Chapter 13
Stress-Induced (Premature) Senescence

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Abstract Three main roads lead to senescence: telomere-dependent replicative senescence, oncogene-induced senescence and stress-induced (premature) senescence. This latter type of senescence appears after exposure of normal, immortalized or transformed cells to stress of chemical or physical nature inducing oxidative stress and/or DNA damage. After these exposures, chronic or acute, single or multiple, stressed cells developed a "senescence-like" phenotype. This "senescence-like" phenotype presents several biomarkers of cellular senescence such as irreversible growth arrest, morphological change, senescence-associated ß-galactosidase (SA-ßgal) activity and senescence-associated secretory phenotype (SASP). However, large-scale studies of transcriptome and proteome of cells in replicative senescence or in stress-induced senescence show that although they share similarities, the two phenotypes are not identical. Different signaling pathways involved in the development of stress-induced senescence are presented as those dependent on TGF-ß1, $p38^{MAPK}$, IGF-R1 and DNA damage. The possible induction of this type of senescence *in vivo* and in cancer treatment is discussed.

Keywords Replicative senescence • Cell cycle • Stress • SIPS • Oxidation • DNA damage • Telomeres

13.1 Introduction

Ageing is characterized by a progressive functional decline, leading to increased risk of developing major human pathologies, such as cardiovascular disorders, cancer, diabetes and neurodegenerative diseases. Nine hallmarks of ageing have been established, including cellular senescence (Lopez-Otin et al. 2013). Cellular senescence, first described *in vitro* in normal human diploid fibroblasts (HDFs)

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by L. Hayflick in the early 1960s, is defined as an irreversible arrest of the cellular divisions and for this reason also called “replicative senescence” (RS) (Hayflick and Moorhead 1961). Almost 30 years later, it was shown that this type of senescence is associated with a « critical » shortening of the telomeres after extensive proliferation in the absence of endogenous telomerase activity (Harley et al. 1990).

After the discovery of cellular senescence, studies were performed to characterize the senescent phenotype and to identify specific biomarkers that allow to distinguish senescent cells from other non-dividing cells (such as quiescent or terminally differentiated cells). Senescence-associated biomarkers are among others typical morphology (Bayreuther et al. 1988), irreversible growth arrest with overexpression of $p21^{WAF-1}$ and $p16^{INK-4a}$ (Alcorta et al. 1996; Sherr and Roberts 1999), senescence-associated beta-galactosidase activity (SA-ßgal) (Dimri et al. 1995), altered gene expression (Dumont et al. 2000b), senescence-associated heterochromatin foci (SAHFs) and senescence-associated DNA-damage foci (SDFs) (d’Adda di Fagagna et al. 2003) and lamin B1 loss (Freund et al. 2012). Using some of these biomarkers such as SA-ßgal and $p16^{INK-4a}$ overexpression, it was demonstrated that senescent cells accumulate in tissues with age (Dimri et al. 1995; Ressler et al. 2006).

The role of senescent cells *in vivo* is still unclear, but from their discovery, it has been speculated that they may be involved in the functional decline associated with ageing. In 2011, Baker et al. (2011) demonstrated that clearance of senescent cells in mice delays ageing-associated disorders as cataract and muscular mass loss, demonstrating the impact of senescent cells on their cellular and matrix environment and on tissue function. This impact is more than probably related to their ability to secrete a variety of factors such as inflammatory cytokines, metalloproteinases and growth factors, identified as the senescence-associated secretory phenotype (SASP) (Coppe et al. 2008).

13.2 Different Types of Senescence

Many studies have shown that multiple ways can lead to senescence or at least to a “senescence-like” phenotype. Senescence can be induced by multiple stimuli such as shortened or dysfunctional telomeres, excessive mitogenic signals (including those produced by oncogenes) and stress. Three major types of senescence may thus be highlighted: replicative senescence or telomere-dependent senescence, oncogene-induced senescence and stress-induced (premature) senescence. These three types of senescence share a common senescent phenotype, as highlighted by the appearance of senescence-associated biomarkers (Fig. 13.1).

