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Mini-review

Senescence and immortality in hepatocellular carcinoma

Mehmet Ozturk [a,b,*], Ayca Arslan-Ergul [a], Sevgi Bagislar [a,b], Serif Senturk [a], Haluk Yuzugullu [a,b]

[a] Department of Molecular Biology and Genetics, Bilkent University, 06800 Ankara, Turkey
[b] Centre de Recherche INSERM-Université Joseph Fourrier U823, Institut Albert Bonniot, 38042 Grenoble, France

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ABSTRACT

Cellular senescence is a process leading to terminal growth arrest with characteristic morphological features. This process is mediated by telomere-dependent, oncogene-induced and ROS-induced pathways, but persistent DNA damage is the most common cause. Senescence arrest is mediated by $p16^{INK4a}$- and $p21^{Cip1}$-dependent pathways both leading to retinoblastoma protein (pRb) activation. p53 plays a relay role between DNA damage sensing and $p21^{Cip1}$ activation. pRb arrests the cell cycle by recruiting proliferation genes to facultative heterochromatin for permanent silencing. Replicative senescence that occurs in hepatocytes in culture and in liver cirrhosis is associated with lack of telomerase activity and results in telomere shortening. Hepatocellular carcinoma (HCC) cells display inactivating mutations of p53 and epigenetic silencing of $p16^{INK4a}$. Moreover, they re-express telomerase reverse transcriptase required for telomere maintenance. Thus, senescence bypass and cellular immortality is likely to contribute significantly to HCC development. Oncogene-induced senescence in premalignant lesions and reversible immortality of cancer cells including HCC offer new potentials for tumor prevention and treatment.

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1. Introduction

Senescence is an evolutionary term meaning "the process of becoming old"; the phase from full maturity to death characterized by accumulation of metabolic products and decreased probability of reproduction or survival [1]. The term "cellular senescence" was initially used by Hayflick and colleagues to define cells that ceased to divide in culture [2]. Today, cellular senescence is recognized as a response of proliferating somatic cells to stress and damage from exogenous and endogenous sources. It is characterized by permanent cell cycle arrest. Senescent cells also display altered morphology and an altered pattern of gene expression, and can be recognized by the presence of senescence markers such as senescence-associated β-galactosidase (SABG), $p16^{INK4A}$, senescence-associated DNA-damage foci and senescence-associated heterochromatin foci (for a review see Ref. [3]). This cellular response has both beneficial (anti-cancer) and probably deleterious (such as tissue aging) effects on the organism. Most of our knowledge of cellular senescence is derived from in vitro studies performed with fibroblasts, and some epithelial cells such as mammary epithelial cells. Animal models are increasingly being used to study cellular senescence in vivo. Telomerase-deficient mouse models lacking RNA subunit (TERC−/−) have been very useful in demonstrating the critical role of telomeres in organ aging and tumor susceptibility [4]. Other mouse models including tumor suppressor gene-deficient and oncogene-expressing mice were also used extensively.

Compared to other tissues and cancer models, the role of senescence in liver cells and its implications in hepatocellular carcinogenesis have been less explored. One of

* Corresponding author. Address: Centre de Recherche INSERM-Université Joseph Fourrier U823, Institut Albert Bonniot, Grenoble 38000, France. Tel.: +33 (0) 4 76 54 94 10; fax: +33 (0) 4 76 54 94 54.
E-mail address: ozturkm@ujf-grenoble.fr (M. Ozturk).

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the main obstacles is the lack of adequate in vitro systems. As hepatocytes can not divide in cell culture, the study of their replicative senescence mechanisms is not easy. Nevertheless, these cells are able to quit their quiescent state in vivo and proliferate massively in response to partial hepatectomy or liver injury [5]. This capacity can be explored to study in vivo senescence of hepatocytes using rodent models. Studies with clinical samples indicate that hepatocyte senescence occurs in vivo in patients with chronic hepatitis, cirrhosis and HCC [6–8]. In contrast to the paucity of studies directly addressing cellular senescence, the critical role of telomere shortening (as a feature associated with replicative senescence) in cirrhosis and HCC development is well established [9]. Telomeres in normal liver show a consistent but slow shortening during aging. In contrast, hepatocyte DNA telomere shortening is accelerated in patients with chronic liver disease with shortest telomeres described in cirrhotic liver and HCC. Telomerase-deficient mice have also been used elegantly to demonstrate the critical roles of telomerase and telomeres in liver regeneration and experimentally induced cirrhosis [10,11]. A major accomplishment in recent years was the demonstration of critical role played by senescence for the clearance of ras-induced murine liver carcinomas following p53 restoration [12].

Despite a relatively important progress, the mechanisms of hepatocellular senescence and the role of cellular immortality in HCC remain ill-known issues. As one of the rare tissues with ample clinical data on senescence-related aberrations, liver may serve as an excellent model to further explore the relevance of cellular senescence in human biology. Moreover, a better understanding of senescence and immortality in hepatic tissues may help to develop new preventive and therapeutic approaches for severe liver diseases such as cirrhosis and HCC. Here we will review recent progress on senescence and immortality mechanisms with a specific emphasis on hepatocellular carcinogenesis.

2. Senescence pathways

Cellular senescence has long been considered as a mechanism that limits the number of cell divisions (or population doublings) in response to progressive telomere shortening. Most human somatic cells are telomerase-deficient because of the repression of telomerase reverse transcriptase (TERT) expression. Therefore, proliferating somatic cells undergo progressive telomere DNA erosion as a function of their number of cell divisions. This form of senescence is now called as replicative or telomere-dependent senescence (Fig. 1).

Human chromosome telomere ends are composed of TTAGGG repeats (5–20 kb) in a DNA-protein complex formed by six telomere-specific proteins, called "shelterin" [13]. Telomeric DNA has a structure called "t-loop" which is formed as a result of invasion of the single stranded G-rich sequence into the double-stranded telomeric tract. Since the 1930s, it has been known that telomeres, with telomere-binding proteins, prevent genomic instability and the loss of essential genetic information by "capping" chromosome ends. They are also indispensable for proper recombination and chromosomal segregation during cell division. Telomeres become shorter with every cell division in somatic cells, because of replication complex's inability to copy the ends of linear DNA, which also makes them a "cell cycle counter" for the cell [14]. Telomeres are added to the end of chromosomes with a complex containing the RNA template TERC and the reverse transcriptase TERT [15]. Most somatic cells lack telomerase activity because the expression of TERT is repressed, in contrast to TERC expression. The lack of sufficient TERT expression in somatic cells is the main cause of telomere shortening during cell replication. This telomerase activity also helps to maintain telomere integrity by telomere capping [15].

The loss of telomeres has long been considered to be the critical signal for senescence induction. It is now well known that telomere-dependent senescence is induced by a change in the protected status of shortened telomeres, whereby the loss of telomere DNA contributes to this change [16]. The loss of telomere protection or any other cause of telomere dysfunction results in inappropriate chromosomal end-to-end fusions through non-homologous end joining or homologous recombination DNA repair pathways [17]. These DNA repair pathways are used principally to repair double-strand DNA breaks (DSBs). Thus, it is highly likely that the open-ended telomere DNA is sensed as a DSB by the cell machinery when telomere structure becomes dysfunctional. Accordingly, dysfunctional telomeres elicit a potent DSB type DNA damage response by recruiting phosphorylated H2AX, 53BP1, NBS1 and MDC1 [18].

Telomere-dependent senescence is not the only form of senescence. At least two other forms of telomere-independent senescence are presently known: (1) oncogene-induced senescence; and (2) reactive oxygen species (ROS)-induced senescence (Fig. 1).

