Protoc 2009; 4: 1798-1806 [PMID: 20010931 DOI: 10.1038/nprot.2009.191]
17. Ito Y, Hare M, Narita M. Spatial and Temporal Control of Senescence. Trends Cell Biol 2017; 27: 820-832 [PMID: 28822679 DOI: 10.1016/j.tcb.2017.07.004]
18. Lu T, Finkel T. Free radicals and senescence. Exp Cell Res 2008; 314: 1918-1922 [PMID: 18282588 DOI: 10.1016/j.yexcr.2008.01.011]
19. Chen Q, Fischer A, Reagan JD, Yan LJ, Ames BN. Oxidative DNA damage and senescence of human diploid fibroblast cells. Proc Natl Acad Sci USA 1995; 92: 4337-4341 [PMID: 7753800]
20. Sherr CJ, McCormick F. The RB and p53 pathways in cancer. Cancer Cell 2002; 2: 103-112 [PMID: 12204530]
21. Wei W, Herbig U, Wei S, Dutriaux A, Sedivy JM. Loss of retinoblastoma but not p16 function allows bypass of replicative senescence in human fibroblasts. EMBO Rep 2003; 4: 1061-1066 [PMID: 14566323 DOI: 10.1038/sj.embor.7400001]
Yang H, Wang H, Ren J, Chen Q, Chen ZJ. GAG5 is essential for cellular senescence. Proc Natl Acad Sci
Huda N et al. Hepatic senescence

U/S 2017; 114: E4612-E4620 [PMID: 28533362 DOI: 10.1073/pnas.1705499114]

Tasdernit N, Banito A, Roe JS, Alonso-Curbelo D, Camilo M, Tschaharganeh DF, Huang CH, Aksoy O, Bolden JE, Chen CC, Fennell M, Thapar V, Chicas A, Vakoc CR, Lowe SW. BRD4 Connects Enhancer Remodeling to Senescence Immune Surveillance. Cancer Discov 2016; 6: 612-629 [PMID: 27099232 DOI: 10.1158/2159-8290.CD-16-0217]

Lahberger RM, Sun Y, Ojralo AV, Patil CK, Freund A, Zhou L, Curran SC, Davalos AR, Wilson-Edell KA, Liu S, Limbad C, Demaria M, Li P, Hubbard IB, Ikento Y, Javors M, Desprez PY, Benz CC, Kapahi P, Nelson PS, Campisi J. MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. Nat Cell Biol 2015; 17: 1049-1061 [PMID: 26147250 DOI: 10.1038/ncb3195]

Young AR, Narita M, Ferreira M, Kirschner K, Sadade M, Darot JF, Tavarez S, Arakawa S, Shimizu S, Watt FM, Narita M. Autophagy mediates the mitotic senescence transition. Genes Dev 2009; 23: 798-803 [PMID: 19279323 DOI: 10.1101/gad.197090]

Kang HT, Lee KB, Kim SY, Choi HR, Park SC. Autophagy impairment induces premature senescence in primary human fibroblasts. PLoS One 2011; 6: e23367 [PMID: 21858089 DOI: 10.1371/journal.pone.0023367]

Garcia-Prat L, Martinez-Vicente M, Perdiguero E, Ortel L, Rodriguez-Ubreva J, Rebollo E, Ruiz-Bonilla V, Guturri S, Ballister E, Serrano AL, Sandri M, Muñoz-Cánoves P. Autophagy maintains senescence by preventing senescence. Nature 2016; 529: 37-42 [PMID: 26738589 DOI: 10.1038/nature161877]

Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4 Science 2015; 349: aaa5612 [PMID: 26404840 DOI: 10.1126/science.aaa5612]

Cao X, Li M. A New Pathway for Senescence Regulation. Genomics Proteomics Bioinformatics 2015; 13: 333-335 [PMID: 26777577 DOI: 10.1016/j.gpb.2015.11.002]