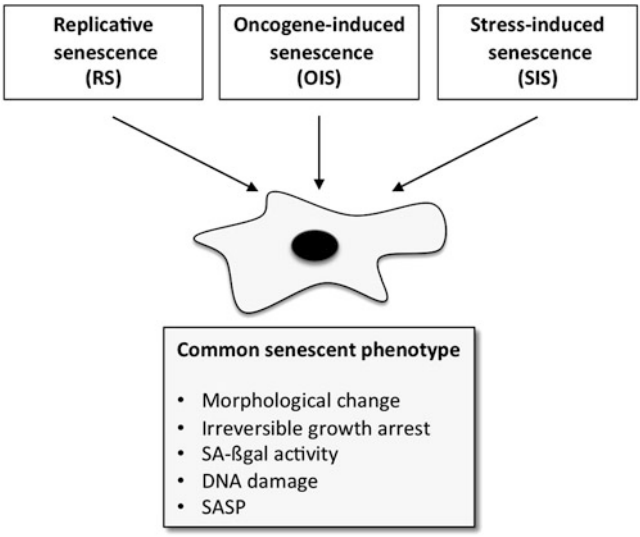

Fig. 13.1 Replicative senescence, oncogene-induced senescence and stress-induced senescence share a common senescent phenotype presenting several of the so-called senescence-associated biomarkers

13.2.1 Replicative Senescence or Telomere-Dependent Senescence

Replicative senescence is intimately linked to telomere shortening. Telomeres are sequences of repetitive DNA (5′-TTAGGG-3′ in vertebrates) located at the extremities of linear chromosomes and associated to telomere-specific protein complex, also known as shelterin/telosome, including TRF1, TRF2, TIN2, POT1, TPP1 and hRAP1 (for a review: Palm and de Lange 2008). Length of telomeres are shortened after each cellular division, this is inherently linked to the activity of the DNA polymerase which is not able to completely replicate one strand of DNA, also referred as the « end-replication problem » (Harley et al. 1990). This leads to critically short and dysfunctional telomeres, which are sensed by the cells as double-strand breaks (DSBs). There follows activation of a classical DNA-damage response (DDR) inducing activation of upstream kinases as ataxia telangectasia mutated (ATM) and ATM and Rad-3 related (ATR), DNA-damage adaptator proteins as MDC1 (mediator of DNA-damage checkpoint protein-1), 53BP1 (p53-binding protein-1), BRCA1 (breast cancer type-1 susceptibility protein) and claspin, downstream kinases such as checkpoint-1 and -2 (CHK1 and CHK2) and finally effectors proteins as p53, CDC25 and SMC1 (structural maintenance of chromosomes protein-1). This activated pathway induces cell cycle arrest, mainly by phosphorylating p53, activating the expression of $p21^{WAF-1}$ inhibiting CDK-cyclin complexes and triggering DNA repair (Campisi and d'Adda di Fagagna 2007; d'Adda di Fagagna et al. 2003).

13.2.2 Oncogene-Induced Senescence

Oncogenes are mutant version of normal genes that have the potential to transform cells in combination with additional mutations, notably leading to inactivation of tumor-suppressor genes such as p53 or $p16^{ink\text{-}4a}$. Oncogenes activation was shown to induce senescence in normal cells. This response was presented as a way to prevent oncogenic transformation. This was first observed by the expression of oncogenic *ras* ($H\text{-}RAS^{G\ V12}$) form in normal fibroblasts leading to growth arrest and SA-ßgal activity (Serrano et al. 1997), independently of the telomere shortening (Wei and Sedivy 1999). Oncogene-induced senescence (OIS) has been displayed by many oncogenes *in vitro* and *in vivo* including members of Ras signalling pathway as *N-Ras*, *Raf*, $BRAF^{E600}$ (for a review: Gorgoulis and Halazonetis 2010). Senescence can also be induced by the expression of mutated form of tumor suppressor genes like *PTEN*, *VHL*, *RB1*, and *NF-1* leading to their inactivation (Nardella et al. 2011).

13.2.3 Stress-Induced Senescence: Premature or Not?

Senescence can finally appear after exposure of normal, immortalized or transformed cells to stress from physical or chemical agents inducing oxidative stress and/or DNA damage. This was called « Stress Induced Premature Senescence » or SIPS (Toussaint et al. 2000). "Premature" refers that senescence following stress occurs at earlier population doublings (PDs) than the maximum number of PDs at which appears usually replicative senescence. "Premature" emphasizes the accelerated nature of the process. However, the use of the "premature" term may be questionable.