Oncogene-induced senescence had initially been identified as a response to expression of Ras oncogene in normal cells ([19], for a recent review see [20]). The expression of oncogenic Ras in primary human or rodent cells results in permanent G1 arrest. The arrest was accompanied by accumulation of p53 and $p16^{INK4a}$, and was phenotypically indistinguishable from cellular senescence. This landmark observation suggested that the onset of cellular senescence does not simply reflect the accumulation of cell divisions, but can be prematurely activated in response to an oncogenic stimulus [19]. In 10 years following this important discovery, telomere-independent forms of senescence have become a new focus of extensive research leading to the recognition of senescence as a common form of stress response. Moreover, oncogene-induced senescence is now recognized as a novel mechanism contributing to the cessation of growth of premalignant or benign neoplasms to prevent malignant cancer development [21]. In addition to Ras, other oncogenes including Raf, Mos, Mek, Myc and Cyclin E also induce senescence [20]. Conversely, the loss of PTEN tumor suppressor gene also leads to senescence [22]. Similar to telomere-dependent senescence, oncogene-induced senescence is also primarily a DNA damage response (Fig. 1). Experimental inactivation of DNA damage response abrogates Ras-induced senescence

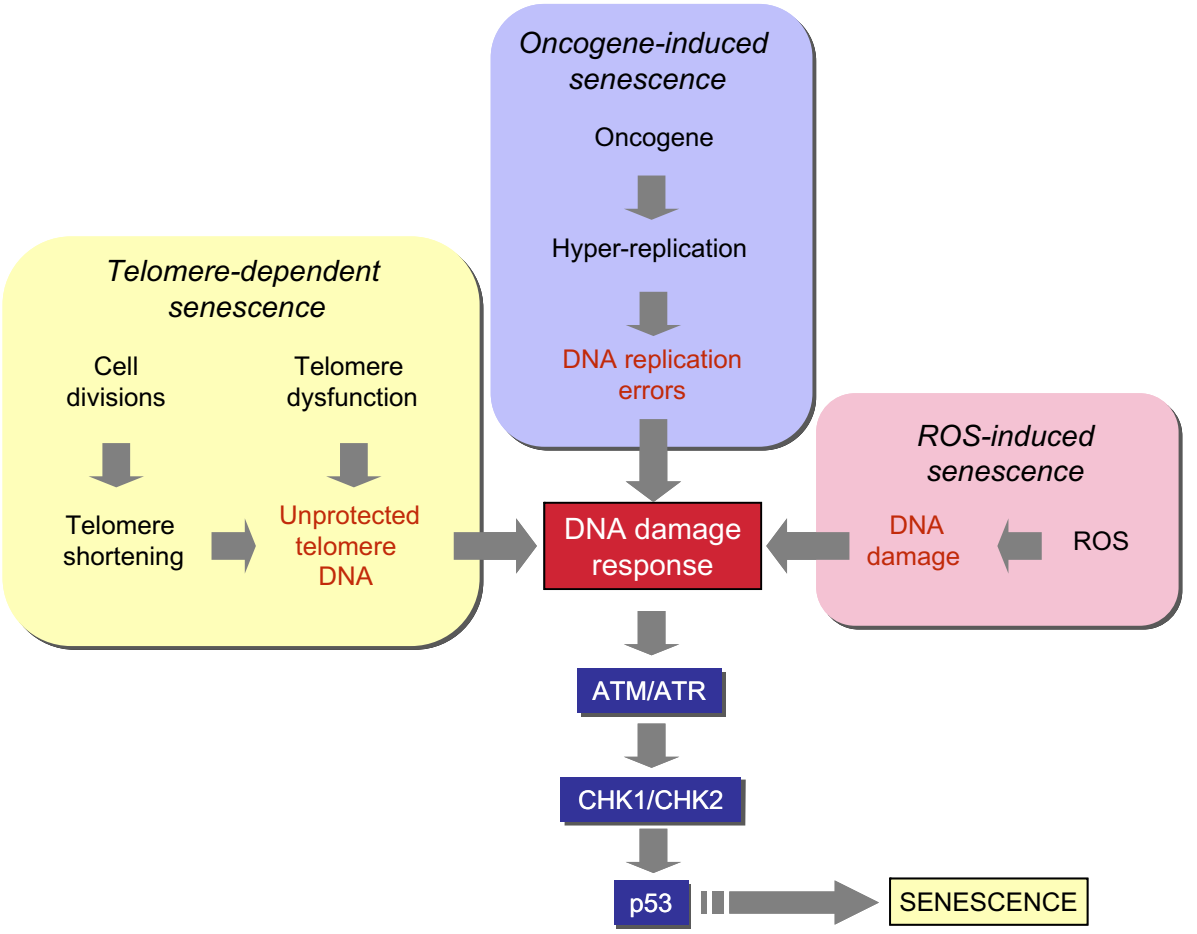

Fig. 1. DNA damage and p53 activation play a central role in different senescence pathways. DNA damage (often in the form of double-strand breaks) activate upstream kinases (ATM and ATR) leading to p53 phosphorylation by CHK1 and CHK2 kinases. Phosphoryated p53 is released from MDM, and stabilized in order to induce senescence arrest or apoptosis (not shown here).

and promotes cell transformation. DNA damage response and oncogene-induced senescence are established following DNA hyper-replication immediately after oncogene expression. Senescent cells arrest with partly replicated DNA, where DNA replication origins have fired multiple times, prematurely terminated DNA replication forks and DNA double-strand breaks are present [23,24].

ROS-induced senescence, the other telomere-independent senescence pathway is gaining importance (for a recent review see Ref. [25]). Mitochondria are the major intracellular sources of ROS which are mainly generated at the respiratory chain. Therefore, ROS have been suspected for many years as cellular metabolites involved in organismal aging [26]. ROS are also generated in the cytoplasm by the NOX family of enzymes [27]. Experimental induction of ROS accumulation in cells (for example by mild H2O2 treatment or glutathione depletion) induces senescence-like growth arrest in different cell types, whereas anti-oxidant treatment can inhibit senescence [25]. More importantly, ROS have been identified as critical mediators of both telomere-dependent and oncogene-induced senescence. Telomere-dependent senescence arrest is accelerated in cells grown under high O_2 conditions. Inversely, cells grown under low O_2 conditions display increased lifespan ([28], see Ref. [25]). ROS also play a critical role in Ras-induced senescence [29,30].

Currently, mechanisms of ROS-induced senescence are not fully understood. It is generally accepted that oxidative stress and ROS eventually cause DNA damage, whereby DNA damage response may contribute to senescence induction. The relationship between mitochondrial dysfunction, ROS, DNA damage and telomere-dependent senescence has recently been demonstrated [31]. However, ROS may also induce modifications in the cellular signaling pathways resulting in senescence arrest. For example, ROS induce senescence in hematopoietic stem cells by activating p38 MAPK [32].

Whether induced by telomere dysfunction, DNA replication stress following oncogene activation, or ROS accumulation, DNA damage is one of the common steps in the generation of senescence arrest via p53 activation (Fig. 1). Upstream checkpoint kinases, such as ATM or ATR are activated in response to DNA damage in the form of double-stand breaks. These kinases phosphorylate

downstream factors including CHK1 and CHK2 that in turn phosphorylate p53. Phosphorylation of p53 results in its activation by the displacement of the MDM2 protein. Critical involvement of this p53 activating pathway has been reported for both telomere-dependent [33], and oncogene-induced senescence [34].

Other mechanisms of senescence that are apparently not driven by DNA damage should also be discussed here. Of particular interest is the INK4 locus encoding two inhibitors of cyclin-dependent kinases ($p16^{INK4a}$, $p15^{INK4b}$), and ARF, a p53 regulatory protein (for a review see Ref. [35]). $p16^{INK4a}$ and $p15^{INK4b}$ connect some senescent initiating signals to the retinoblastoma (Rb) pathway, independent of p53 activation. These proteins are easily activated in cell culture and induce senescence arrest. Cells that escape senescence often display inactivation of $p16^{INK4a}$, and sometimes $p15^{INKb}$ and ARF either by homozygous deletion or by shutting-down gene expression. A prominent role for $p16^{INK4a}$ in senescence and tumor suppression in humans has emerged, despite some confusion due to the fact that a relatively small DNA segment encodes the 3 proteins of the INK4 locus. $p16^{INK4a}$ is activated during telomere-dependent and oncogene-induced senescence [19,36]. Moreover, its expression is induced in aging tissues [37]. The mechanisms of regulation of $p16^{INK4a}$ expression are not well known. Although individual components of INK4 locus can respond independently to positively – (for example to Ras) or negatively – (for example c-Myc) acting signals, the entire INK4 locus might be coordinately regulated by epigenetic mechanisms (reviewed in Ref. [35]).