Perman H, Suzuki E, Simonson M, Smith WC, Rak J. GATA-4 induces p21 expression and G1 cell cycle arrest. J Biol Chem 1998; 273: 13713-13718 [PMID: 9593712]

Zhang Z, Yao Z, Zhao S, Shao J, Chen A, Zhang F, Zheng S. Interaction between autophagy and senescence is required for dihydroorotammin to alleviate liver fibrosis. Cell Dis 2017; 8: e2886 [PMID: 28617435 DOI: 10.1016/j.celdis.2017.255]

Ferreira-Gonzalez S, Lu YY, Raven A, Dwyer B, Man TY, O'Dubhlaí E, Lewis PJS, Campana L, Kendall TJ, Bird TG, Tarrats N, Acosta JC, Boutlet L, Forbes SJ. Paracrine cellular senescence exacerbates biliary injury and impairs regeneration. Nat Commun 2018; 9: 1020 [PMID: 25293787 DOI: 10.1038/s41467-018-03299-5]

Paradis V, Youssef N, Dargère D, Bä N, Bonvoust F, Deschatrette J, Bedossa P. Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. Hum Pathol 2001; 32: 327-332 [PMID: 11274643 DOI: 10.1016/s0363-4619(01)00274-7]

Zlotnik A, Kershenobich D. Cellular senescence in livers from children with end stage liver disease. Lab Invest 2003; 83: 102-109 [PMID: 12563309 DOI: 10.1038/nrc991]

Chène P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. Nat Rev Cancer 2014; 15: 99-110 [PMID: 23935225 DOI: 10.1038/nrc3707]

Frey N, Venturrelli S, Zender L, Bitzer M. Cellular senescence in gastrointestinal diseases: from pathogenesis to therapeutics. Nat Rev Gastroenterol Hepatol 2018; 15: 81-95 [PMID: 29184185 DOI: 10.1038/nrgastro.2017.146]

Krizhanovsky V, Yon M, Dickins RA, Heam S, Simon J, Miething C, Yee H, Zender L, Lowe SW. Senescence of activated stellate cells limits liver fibrosis. Cell 2008; 134: 657-667 [PMID: 18724938 DOI: 10.1016/j.cell.2008.06.049]

Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Lahberger RM, Viji J, Van Steeg H, Dolle ME, Hoeijmakers JH, de Bruijn A, Hara E, Campisi J. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. Dev Cell 2014; 31: 722-733 [PMID: 23999410 DOI: 10.1016/j.devcel.2014.11.012]

Cell P. Inhibiting the p53-MDM2 interaction: an important target for cancer therapy. Nat Rev Cancer 2003; 3: 102-109 [PMID: 12663309 DOI: 10.1038/nrc991]

Zhao T, Wu N, Meng F, Venter J, Jiang TK, Francis H, Kiritri K, Wu C, Franchitto A, Alvaro D, Mazzoni M, Onori P, Mancinelli R, Guaduo E, Glaser S, Alpini G. Knockout of secretin receptor reduces biliary damage and liver fibrosis in Mdr2−/− mice by diminishing senescence of cholangiocytes. Lab Invest 2018; 98: 1449-1464 [PMID: 29977037 DOI: 10.1038/s41374-018-0093.9]

Chan W, Meng F, Wu N, Zhou T, Venter J, Francis H, Kennedy L, Glaser T, Bernuzzi F, Invernizzi P, Glaser S, Huang Q, Alpini G. Substance P increases liver fibrosis by differential changes in senescence of cholangiocytes and hepatic stellate cells. Hepatology 2017; 66: 528-541 [PMID: 28256736 DOI: 10.1002/hep.29138]

McDaniel K, Meng F, Wu N, Sato K, Venter J, Bernuzzi F, Invernizzi P, Zhou T, Kiritri K, Wan Y, Huang Q, Onori P, Francis H, Guaduo E, Glaser S, Alpini G. Forkhead box A2 regulates bile ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology 2017; 65: 544-559 [PMID: 27639079 DOI: 10.1002/hep.28831]