First because the *in vitro* cell culture conditions are still not ideal and that the definition of a "classical" number of passages at which cells enter in senescence is dependent of many variables of cell culture conditions. For instance current cell culture conditions consist in maintaining cells at atmospheric oxygen concentration (21 %). However, *in vivo*, cells are exposed to a reduced oxygen concentration ranging between tissues from 3 to 4 % in the brain (Dings et al. 1998), 3–7 % in the muscles (Vedsted et al. 2006), 5–7 % in the dermis and 16–19 % in the epidermis (Stucker et al. 2002). HDFs maintained in culture at a more physiological/reduced oxygen pressure, are able to achieve more passages before undergoing replicative senescence than cells maintained at atmospheric partial oxygen pressure (Packer and Fuehr 1977). From then on, one may wonder what the standard is and if the cells maintained at 21 % oxygen are not themselves subject to some oxidative stress (Toussaint et al. 2011). We understand here the difficulty of setting a classical or a premature onset of senescence. However, the culture conditions are identical between stressed and control cells, "premature" insists on the quickening of the process between the two conditions.

Second, transformed cells are also able to undergo senescence after stress. The mechanisms of growth arrest might be extremely different between cancer cells,

whose cycle is by definition deregulated for many possible reasons, and normal cells exposed to stressful agents. In that case, can we really speak of "premature" senescence, since these cells, by nature, do not enter in senescence?

These examples, beyond the debate on whether the use of the term "premature" is adequate, demonstrate the multiplicity of senescence-like phenotypes. To encompass all forms of senescence induced by stress, it therefore seems more prudent in this review to talk about "stress-induced senescence" or SIS (Dierick et al. 2003; Hornsby 2010).

13.3 Models of Stress-Induced Senescence

In vivo, cells are continuously exposed to different types of stress. Depending on the nature of the stress, its intensity and the cell type, stressed cells can select one of the three main tracks: repair, die or senesce. If the intensity of the stress is low, the cell can repair damage and after a transient cell cycle arrest, resumes its growth. If the stress is intense, apoptosis will be privileged. There is therefore a "moderate" stress zone for which cells exposed to high stress, but still in the subcytotoxic range, will not be able to repair all damage caused by the stress. With reference to the thermodynamic theory of far from equilibrium open systems, cells will drop to a lower steady state of internal entropy (with lower metabolic activity) and if exceeded, its cell cycle will be irreversibly arrested and cells will enter in premature senescence (Toussaint et al. 2002).

Potentially almost any stress from chemical or physical nature can induce cells to undergo senescence by provoking increased oxidative stress and/or DNA damage. Per se, the types of stress that can induce senescence seem infinite and many models of SIS exist which will not be possible to list here. However, we can describe the main classes of stress models that can induce stress-induced senescence: physical stress (UV and X-irradiation), oxidizing agents and hyperoxia, cytokines/adipokines, chemotherapeutic drugs and copper (Fig. 13.2). In the different models, the conditions used reduce the growth rate and the maximum obtainable cumulative population doublings (CPDs) of cells and induce the appearance of senescence-associated biomarkers.

Cellular senescence can be induced by both chronic or acute stress protocols in different cell types such as normal HDFs, endothelial cells, melanocytes and transformed cells. Both protocols use sublethal concentrations of stressors, in accordance with theoretical studies based on the stability of cellular systems (Toussaint et al. 1991).

Chronic stress protocols consist of treating different human cell types with prolonged exposure (several weeks) to a stressor like mild hyperoxia in HDFs (with oxygen partial concentration around 40 %) (von Zglinicki et al. 1995, 2000), chronic exposure of endothelial cells to homocysteine (Xu et al. 2000), prolonged culture of human umbilical vein endothelial cells on glycated collagen (Chen et al. 2002) and chronic exposure of colon fibroblasts to selenite (Rudolf et al. 2014).