A very recent addition to the list of senescence mechanisms is to be qualified as "senescence induced by secreted proteins". It was reported many years ago that TGF-β is a mediator of oncogene-induced senescence [38]. This mechanism of induction is of particular interest, because it suggests that not only intrinsic cellular factors, but also extracellular or secreted proteins can induce senescence. Recent discovery of several other secreted proteins, including IGFBP7 and IL6 as autocrine/paracrine mediators of oncogene-induced senescence arrest, provide strong support for an extracellularly induced form of senescence [39–41]. This new form of senescence regulation is reminiscent of the so called active apoptosis induction by death ligands. Thus, an active form of cellular senescence induced by "aging ligands" could be a major physiological regulator of tissue/organism aging.

3. Cyclin-dependent inhibitors as common mediators of senescence arrest

We have already stated that senescence and apoptosis share interesting similarities. Another similarity between these cellular processes is the convergence of different pathways in a common place to induce the same cell fate, independent of the initial signal. Similarly to caspase activation, prior to apoptosis induction by different stimuli, most if not all senescence pathways result in the activation of cyclin-dependent kinase inhibitors (CDKIs) in order to induce permanent cell cycle arrest. Senescent cells accumulate at G1 phase of the cell cycle due to an inability to enter into S phase in order to initiate DNA synthesis. The transition of proliferating cells from G1 to S phase requires the release of E2F factors from their inhibitory partner retinoblastoma protein (pRb) following phosphorylation by cyclin-dependent kinases (CDKs), in particular by CDK4/CDK6 and CDK2 at this stage of the cycle [35]. The senescence arrest is mediated by inhibition of pRb phosphorylation by CDK4 and CDK2. The activities of these enzymes are controlled by different mechanisms, but the major proteins involved in the control of senescence arrest are CDKIs. Almost all known CDKIs have been reported to be implicated in senescence arrest, but three of them are best characterized: $p16^{INK4a}$ and $p15^{INK4b}$ which inhibit CDK4/CDK6, and $p21^{Cip1}$ which inhibits CDK2 (Fig. 2).

$p21^{Cip1}$ is one of the main targets of p53 for the induction of cell cycle arrest following DNA damage [42]. Pathways that generate DNA damage response and p53 activation use $p21^{Cip1}$ as a major mediator of cellular senescence to control pRb protein [43]. Exceptionally, $p21^{Cip1}$ can be activated by p53-independent pathways to induce senescence [44].

The Rb protein plays two important and complementary roles that are necessary to initiate and to permanently maintain the cell cycle arrest in senescent cells. pRb proteins firstly contribute to the exit from the cell cycle by arresting cells at G1 phase, as expected [45]. In senescent cells, this exit is complemented with a dramatic remodeling of chromatin through the formation of domains of facultative heterochromatin called SAHF [46–48]. SAHF contain modifications and associated proteins characteristic of transcriptionally silent heterochromatin. Proliferation-promoting genes, such as E2F target genes are recruited into SAHF in a pRb protein-dependent manner. This recruitment is believed to contribute to irreversible silencing of these proliferation-promoting genes [49].

4. Senescence of hepatocytes and chronic liver disease

Hepatocytes in the adult liver are quiescent cells, they are renewed slowly, approximately once a year, as estimated by telomere loss which is 50–120 bp per year in healthy individuals [50,51]. However, the liver has an extremely powerful regenerative capacity, as demonstrated experimentally in rodents, and as observed in patients with chronic liver diseases [5]. This regenerative capacity is due mostly to the ability of mature hepatocytes to proliferate in response to a diminution of total liver mass either experimentally, or following exposure to viral and non viral hepatotoxic agents. In addition, the adult liver seems to harbor hepatocyte-progenitor cells (<0.10% of total hepatocyte mass) that are able to restore liver hepatocyte populations [52]. However, hepatocytes, like any other somatic cells, do not have unlimited replicative capacity, due to the lack of telomerase activity that is needed to avoid telomere shortening during successive cell divisions. This is best exemplified by decreased hepatocyte proliferation in liver cirrhosis stage of chronic liver diseases [53], providing in vivo evidence for the exhaustion of hepatocyte proliferation capacity. Senescence mechanisms in hepatocytes and in liver tissue are not well known. However, a limited

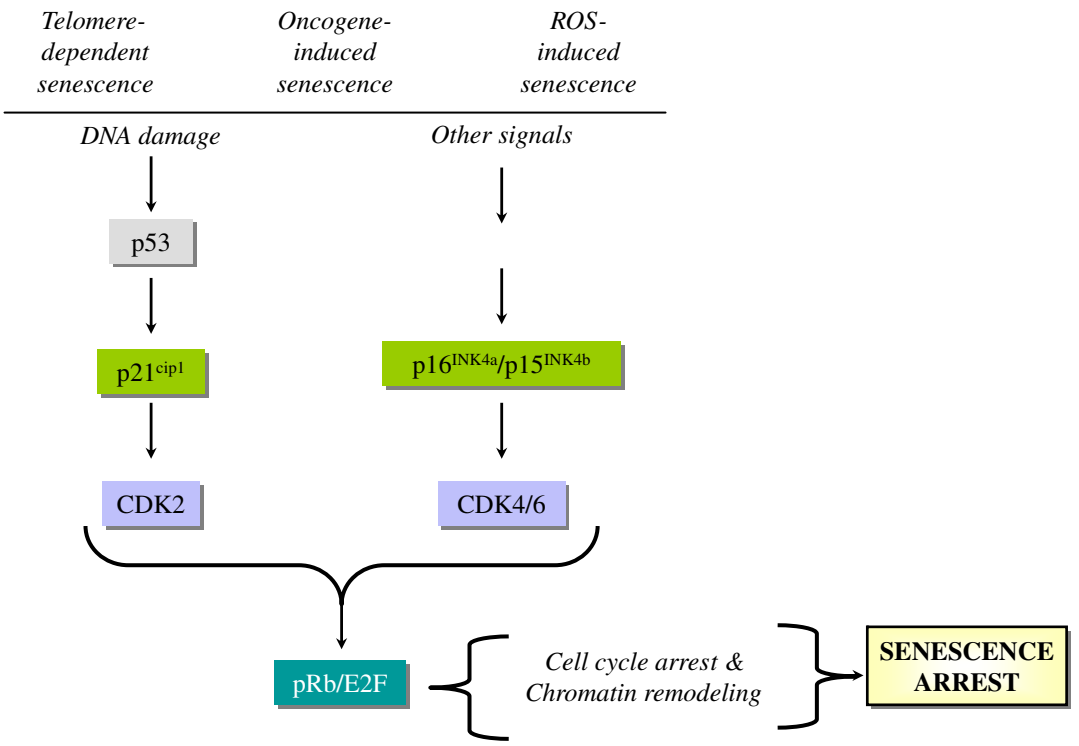

Fig. 2. All known senescence pathways converge at the level of activation of CDKIs ($p15^{INK4b}$, $p16^{INK4a}$ and $p21^{Cip1}$) that keep the pRb protein under the active form. The pRb protein inhibits E2F action and prevents the expression of growth-promoting genes for cell cycle exit. Furthermore, pRb recruits growth-promoting genes into a facultative chromatin structure for permanent silencing and growth arrest.

number of in vitro studies with hepatocytes, as well as numerous descriptive in vivo studies in liver tissue provide sufficient evidence that hepatocytes can undergo senescence type changes.

In vitro senescence in hepatocytes: as stated earlier, limited proliferative capacity of somatic cells is controlled by replicative senescence. The experimental study of replicative senescence is done traditionally by serial culture of primary cells. Initially observed in fibroblasts, this phenomenon has also been well understood in some epithelial cells, mammary epithelial cells in particular [54]. On the other hand, our knowledge of hepatocyte replicative senescence is highly limited. In contrast to in vivo conditions, mature hepatocytes are extremely resistant to cell proliferation in cell culture. Usually, more than 99.9% of adult liver hepatocytes do not divide and can only be maintained in culture for a few weeks at most. A small progenitor-type cell population (so called small hepatocytes) has been shown to proliferate in vitro, but they usually stop growing at passages 5–7, with an ill-defined senescence-like phenotype [55].

Fetal hepatocytes display better proliferation capacity in culture. A few studies have shown that these fetal cells enter replicative senescence, as shown by senescence-associated β-galactosidase assay (SABG) at population doubling (PD) 30–35 [55]. This is accompanied by progressive shortening of telomeres down to ~6 kbp, as these cells like adult hepatocytes lack telomerase activity. However, it was possible to immortalize these fetal hepatocytes by stable expression of TERT [55]. Such immortalized cells have been expanded beyond known senescence barriers (>300 PD).