Liu Y, He J, Ji S, Wang Q, Pu H, Jiang T, Meng L, Yang X, Ji J. Comparative studies of early liver dysfunction in senescence-accelerated mouse using mitochondrial proteomics approaches. Mol Cell Proteomics 2008; 7: 1737-1747 [PMID: 18515266 DOI: 10.1074/mcp.M800109-MCP200]

Cheonston AD, Powell EE, Walsh MJ, Richardson MM, Demetris AJ, Jonson JR. Fibrosis correlates with a ductular reaction in hepatitis C: roles of impaired replication, progenitor cells and steatosis. Hepatology 2005; 41: 809-818 [PMID: 15793848 DOI: 10.1002/hep.20650]

Cieslak KP, Baur O, Verheij J, Bernink RJ, van Gulik TM. Liver function declines with increased age. HPB (Oxford) 2016; 18: 691-696 [PMID: 27485064 DOI: 10.1111/1365-3016.12631]

Schmucker DL. Age-related changes in liver structure and function: Implications for disease? Exp Gerontol 2005; 40: 650-659 [PMID: 16102930 DOI: 10.1016/j.exger.2005.06.009]

Zhang HJ, Xu L, Drake VJ, Xie L, Oberley LW, Kregel KC. Heat-induced liver injury in old rats is associated with exaggerated oxidative stress and altered transcription factor activation. FASEB J 2003; 17: 2293-2295 [PMID: 14590545 DOI: 10.1096/fj.03-3159ec]

Intorno GW, Cho EJ, McMahan CA, Walter CA. Age-related base excision repair activity in mouse brain
Huda N et al. Hepatic senescence and liver nuclear extracts. *J Gerontol A Biol Sci Med Sci* 2003; 58: 205-211 [PMID: 12634285 DOI: 10.1093/gerona/58.3.b205]

77 Xiao M, Chen W, Wang C, Wu Y, Zhu S, Zeng C, Cai Y, Liu C, He Z. Senescence and cell death in chronic liver injury: roles and mechanisms underlying hepatocarcinogenesis. *Oncotarget* 2017; 9: 8772-8784 [PMID: 29492237 DOI: 10.18632/oncotarget.23622]

78 Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science* 2000; 287: 1253-1258 [PMID: 10678830 DOI: 10.1126/science.287.5456.1253]

79 Xiao M, Chen W, Wang C, Wu Y, Zhu S, Zeng C, Cai Y, Liu C, He Z. Senescence and cell death in chronic liver injury: roles and mechanisms underlying hepatocarcinogenesis. *Gastroenterology* 2010; 138: 1155-1165.e1-2 [PMID: 19919837 DOI: 10.1053/j.gastro.2009.11.008]

80 Rudolph KL, Chang S, Millard M, Schreiber-Agus N, DePinho RA. Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery. *Science* 2000; 287: 1253-1258 [PMID: 10678830 DOI: 10.1126/science.287.5456.1253]

81 Xiao M, Chen W, Wang C, Wu Y, Zhu S, Zeng C, Cai Y, Liu C, He Z. Senescence and cell death in chronic liver injury: roles and mechanisms underlying hepatocarcinogenesis. *Oncotarget* 2017; 9: 8772-8784 [PMID: 29492237 DOI: 10.18632/oncotarget.23622]

82 Xiao M, Chen W, Wang C, Wu Y, Zhu S, Zeng C, Cai Y, Liu C, He Z. Senescence and cell death in chronic liver injury: roles and mechanisms underlying hepatocarcinogenesis. *Gastroenterology* 2010; 138: 1155-1165.e1-2 [PMID: 19919837 DOI: 10.1053/j.gastro.2009.11.008]

83 Xiao M, Chen W, Wang C, Wu Y, Zhu S, Zeng C, Cai Y, Liu C, He Z. Senescence and cell death in chronic liver injury: roles and mechanisms underlying hepatocarcinogenesis. *Gastroenterology* 2010; 138: 1155-1165.e1-2 [PMID: 19919837 DOI: 10.1053/j.gastro.2009.11.008]