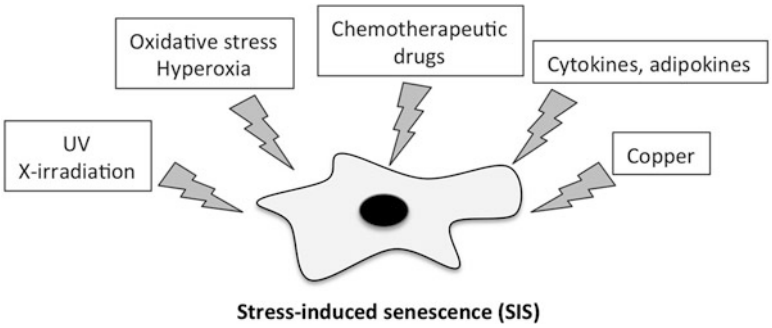

Fig. 13.2 Several types of stress can induce stress-induced senescence (SIS)

In the second type of protocol, cells, such as HDFs, at early CPDs are exposed once or several times to acute sublethal stress such as a unique H_2O_2 exposure (Chen and Ames 1994) or a repeated exposure to *tert*-butylhydroperoxide (*t*-BHP) (Dumont et al. 2000b). The use of repeated sublethal stresses allow reducing the concentration of the stressor necessary to trigger stress-induced senescence. The major advantage of this type of protocol is that the induction of senescence can be studied relatively independently from purely adaptive responses, if the cells are allowed to recover for at least 3 days after the stress before analysis of biomarkers of senescence and if the repetition of stresses increases the fraction of cells undergoing stress-induced senescence (Toussaint et al. 2000).

Among the different models of existing SIS, some are based on physical stress such as ionizing radiation of endothelial cells (ECs) (Igarashi et al. 2007; Panganiban et al. 2013), exposure of HDFs or melanocytes to UV light (Rodemann 1989; Debacq-Chainiaux et al. 2005; Medrano et al. 1995), to psoralen pre-treatment and UVA exposure (pUVA) (Ma et al. 2002) and to electromagnetic fields (Rodemann et al. 1989b) and exposure of human mesenchymal stem cells to sublethal heat shock (Alekseenko et al. 2014).

Other models induce oxidative stress as in HDFs exposed to chronic hyperoxia or to acute subcytotoxic exposure to oxidizing agents such as H_2O_2 or *t*-BHP (Chen and Ames 1994; Dumont et al. 2000a; von Zglinicki et al. 1995).

Stress-induced senescence can also be provoked by stimulating cells with cytokines or adipokines like repeated stimulation of lung HDFs or endothelial cells with interleukin-1α (IL-1α), tumor-necrosis factor-α (TNF-α) (Dumont et al. 2000a), transforming growth factor-beta 1 (TGF-ß1) (Debacq-Chainiaux et al. 2005, 2008; Frippiat et al. 2001) and visfatin/Nampt (Villalobos et al. 2014).

Senescence can also be induced by subcytotoxic doses of drugs and in particular chemotherapeutic drugs such as mitomycin C (Rodemann 1989), cisplatin (Berndtsson et al. 2007), etoposide (Chiu et al. 2005) and doxorubicin (Maejima et al. 2008; Piegari et al. 2013).

Finally, premature senescence appears by incubating HDFs with copper (Cu), which itself accumulates in cells during replicative senescence. Incubation of HDFs with copper sulphate ($CuSO_4$) for 16 h induces the appearance of several senescence-associated biomarkers (Boilan et al. 2013; Matos et al. 2012). Conversely, iron-chelation using desferroxamine mesylate induces growth arrest and the appearance of senescence-associated biomarkers in hepatocyte cell lines (Yoon et al. 2002).

13.4 Damage Induced by SIS

13.4.1 DNA Damage

DNA is a favourite target of stress, directly or indirectly. Many types of damage can be produced on DNA, but the most critical for the induction of senescence is double strand breaks (DSBs). Shortened and dysfunctional telomeres form similar structures of DSBs, inducing DDR pathway (Takai et al. 2003).

Several models of stress-induced senescence induced in normal or immortal cells by chemotherapeutic drugs (mitomycin C, bleomycin, actinomycin D) (Robles and Adami 1998) or ionizing radiation (X-irradiation) (Kim et al. 2014) generate DNA damage, including DSBs leading to senescence. Once DNA repair machinery detects DSBs, large protein complexes including in particular γH2Ax and 53BP1 are recruited at the cleavage site and help to stabilize the damaged strands and to activate the repair process. If the damage is too important for repair, DDR pathway is activated and induces activation of p53 transcription factor and expression of $p21^{WAF\text{-}1}$. The overexpression of $p21^{WAF\text{-}1}$ is transient and followed by a delayed induction of $p16^{INK\text{-}4a}$, as it has been demonstrated in RS. The pathway leading to $p16^{INK\text{-}4a}$ overexpression after DNA damage is still unclear (Robles and Adami 1998) but could be linked to oxidative stress.