In vivo senescence in liver tissue: in contrast to in vitro studies, in vivo senescence of human hepatocytes is better known. Indeed, the liver is one of the rare tissues where in vivo evidence for senescence has been convincingly and independently demonstrated by different investigators [6–9]. Replicative senescence (as tested by SABG assay) displayed a gradual increase from 10% in normal liver, to 84% in cirrhosis ([6,7]. It was also detected in 60% HCCs [6]. It has also been demonstrated that telomere shortening in cirrhosis is restricted to hepatocytes and this hepatocyte-specific shortening was correlated with SABG staining [7].

Potential mechanisms of senescence in hepatocytes and the liver: as presented in detail in the previous section, multiple pathways of senescence have been described in different experimental systems. Key molecules that are already involved in senescence arrest have also been summarized. The published data on different senescence pathways in the liver is fragmented and control mechanisms involved in hepatocyte senescence are not completely understood. Therefore, existing data on hepatocellular senescence together with potential mechanisms that may be involved in this process will be presented.

For reasons previously described, almost nothing is known about molecular mechanisms involved in replicative senescence and immortalization of hepatocytes in cul-

ture. There are only a few demonstrations of hepatocyte immortalization in vitro. Thus, ectopically expressed TERT may induce hepatocyte immortalization. However, as the published data using TERT immortalization is scarce, it is highly likely that the immortalization of hepatocytes is not an easy task even with a well-established protocol that works with other epithelial cell types such as mammary epithelial cells. The mechanisms of in vitro senescence induction in hepatocytes are also mostly unknown. Rapid induction of a senescence arrest in cultured hepatocytes suggests that these cells display robust telomere-independent senescence-inducing systems that are functional in vitro. However, they remain to be discovered. It is highly likely that, similar to other somatic cells, p53 and RB pathways in general, and some CDKIs in particular are also involved in hepatocyte senescence, but the evidence is lacking for the time being.

Telomere shortening during aging is slow (55–120 base pairs per year) and stabilizes at mid age in healthy liver, so that the loss of telomeric DNA does not reach a level to induce telomere dysfunction and DNA damage response [50,51]. Other forms of telomere-independent senescence such as ROS-induced senescence may also be rare under normal physiological conditions. On the other hand, telomere loss is accelerated in chronic liver disease to reach lowest levels in the cirrhotic liver [7,51]. Therefore, one plausible mechanism involved in cirrhosis is probably telomere-dependent senescence, or replicative senescence. The relevance of replicative senescence to liver tissue aging has been demonstrated experimentally using telomerase-deficient mice. Late generation telomerase-deficient mice display critically shortened telomeres and an impaired liver growth response to partial hepatectomy. A subpopulation of telomere-shortened hepatic cells displayed impaired proliferative capacity that is associated with SABG activity [11,56]. On the other hand, it has been reported that mouse liver cells are highly resistant to extensive telomere dysfunction. Conditional deletion of the telomeric protein TRF2 in hepatocytes resulted in telomeric accumulation of phospho-H2AX and frequent telomere fusions, indicating loss of telomere protection. However, there was no induction of p53 and liver function appeared unaffected. The loss of TRF2 did not compromise liver regeneration after partial hepatectomy. Liver regeneration occurred without cell division involving endoreduplication and cell growth, thereby circumventing the chromosome segregation problems associated with telomere fusions. Thus, it appears that hepatocytes display intrinsic resistance to telomere dysfunction, although they are apparently vulnerable to severe telomere loss [57].

Hepatocyte senescence that is observed in severe chronic liver diseases such as cirrhosis may also be induced by telomere-independent pathways. Chronic liver injury observed under such conditions is accompanied with inflammation, cell death, and oxidative stress [58–60]. Some of the etiological factors such as HCV and alcohol induce mitochondrial dysfunction may result in ROS accumulation [61,62]. Thus, ROS-induced senescence may also occur during cirrhosis, although this has not yet been reported. The status of DNA damage in chronic liver disease is less well-known. 8-Hydroxydeoxyguanosine, an indicator of DNA lesions produced by ROS, was reported to be increased in chronic liver disease [63]. On the other hand, the upregulation of DNA repair enzymes in cirrhosis has also been reported [64]. Increased DNA repair activity in cirrhosis which may reflect increased DNA damages as a consequence of chronic liver injury, but also inhibition of DNA damage responses such as senescence were observed. Taken together, these observations suggest that the primary cause of senescence in cirrhotic patients is telomere dysfunction and that ROS may also play additional roles.

Among senescence-related proteins, $p16^{INK4a}$ and $p21^{Cip1}$ expression was found to be high in cirrhosis, as compared to normal liver and tumor tissues [65], suggesting that these major senescence-inducing proteins accumulate in the cirrhotic liver. Promoter methylation of these CDKIs was also studied. Chronic liver disease samples displayed lower levels of methylation as compared to HCCs [66]. Thus, the progression of chronic liver disease towards cirrhosis is accompanied with a progressive activation of different CDKIs, as expected.

5. Senescence pathway aberrations and telomerase reactivation in hepatocellular carcinoma

As stated earlier, p53 and retinoblastoma (Rb) pathways play a critical role in senescence arrest as observed in different in vitro and in vivo models. Indirect evidence suggests that these pathways may also be important in hepatocellular senescence. The accumulation of p21 and p16 in cirrhotic liver tissues has been reported independently by different reports. On the other hand, HCC rarely develops in liver tissues absent of chronic liver disease. More than 80% of these cancers are observed in patients with cirrhosis [9]. As the appearance of proliferating malignant cells from this senescence stage requires the bypass of senescence, the status of both p53 and RB pathways in HCC is of great importance in terms of molecular aspects of hepatocellular carcinogenesis.

HCC is one of the major tumors displaying frequent p53 mutations [67,68]. The overall p53 mutation frequency in HCC is around 30%. Both the frequency and the spectrum of p53 mutations show great variations between tumors from different geographical areas of the World. A hotspot mutation (codon 249 AGG → AGT) has been linked to exposure to aflatoxins which are known to be potent DNA damaging agents (for a review see Ref. [67]). Although, it is unknown whether aflatoxins are able to generate a DNA damage-dependent senescence response in hepatocytes, their association with DNA damage and p53 mutation provides indirect evidence for such an ability. Other p53 mutations described in HCCs from low aflatoxin areas may similarly be correlated with other DNA damaging agents, such as ROS which are known to accumulate in the livers of patients with chronic liver diseases, including cirrhosis.

Another player of senescence arrest, the p16 gene is rarely mutated in HCC, but its epigenetic silencing by promoter methylation is highly frequent in this cancer. More than 50% of HCCs display de novo methylation of the promoter of CDKN2A gene, encoding p16 protein, resulting in

loss of gene expression [67]. Major components of p53 and Rb pathways in the same set of HCCs with different etiologies have been analyzed [69]. Retinoblastoma pathway alterations (p16 ^{INK4a}, p15^{INK4b} or RB1 genes) were present in 83% of HCCs, whereas p53 pathway alterations (p53 or ARF genes) were detected in only 31% of tumors. Alterations in both Rb and p53 pathways were present in 30% of HCCs. Thus, it appears that either the Rb and/or the p53 pathway are affected in the great majority of HCCs, and that both pathways are affected in at least one third of these tumors. Unfortunately, p53 and p16^{INK4a} aberrations observed in HCC have not yet been studied in relation to senescence aberrations. However, these observations provide supporting evidence on the critical role of senescence-controlling pathways in the development of HCC.

The lack of telomerase activity in normal and cirrhotic liver correlates with progressive loss of telomere sequences ending up with a senescence arrest. The emergence of malignant hepatocytes from this senescence-dominated cirrhotic milieu would require not only the bypass of senescence, but also a way of survival despite critically shortened telomeres. Additionally, the proliferative expansion of neoplastic cells in order to form sustained tumor masses would require telomeres at a minimal length required to maintain intact chromosomal structures.