13.4.2 Oxidative Stress

Oxidative stress is probably the most often used inducer of stress-induced senescence. The link between replicative senescence and oxidative stress was clearly demonstrated by comparing the number of passages in culture performed under conditions of hypoxia or hyperoxia, and by the use of antioxidants and/or free radical scavengers as, for instance *N-tert*-butyl-alpha-phenylnitrone (PBN). PBN is a biphasic antioxidant that is soluble in lipid and water, and is stable in cell culture media. Presenescent HDFs incubated with PBN are able to achieve between four and seven additional CPDs than untreated cells (von Zglinicki et al. 2000).

An increase of radical oxygen species (ROS) is highlighted in HDFs exposed to H_2O_2, UVB and copper (Boilan et al. 2013; Borlon et al. 2007; Zdanov et al. 2006b). The use of antioxidants as mannitol or trolox significantly reduces the appearance of senescence-associated biomarkers following copper incubation of HDFs (Boilan et al. 2013). Nrf-2 transcription factor is also activated in HDFs incubated with copper (Boilan et al. 2013).

An hypothesis to explain the expression of $p16^{INK-4a}$ after DNA damage is the induction of a second phase of stress after direct DNA damage via oxidative stress inducing accumulation of ROS, activation of $p38^{MAPK}$ and $p16^{INK-4a}$ overexpression (Hornsby 2010).

13.5 Cellular and Molecular Characteristics of SIS

13.5.1 Biomarkers of Senescence

Cells in stress-induced senescence share common features with replicative senescence as it was highlighted by the study of biomarkers of senescence.

Senescent cells are characterized by morphological change, they generally enlarge, often doubling in volume, and, if adherent, adopt a flattened morphology. Using the morphotypes classification of HDFs (Rodemann et al. 1989a), it was shown that after sublethal stress under H_2O_2, *t*-BHP, UV light, mitomycin C, etc., the treated HDFs acquired the morphological features of senescent HDFs (Chen and Ames 1994; Debacq-Chainiaux et al. 2005; Dumont et al. 2000b; Rodemann et al. 1989a).

Their growth is irreversibly arrested at the G1/S phase of the cell cycle due to the overexpression of several cyclin-dependant kinase inhibitors as $p21^{waf-1}$ and $p16^{ink-4a}$ and hypophosphorylation of the retinoblastoma protein (pRb) (Chen et al. 1998).

Senescence-associated ß-galactosidase (SA-ßgal) is a commonly used marker of senescent cells allowing to identify easily senescent cells both *in vitro* and *in vivo* (Dimri et al. 1995). This activity derives from the lysosomal ß-galactosidase, overexpressed in senescent cells, and then detectable at a suboptimal pH (Kurz et al. 2000). After exposure to stress inducing senescence, proportion of cells positive for SA-ßgal activity increases.

Mitochondrial DNA undergoes many changes during replicative senescence among which a 4977 bp « common » mitochondrial DNA deletion. This deletion is clearly detected in HDFs exposed to sublethal stresses with *t*-BHP or UVB (Debacq-Chainiaux et al. 2005; Dumont et al. 2000b).

HDFs in RS and in SIS induced by X-irradiation or by bleomycin display a highly correlated SASP including increased expression of IL-1ß, IL-8, GROα and MMP-1 (Coppe et al. 2008; Bavik et al. 2006).

13.5.2 Gene and Protein Expression: Are RS and SIS Identical Phenotypes?

Senescent cells show striking changes in gene expression, including gene expression changes of cell-cycle effectors. Two cyclin-dependent kinase inhibitors (CDKIs) are often overexpressed in senescent cells: $p21^{waf\text{-}1}$ (*CDKN1a*) and $p16^{ink\text{-}4a}$ (*CDKN2a*). On the contrary, senescent cells can also repress genes that encode for cell-cycle activators as *c-fos*, *cyclin B* and *PCNA* (*proliferating cell nuclear antigen*).