Many studies showed that telomerase activity is a hallmark of all human cancers, including 80–90% of HCCs [70–72]. It is currently unclear how the TERT expression is repressed and released in normal hepatocytes and HCC cells, respectively. The integration of HBV DNA sequences into TERT gene provides evidence for a virus-induced deregulation of TERT expression, but this appears to rarely occur, as only four cases have been reported thus far [73–75]. Hbx and PreS2 proteins may upregulate TERT expression [76,77]. The molecular mechanisms involved in TERT suppression in somatic cells and its reactivation in cancer cells are ill-known. The TERT promoter displays binding sites for a dozen of transcriptional regulators: estrogen receptor, Sp1, Myc and ER81 acting positively, and vitamin D receptor, MZF-2, WT1, Mad, E2F1 and SMAD interacting protein-1 (SIP1, also called ZEB-2 or ZFHX1B) acting negatively [78]. Despite high telomerase activity, telomeres in HCC were repeatedly found to be highly shortened [65,79,80]. However, 3′ telomere overhangs were found to be increased in nearly 40% HCCs [80]. Moreover, the expression of several telomeric proteins is increased in HCC [80,81].

Another ill-known aspect of TERT activity in HCC cells is the cellular origin of these malignant cells. It is presently unclear whether HCC arises from mature hepatocytes which lack telomerase activity, or stem/progenitor cell-like cells that may already express TERT at sufficient levels to maintain telomere integrity. In the non-tumor area surrounding the cancer tissue, telomerase activity could not be detected, or was detected at very low levels.

The importance of telomerase activity in HCC development has been studied experimentally using telomerase-deficient mouse model. These mice show increased susceptibility to adenoma development (tumor initiation), but they are quite resistant to fully malignant tumor development [82]. Likewise, telomerase deletion limits the progression of p53-mutant HCCs with short telomeres [83]. These observations suggest that the aberrations affecting telomerase activity and senescence controlling genes such as p53 may cooperate during hepatocellular carcinogenesis.

In summary, HCC is characterized by mutational inactivation of p53, a major player in DNA damage-induced senescence. In addition, p15^{INK4b}, p16^{INK4a}, p21^{Cip1} CDKIs are often inactivated in this cancer mostly by epigenetic mechanisms involving promoter methylation. These changes may play a critical role in the bypass of senescence that is observed in most cirrhosis cases, allowing some initiated cells to escape senescence control and proliferate. In the absence of telomerase activity such cells would probably not survive due to telomere loss. However, since more than 80% of HCCs display telomerase activity, it is highly likely that the telomerase reactivation, together with the inactivation of major CDKIs, plays a critical role in HCC development by conferring premalignant or malignant cells the ability to proliferate indefinitely (Fig. 3). However, cellular immortality is not sufficient for full malignancy [84]. Thus, senescence-related aberrations that are observed in HCC cells, may confer a partial survival advantage that would need to be complemented by other genetic or epigenetic alterations.

6. Senescence as an anti-tumor mechanism in hepatocellular carcinoma

Senescence in normal somatic cells and tissues is expected. How about cancer cells and tumors? Initial studies using different cancer cell lines provided ample evidence for the induction of senescence by different genetic as well as chemical or biological treatments [85]. Thus, it appeared that cancer cells, immortalized by definition, do have a hidden senescence program that can be revealed by different senescence-inducing stimuli. These studies provided preliminary evidence for considering senescence induction as an anti-cancer therapy. The in vivo relevance of these observations and expectations became evident only very recently. Senescence was observed in tumors or pre-neoplastic lesions. SABG activity as well as several other senescence markers were detected in lung adenomas, but not in adenocarcinomas observed in oncogenic Ras "knock-in" mice [86]. Ras-driven mouse T-cell lymphomas entered senescence after drug therapy, when apoptosis was blocked [87]. The first direct evidence of cellular senescence in humans was reported for the melanocytic nevus [88].

Senescence response of HCC cells was not the subject of intensive study until very recently. Therefore the potential role of senescence in these tumors is less well understood. Treatment of HCC cell lines with 5-aza-2-deoxycytidine induced the expression of p16^{INK4a}, hypophosphorylation of pRb and G1 arrest associated with positive SABG staining [89]. Recent findings indicate that senescence induction is a powerful mechanism of HCC regression. Xue et al. expressed H-ras oncogene and suppressed endogenous p53 expression in mouse hepatoblasts which produced massive

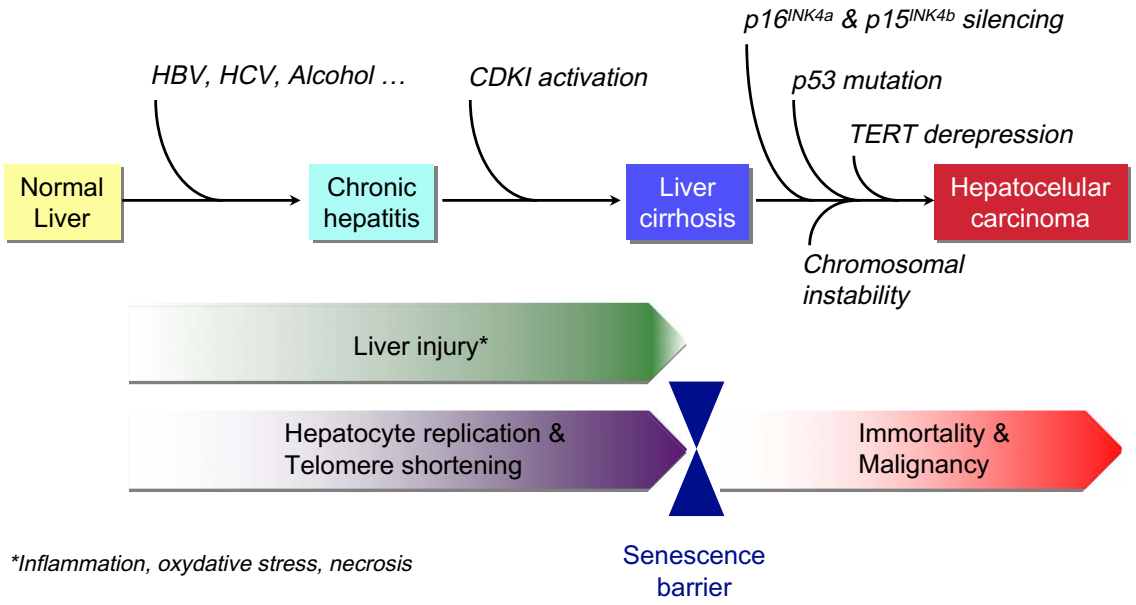

Fig. 3. Role of cellular senescence and immortalization in hepatocellular carcinogenesis. Chronic liver injury (triggered by major etiological factors HBV, HCV and alcohol) leading to cirrhosis is a common cause of HCC. Hepatocytes having no telomerase activity undergo progressive telomere shortening and DNA damage during this process. Consequently, CDKIs (primarily $p16^{INK4a}$ and $p21^{Cip1}$) are activated gradually to induce senescence in the preneoplastic cirrhosis stage. Mutation and expression analyses in HCC strongly suggest that neoplastic cells bypass the senescence barrier by inactivating major senescence-inducing genes (p53, $p16^{INK4a}$ and $p15^{INK4b}$). Moreover, they acquire the ability of unlimited proliferation (immortality) by re-expressing the TERT enzyme. Chromosomal instability that is generated by telomere erosion may contribute to additional mutations necessary for tumor progression.

HCCs upon implantation into livers of athymic mice [12]. However, these tumors regressed rapidly upon restoration of p53 expression. Tumor regression was due to differentiation and massive senescence induction, followed by immune-mediated clearance of senescent cells. These observations may indicate that oncogene-induced senescence is also involved in HCC. On the other hand, HCCs induced by tet-regulated c-Myc activation in mouse liver cells differentiate into mature hepatocytes and biliary cells or undergo senescence [90]. Thus, senescence induction may also be relevant to oncogene inactivation in HCC. In this regard, c-Myc down-regulation and senescence induction in several HCC cell lines as a response to TGF-β was observed (S. Senturk, M. Ozturk, unpublished data).

So far, all the reported examples of senescence induction in HCC cells are in the form of a telomere-independent permanent cell cycle arrest. Until recently, it was unknown whether replicative senescence could also be induced in immortal cancer cells. Ozturk et al. reported recently that immortal HCC cells can revert spontaneously to a replicative senescence phenotype [91]. Immortal HCC cells generated progeny that behaved, in vitro, similar to normal somatic cells. Such senescence-programmed progeny lacked telomerase activity due to TERT repression (probably mediated by SIP1 gene), and displayed progressive telomere shortening in cell culture, resulting in senescence arrest. It will be interesting to test whether such spontaneous reversal of replicative immortality is involved in well known tumor dormancy and/or spontaneous tumor regression.

7. Concluding remarks

Cellular senescence has gained great interest in recent years following the demonstration that it also occurs in vivo. It is also highly interesting that senescence can be mediated by a large number of pathways and molecules, as is the case for apoptosis. Recent findings that implicate secreted molecules in senescence induction strongly suggest that cellular senescence is not just a cellular event, but also a physiologically relevant process for the whole organism. In terms of tumor biology, oncogene-induced senescence that may serve as anti-tumor mechanism in pre-neoplastic lesions underlines its clinical relevance. On the other hand, induced or spontaneous senescence that is observed in cancer cells is promising to explore new approaches for tumor prevention and treatment. The role of senescence bypass and cellular immortality in hepatocellular carcinogenesis is not well defined. But, many findings (inactivation of senescence-mediator genes such as p53, $p16^{INK4a}$ and $p15^{INK4b}$, as well as reactivation of TERT) indicate that senescence mechanisms and their aberrations are critically involved in HCC. We may expect that this field will attract more attention in coming years for a better definition of senescence implications in hepatocellular carcinogenesis.

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References

[1] D.E. Crew (Ed.), Human senescence-evolutionary and biocultural perspectives, Cambridge University Press, Cambridge, 2003.
[2] L. Hayflick, The limited in vitro lifetime of human diploid cell strains, Exp. Cell Res. 37 (1965) 614–636.
[3] J. Campisi, F. d'Adda di Fagagna, Cellular senescence: when bad things happen to good cells, Nat. Rev. Mol. Cell Biol. 8 (2007) 729–740.
[4] K.L. Rudolph, S. Chang, H.W. Lee, M. Blasco, G.J. Gottlieb, C. Greider, R.A. DePinho, Longevity, stress response, and cancer in aging telomerase-deficient mice, Cell 96 (1999) 701–712.
[5] G.K. Michalopoulos, Liver regeneration, J. Cell Physiol. 213 (2007) 286–300.
[6] V. Paradis, N. Youssef, D. Dargere, N. Ba, F. Bonvoust, J. Deschatrette, P. Bedossa, Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas, Hum. Pathol. 32 (2001) 327–332.
[7] S.U. Wiemann, A. Satyanarayana, M. Tsahuridu, H.L. Tillmann, L. Zender, J. Klempnauer, P. Flemming, S. Franco, M.A. Blasco, M.P. Manns, K.L. Rudolph, Hepatocyte telomere shortening and senescence are general markers of human liver cirrhosis, FASEB J. 16 (2002) 935–942.
[8] V. Trak-Smayra, J. Contreras, F. Dondero, F. Durand, S. Dubois, D. Sommacale, P. Marcellin, J. Belghiti, C. Degott, V. Paradis, Role of replicative senescence in the progression of fibrosis in hepatitis C virus (HCV) recurrence after liver transplantation, Transplantation 77 (2004) 1755–1760.
[9] H.B. El-Serag, K.L. Rudolph, Hepatocellular carcinoma: epidemiology and molecular carcinogenesis, Gastroenterology 132 (2007) 2557–2576.
[10] K.L. Rudolph, S. Chang, M. Millard, N. Schreiber-Agus, R.A. DePinho, Inhibition of experimental liver cirrhosis in mice by telomerase gene delivery, Science 287 (2000) 1253–1258.
[11] A. Satyanarayana, S.U. Wiemann, J. Buer, J. Lauber, K.E. Dittmar, T. Wüstefeld, M.A. Blasco, M.P. Manns, K.L. Rudolph, Telomere shortening impairs organ regeneration by inhibiting cell cycle re-entry of a subpopulation of cells, EMBO J. 22 (2003) 4003–4013.
[12] W. Xue, L. Zender, C. Miething, R.A. Dickins, E. Hernando, V. Krizhanovsky, C. Cordon-Cardo, S.W. Lowe, Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas, Nature 445 (2007) 656–660.
[13] T. de Lange, Shelterin: the protein complex that shapes and safeguards human telomeres, Genes Dev. 19 (2005) 2100–2110.
[14] E.H. Blackburn, Structure and function of telomeres, Nature 350 (1991) 569–573.
[15] Y.S. Cong, W.E. Wright, J.W. Shay, Human telomerase and its regulation, Microbiol. Mol. Biol. Rev. 66 (2002) 407–425.
[16] J. Karlseder, A. Smogorzewska, T. de Lange, Senescence induced by altered telomere state, Not telomere loss, Science 295 (2002) 2446–2449.
[17] R.E. Verdun, J. Karlseder, Replication and protection of telomeres, Nature 447 (2007) 924–931.
[18] F. d'Adda di Fagagna, P.M. Reaper, L. Clay-Farrace, H. Fiegler, P. Carr, T. Von Zglinicki, G. Saretzki, N.P. Carter, S.P. Jackson, A DNA damage checkpoint response in telomere-initiated senescence, Nature 426 (2003) 194–198.
[19] M. Serrano, A.W. Lin, M.E. McCurrach, D. Beach, S.W. Lowe, Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a, Cell 88 (1997) 593–602.
[20] R. Di Micco, M. Fumagalli, d'Adda F. di Fagagna, Breaking news: high-speed race ends in arrest-how oncogenes induce senescence, Trends Cell Biol. 17 (2007) 529–536.
[21] W.J. Mooi, D.S. Peeper, Oncogene-induced cell senescence-halting on the road to cancer, N. Engl. J. Med. 355 (2006) 1037–1046.
[22] Z. Chen, L.C. Trotman, D. Shaffer, H.K. Lin, Z.A. Dotan, M. Niki, J.A. Koutcher, H.I. Scher, T. Ludwig, W. Gerald, C. Cordon-Cardo, P.P. Pandolfi, Crucial role of p53-dependent cellular senescence in suppression of Pten-deficient tumorigenesis, Nature 436 (2005) 725–730.
[23] R. Di Micco, M. Fumagalli, A. Cicalese, S. Piccinin, P. Gasparini, C. Luise, C. Schurra, M. Garre', P.G. Nuciforo, A. Bensimon, R. Maestro, P.G. Pelicci, F. d'Adda di Fagagna, Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication, Nature 444 (2006) 638–642.
[24] J. Bartkova, N. Rezaei, M. Liontos, P. Karakaidos, D. Kletsas, N. Issaeva, L.V. Vassiliou, E. Kolettas, K. Niforou, V.C. Zoumpourlis, M. Takaoka, H. Nakagawa, F. Tort, K. Fugger, F. Johansson, M. Sehested, C.L. Andersen, L. Dyrskjot, T. Ørntoft, J. Lukas, C. Kittas, T. Helleday, T.D. Halazonetis, J. Bartek, V.G. Gorgoulis, Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints, Nature 444 (2006) 633–637.
[25] T. Lu, T. Finkel, Free radicals and senescence, Exp. Cell Res. 314 (2008) 1918–1922.
[26] M. Giorgio, M. Trinei, E. Migliaccio, P.G. Pelicci, Hydrogen peroxide: a metabolic by-product or a common mediator of ageing signals?, Nat Rev. Mol. Cell Biol. 8 (2007) 722–728.
[27] C. Blanchetot, J. Boonstra, The ROS-NOX connection in cancer and angiogenesis, Crit. Rev. Eukaryot. Gene Expr. 18 (2008) 35–45.
[28] T. von Zglinicki, G. Saretzki, W. Döcke, C. Lotze, Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence?, Exp Cell Res. 220 (1995) 186–193.
[29] A.C. Lee, B.E. Fenster, H. Ito, K. Takeda, N.S. Bae, T. Hirai, Z.X. Yu, V.J. Ferrans, B.H. Howard, T. Finkel, Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species, J. Biol. Chem. 274 (1999) 7936–7940.
[30] S. Courtois-Cox, S.L. Jones, K. Cichowski, Many roads lead to oncogene-induced senescence, Oncogene 27 (2008) 2801–2809.
[31] J.F. Passos, G. Saretzki, S. Ahmed, G. Nelson, T. Richter, H. Peters, I. Wappler, M.J. Birket, G. Harold, K. Schaeuble, M.A. Birch-Machin, T.B. Kirkwood, T. von Zglinicki, Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence, PLoS Biol. 5 (2007) e110.
[32] K. Ito, A. Hirao, F. Arai, K. Takubo, S. Matsuoka, K. Miyamoto, M. Ohmura, K. Naka, K. Hosokawa, Y. Ikeda, T. Suda, Reactive oxygen species act through p38 MAPK to limit the lifespan of hematopoietic stem cells, Nat. Med. 12 (2006) 446–451.
[33] U. Herbig, W.A. Jobling, B.P. Chen, D.J. Chen, J.M. Sedivy, Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a) but not p16(INK4a), Mol. Cell. 14 (2004) 501–513.
[34] F.A. Mallette, M.F. Gaumont-Leclerc, G. Ferbeyre, The DNA damage signaling pathway is a critical mediator of oncogene-induced senescence, Genes Dev. 21 (2007) 43–48.
[35] J. Gil, G. Peters, Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all, Nat. Rev. Mol. Cell Biol. 7 (2006) 667–677.
[36] D.A. Alcorta, Y. Xiong, D. Phelps, G. Hannon, D. Beach, J.C. Barrett, Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts, Proc. Natl. Acad. Sci. U S A 93 (1996) 13742–13747.
[37] F. Zindy, D.E. Quelle, M.F. Roussel, C.J. Sherr, Expression of the p16INK4a tumor suppressor versus other INK4 family members during mouse development and aging, Oncogene 15 (1997) 203–211.
[38] R. Tremain, M. Marko, V. Kinnimulki, H. Ueno, E. Bottinger, A. Glick, Defects in TGF-beta signaling overcome senescence of mouse keratinocytes expressing v-Ha-ras, Oncogene 19 (2000) 1698–1709.
[39] N. Wajapeyee, R.W. Serra, X. Zhu, M. Mahalingam, M.R. Green, Oncogenic BRAF induces senescence and apoptosis through pathways mediated by the secreted protein IGFBP7, Cell 132 (2008) 363–374.
[40] J.C. Acosta, A. O'Loghlen, A. Banito, M.V. Guijarro, A. Augert, S. Raguz, M. Fumagalli, M. Da Costa, C. Brown, N. Popov, Y. Takatsu, J. Melamed, F. d'Adda di Fagagna, D. Bernard, E. Hernando, J. Gil, Chemokine signaling via the CXCR2 receptor reinforces senescence, Cell 133 (2008) 1006–1018.
[41] T. Kuilman, C. Michaloglou, L.C. Vredeveld, S. Douma, R. van Doorn, C.J. Desmet, L.A. Aarden, W.J. Mooi, D.S. Peeper, Oncogene-induced senescence relayed by an interleukin-dependent inflammatory network, Cell 133 (2008) 1019–1031.
[42] W.S. el-Deiry, T. Tokino, V.E. Velculescu, D.B. Levy, R. Parsons, J.M. Trent, D. Lin, W.E. Mercer, K.W. Kinzler, B. Vogelstein, WAF1, a potential mediator of p53 tumor suppression, Cell 75 (1993) 817–825.
[43] Y. Deng, S.S. Chan, S. Chang, Telomere dysfunction and tumor suppression: the senescence connection, Nat. Rev. Cancer 8 (2008) 450–458.
[44] L. Fang, M. Igarashi, J. Leung, M.M. Sugrue, S.W. Lee, S.A. Aaronson, p21Waf1/Cip1/Sdi1 induces permanent growth arrest with markers