But many changes in gene expression are unrelated to growth arrest. Senescent cells overexpress genes encoding secreted proteins that can alter the tissue microenvironment, for instance to remodel extracellular matrix or to mediate local inflammation (Campisi et al. 1996).

If similar gene expression change is observed in stress-induced senescence as overexpression of $p21^{waf\text{-}1}$, $p16^{ink\text{-}4a}$, *apolipoprotein J*, *osteonectin* and *fibronectin* (Debacq-Chainiaux et al. 2005; Dumont et al. 2000b; Pascal et al. 2005), large-scale studies also show specific gene expression change associated either to replicative senescence or to stress-induced senescence (Pascal et al. 2005).

The study of the different biomarkers tends to show the similarities between the two phenotypes of RS and SIS. But are they identical phenotypes? To answer this question, the simple study of the presence of replicative senescence biomarkers after SIS was not enough. It was therefore necessary to achieve a more global comparison between these two phenotypes, through larger studies of gene and protein expression in both phenotypes.

Studies on global gene expression using differential display or cDNA microarray firstly confirmed the presence of genes whose expression is identically modified between RS and SIS, but also demonstrated that specific changes were associated either to RS or to SIS induced by *t*-BHP, ethanol, H_2O_2 or bleomycin in HDFs (Pascal et al. 2005; Debacq-Chainiaux et al. 2008; Bavik et al. 2006). In parallel, a proteomic identification by mass spectrometry after two-dimensional gel electrophoresis (2DGE) came to the same conclusion on the comparison of the same models (Dierick et al. 2002). This observation was confirmed by a proteomic analysis of HDFs in RS and in H_2O_2-induced senescence (Aan et al. 2013). These results reinforced that despite displaying the so-called common biomarkers of senescence, RS and SIS are not alike.

These studies demonstrated that gene and protein expression changes of HDFs in SIS can be classified in three groups: firstly, the changes common with RS, secondly, the changes specific to each kind of stressor and thirdly, changes common to SIS induced by different stressors.

The specific changes related to one of several SIS models were presented as long-term effects of the stress and named “molecular scars”. These “molecular scars” might occur from a few days after the stress and be maintained at long term, after many types of stress (oxidative stress and/or DNA damage, inflammation) *in vitro* and *in vivo* (Dierick et al. 2003).

13.6 SIS, Telomeres and Telomerase

Telomere shortening, intimately linked to replicative senescence, is not always related to the induction of senescence after stress.

After chronic exposure to hyperoxia, telomeres are shortened five to ten times faster than normal (von Zglinicki et al. 1995). This increased shortening is due to accumulation of single-strand breaks in telomeres with hyperoxia, leading to faster telomere loss during DNA replication (von Zglinicki et al. 2000). Surprisingly, the presence of telomerase does not prevent telomere shortening due to oxidative stress, as shown in human endothelial cells with ectopically overexpressed telomerase (Kurz et al. 2004). This is linked to the export of *TERT*, the catalytic subunit of human telomerase, from the nucleus to mitochondria. *TERT*, once localized in mitochondria, protects mtDNA integrity (Ahmed et al. 2008).

In other models of SIS induced by H_2O_2, *t*-BHP or ionizing radiation, no increased telomere loss was brought out after stress whereas senescence biomarkers were well detected (Chen et al. 2001; Dumont et al. 2001; Suzuki et al. 2001).

Moreover, several HDFs strains expressing telomerase and exposed to subcytotoxic doses of H_2O_2, UV, UVB or γ-irradiation in conditions inducing senescence, displayed biomarkers of senescence (de Magalhaes et al. 2002; Gorbunova et al. 2002). This shows that telomere shortening is not detected systematically in all models of SIS and that SIS could be induced independently of telomere erosion.