of replicative senescence in human tumor cells lacking functional p53, Oncogene 18 (1999) 2789–2797.
[45] C. Giacinti, A. Giordano, RB and cell cycle progression, Oncogene 25 (2006) 5220–5227.
[46] M. Narita, S. Nunez, E. Heard, A.W. Lin, S.A. Hearn, D.L. Spector, G.J. Hannon, S.W. Lowe, Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence, Cell 113 (2003) 703–716.
[47] R. Zhang, M.V. Poustovoitov, X. Ye, H.A. Santos, W. Chen, S.M. Daganzo, J.P. Erzberger, I.G. Serebriiskii, A.A. Canutescu, R.L. Dunbrack, J.R. Pehrson, J.M. Berger, P.D. Kaufman, P.D. Adams, Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA, Dev. Cell 8 (2005) 19–30.
[48] M. Narita, M. Narita, V. Krizhanovsky, S. Nunez, A. Chicas, S.A. Hearn, M.P. Myers, S.W. Lowe, A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation, Cell 126 (2006) 503–514.
[49] P.D. Adams, Remodeling of chromatin structure in senescent cells and its potential impact on tumor suppression and aging, Gene 397 (2007) 84–93.
[50] K. Takubo, N. Izumiyama-Shimomura, N. Honma, M. Sawabe, T. Arai, M. Kato, M. Oshimura, K. Nakamura, Telomere lengths are characteristic in each human individual, Exp. Gerontol. 37 (2002) 523–531.
[51] H. Aikata, H. Takaishi, Y. Kawakami, S. Takahashi, M. Kitamoto, T. Nakanishi, Y. Nakamura, F. Shimamoto, G. Kajiyama, T. Ide, Telomere reduction in human liver tissues with age and chronic inflammation, Exp. Cell Res. 256 (2000) 578–582.
[52] R. Utoh, C. Tateno, C. Yamasaki, N. Hiraga, M. Kataoka, T. Shimada, K. Chayama, K. Yoshizato, Susceptibility of chimeric mice with livers repopulated by serially subcultured human hepatocytes to hepatitis B virus, Hepatology 47 (2008) 435–446.
[53] M. Delhaye, H. Louis, C. Degraef, O. Le Moine, J. Devière, B. Gulbis, D. Jacobovitz, M. Adler, P. Galand, Relationship between hepatocyte proliferative activity and liver functional reserve in human cirrhosis, Hepatology 23 (1996) 1003–1011.
[54] M.R. Stampfer, P. Yaswen, Human epithelial cell immortalization as a step in carcinogenesis, Cancer Lett. 194 (2003) 199–208.
[55] H. Wege, H.T. Le, M.S. Chui, L. Lui, J. Wu, G. Giri, H. Malhi, B.S. Sappal, V. Kumaran, S. Gupta, M.A. Zern, Telomerase reconstitution immortalized human fetal hepatocytes without disrupting their differentiation potential, Gastroenterology 124 (2003) 432–444.
[56] A. Lechel, A. Satyanarayana, Z. Ju, R.R. Plentz, S. Schaetzlein, C. Rudolph, L. Wilkens, S.U. Wiemann, G. Saretzki, N.P. Malek, M.P. Manns, J. Buer, K.L. Rudolph, The cellular level of telomere dysfunction determines induction of senescence or apoptosis in vivo, EMBO Rep. 6 (2005) 275–281.
[57] E. Lazzerini Denchi, G. Celli, T. de Lange, Hepatocytes with extensive telomere deprotection and fusion remain viable and regenerate liver mass through endoreduplication, Genes Dev. 20 (2006) 2648–2653.
[58] G. Szabo, P. Mandrekar, A. Dolganiuc, Innate immune response and hepatic inflammation, Semin. Liver Dis. 27 (2007) 339–350.
[59] H. Malhi, G.J. Gores, Cellular and molecular mechanisms of liver injury, Gastroenterology 134 (2008) 1641–1654.
[60] D. Schuppan, N.H. Afdhal, Liver cirrhosis, Lancet 371 (2008) 838–851.
[61] F. Farinati, R. Cardin, M. Bortolami, P. Burra, F.P. Russo, M. Rugge, M. Guido, A. Sergio, R. Naccarato, Hepatitis C virus: from oxygen free radicals to hepatocellular carcinoma, J. Viral Hepat. 14 (2007) 821–829.
[62] A. Reuben, Alcohol and the liver, Curr. Opin. Gastroenterol. 24 (2008) 328–338.
[63] R. Shimoda, M. Nagashima, M. Sakamoto, N. Yamaguchi, S. Hirohashi, J. Yokota, H. Kasai, Increased formation of oxidative DNA damage, 8-hydroxydeoxyguanosine, in human livers with chronic hepatitis, Cancer Res. 54 (1994) 3171–3172.
[64] P. Zindy, L. Andrieux, D. Bonnier, O. Musso, S. Langouët, J.P. Campion, B. Turlin, B. Clément, N. Théret, Upregulation of DNA repair genes in active cirrhosis associated with hepatocellular carcinoma, FEBS Lett. 579 (2005) 95–99.
[65] R.R. Plentz, Y.N. Park, A. Lechel, H. Kim, F. Nellessen, B.H. Langkopf, L. Wilkens, A. Destro, B. Fiamengo, M.P. Manns, M. Roncalli, K.L. Rudolph, Telomere shortening and inactivation of cell cycle checkpoints characterize human hepatocarcinogenesis, Hepatology 45 (2007) 968–976.
[66] M. Roncalli, P. Bianchi, B. Bruni, L. Laghi, A. Destro, S. Di Gioia, L. Gennari, M. Tommasini, A. Malesci, G. Coggi, Methylation framework of cell cycle gene inhibitors in cirrhosis and associated hepatocellular carcinoma, Hepatology 36 (2002) 427–432.
[67] M. Ozturk, Genetic aspects of hepatocellular carcinogenesis, Semin. Liver Dis. 19 (1999) 235–242.
[68] T. Soussi, p53 alterations in human cancer: more questions than answers, Oncogene 26 (2007) 2145–2156.
[69] Y. Edamoto, A. Hara, W. Biernat, L. Terracciano, G. Cathamos, H.M. Riehle, M. Matsuda, H. Fuji, J.M. Scoazec, H. Ohgaki, Alterations of RB1, p53 and Wnt pathways in Hepatocellular carcinomas associated with HCV, HBV and alcoholic liver cirrhosis, Int. J. Cancer 106 (2003) 334–341.
[70] H. Tahara, T. Nakanishi, M. Kitamoto, R. Nakashio, J.W. Shay, E. Tahara, G. Kajiyama, T. Ide, Telomerase activity in human liver tissues: Comparison between chronic liver disease and hepatocellular carcinomas, Cancer Res. 55 (1995) 2734–2736.
[71] H. Kojima, O. Yokosuka, F. Imazeki, H. Saisho, M. Omata, Telomerase activity and telomere length in hepatocellular carcinoma and chronic liver disease, Gastroenterology 112 (1997) 493–500.
[72] J. Nakayama, H. Tahara, E. Tahara, M. Saito, K. Ito, H. Nakamura, T. Nakanishi, E. Tahara, T. Ide, F. Ishikawa, Telomerase activation by hTERT in human normal fibroblasts and hepatocellular carcinomas, Nat. Genet. 18 (1998) 65–68.
[73] D. Gozuacik, Y. Murakami, K. Saigo, M. Chami, C. Mugnier, D. Lagorce, T. Okanoue, T. Urashima, C. Bréchot, P. Paterlini-Bréchot, Identification of human cancer-related genes by naturally occurring Hepatitis B Virus DNA tagging, Oncogene 20 (2001) 6233–6240.
[74] I. Horikawa, JC. Barrett, Transcriptional regulation of the telomerase hTERT gene as a target for cellular and viral oncogenic mechanisms, Carcinogenesis 24 (2003) 1167–1176.
[75] Y. Murakami, K. Saigo, H. Takashima, M. Minami, T. Okanoue, C. Bréchot, P. Paterlini-Bréchot, Large scaled analysis of hepatitis B virus (HBV) DNA integration in HBV related hepatocellular carcinomas, Gut 54 (2005) 1162–1168.
[76] Z.L. Qu, S.Q. Zou, N.Q. Cui, X.Z. Wu, M.F. Qin, D. Kong, Z.L. Zhou, Upregulation of human telomerase reverse transcriptase mRNA expression by in vitro transfection of hepatitis B virus X gene into human hepatocarcinoma and cholangiocarcinoma cells, World J. Gastroenterol. 11 (2005) 5627–5632.
[77] H. Liu, F. Luan, Y. Ju, H. Shen, L. Gao, X. Wang, S. Liu, L. Zhang, W. Sun, C. Ma, In vitro transfection of the hepatitis B virus PreS2 gene into the human hepatocarcinoma cell line HepG2 induces upregulation of human telomerase reverse transcriptase, Biochem. Biophys. Res. Commun. 355 (2007) 379–384.
[78] R. Janknecht, On the road to immortality: hTERT upregulation in cancer cells, FEBS Lett. 564 (2004) 9–13.
[79] N. Miura, I. Horikawa, A. Nishimoto, H. Ohmura, H. Ito, S. Hirohashi, J.W. Shay, M. Oshimura, Progressive telomere shortening and telomerase reactivation during hepatocellular carcinogenesis, Cancer Genet. Cytogenet. 93 (1997) 56–62.
[80] J.E. Lee, B.K. Oh, J. Choi, Y.N. Park, Telomeric 3' overhangs in chronic HBV-related hepatitis and hepatocellular carcinoma, Int. J. Cancer 123 (2008) 264–272.
[81] B.K. Oh, Y.J. Kim, C. Park, Y.N. Park, Up-regulation of telomere-binding proteins, TRF1, TRF2, and TIN2 is related to telomere shortening during human multistep hepatocarcinogenesis, Am. J. Pathol. 166 (2005) 73–80.
[82] A. Satyanarayana, M.P. Manns, K.L. Rudolph, Telomeres and Telomerase: a Dual role in hepatocarcinogenesis, Hepatology 40 (2004) 276–283.
[83] A. Lechel, H. Holstege, Y. Begus, A. Schienke, K. Kamino, U. Lehmann, S. Kubicka, P. Schirmacher, J. Jonkers, K.L. Rudolph, Telomerase deletion limits progression of p53-mutant hepatocellular carcinoma with short telomeres in chronic liver disease, Gastroenterology 132 (2007) 1465–1475.
[84] W.C. Hahn, C.M. Counter, A.S. Lundberg, R.L. Beijersbergen, M.W. Brooks, R.A. Weinberg, Creation of human tumor cells with defined genetic elements, Nature 400 (1999) 464–468.
[85] J.W. Shay, I.B. Roninson, Hallmarks of senescence in carcinogenesis and cancer therapy, Oncogene 23 (2004) 2919–2933.
[86] M. Collado, J. Gil, A. Efeyan, C. Guerra, A.J. Schuhmacher, M. Barradas, A. Benguría, A. Zaballos, J.M. Flores, M. Barbacid, D. Beach, M. Serrano, Tumour biology: senescence in premalignant tumours, Nature 436 (2005) 642.
[87] M. Braig, S. Lee, C. Loddenkemper, C. Rudolph, A.H. Peters, B. Schlegelberger, H. Stein, B. Dörken, T. Jenuwein, C.A. Schmitt, Oncogene-induced senescence as an initial barrier in lymphoma development, Nature 436 (2005) 660–665.

[88] C. Michaloglou, L.C. Vredeveld, M.S. Soengas, C. Denoyelle, T. Kuilman, C.M. van der Horst, D.M. Majoor, J.W. Shay, W.J. Mooi, D.S. Peeper, BRAFE600-associated senescence-like cell cycle arrest of human naevi, Nature 436 (2005) 720–724.
[89] S.I. Suh, H.Y. Pyun, J.W. Cho, W.K. Baek, J.B. Park, T. Kwon, J.W. Park, M.H. Suh, D.A. Carson, 5-Aza-2'-deoxycytidine leads to down-regulation of aberrant p16 RNA transcripts and restores the functional retinoblastoma protein pathway in hepatocellular carcinoma cell lines, Cancer Lett. 160 (2000) 81–88.
[90] C.H. Wu, J. van Riggelen, A. Yetil, A.C. Fan, P. Bachireddy, D.W. Felsher, Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation, Proc. Natl. Acad. Sci. USA 104 (2007) 13028–13033.
[91] N. Ozturk, E. Erdal, M. Mumcuoglu, K.C. Akcali, O. Yalcin, S. Senturk, A. Arslan-Ergul, B. Gur, I. Yulug, R. Cetin-Atalay, C. Yakicier, T. Yagci, M. Tez, M. Ozturk, Reprogramming of replicative senescence in hepatocellular carcinoma-derived cells, Proc. Natl. Acad. Sci. USA 103 (2006) 2178–2183.