13.7 Molecular Pathways

13.7.1 TGF-ß1 Pathway

TGF-ß1 (Transforming Growth Factor-ß1) is overexpressed in skin and lung fœtal HDFs after a single exposure to H_2O_2 (Frippiat et al. 2001) and after a series of exposures to UVB (Debacq-Chainiaux et al. 2005), *t*-BHP or ethanol (Pascal et al. 2005). TGF-ß1 overexpression is necessary for the overexpression of *apolipoprotein J*, *osteonectin*, *fibronectin* and *TGF-ß1* itself, namely via a positive feedback on the activation of $p38^{MAPK}$ (Frippiat et al. 2002). Incubation of lung or skin HDFs with TGF-ß1 for 3 days induces the same phenotype. If neutralizing antibodies directed against TGF-ß1 or its receptor II (TßRII) are incubated with cells after the last stress inducing senescence, this blocks the appearance of the biomarkers of senescence (Frippiat et al. 2001). This has been confirmed in lung or skin HDFs in various models of SIS (*t*-BHP, ethanol (Debacq-Chainiaux et al. 2008) and UVB (Debacq-Chainiaux et al. 2005)), reinforcing the major role played by TGF-ß1 activation in the SIS phenotype.

P38 Mitogen-activated protein kinase (MAPK) is activated in RS, in OIS induced by oncogenic Ras and in SIS induced by H_2O_2, UVB and X-irradiation (Debacq-Chainiaux et al. 2010; Wang et al. 2002; Freund et al. 2011). Activation of $p38^{MAPK}$ by phosphorylation is rapidly detected after the stress. Inhibition of $p38^{MAPK}$

activity attenuates the increase of SA-ßgal positive cells and modifies the profiles of senescence-associated gene expression in H_2O_2-induced senescence (Zdanov et al. 2006a) and reduces the secretion of SASP factors in X-irradiation-induced senescence (Freund et al. 2011).

Once activated, $p38^{MAPK}$ phosphorylates and activates ATF-2 transcription factor. ATF-2 is then responsible of *TGF-ß1* overexpression and interacts with pRb (Frippiat et al. 2002). TGF-ß1 protein is overexpressed both in latent and active forms and induces several biomarkers of senescence such as senescent morphology, SA-ßgal and senescence-associated gene expression (Debacq-Chainiaux et al. 2005; Frippiat et al. 2001), probably via downstream proteins as IGFBP-3 (Debacq-Chainiaux et al. 2008) (Fig. 13.3).

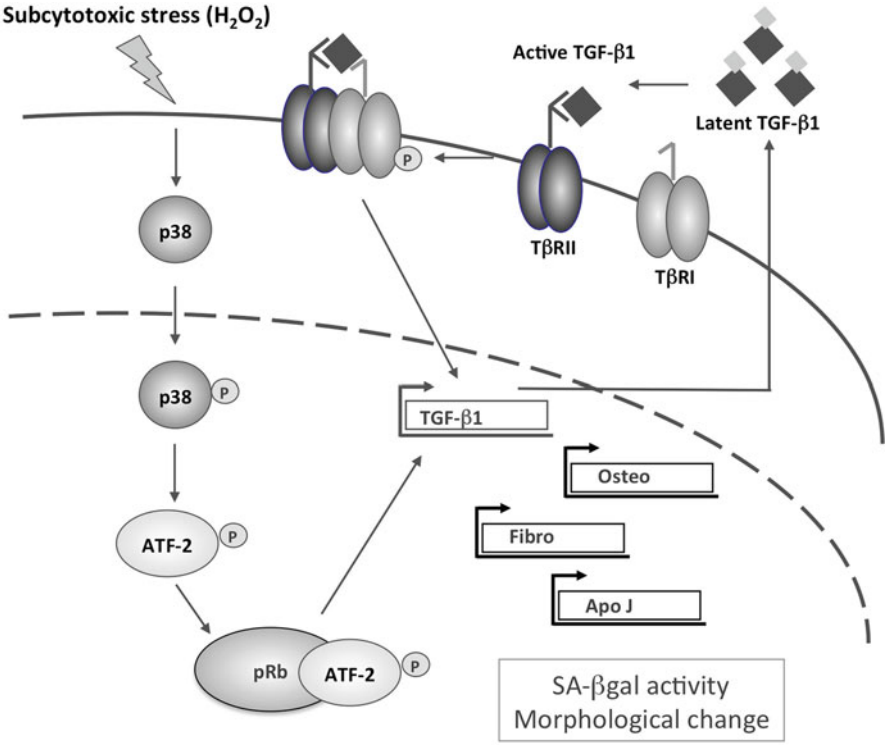

Fig. 13.3 Activation of $p38^{MAPK}$, ATF-2 and TGF-ß1 following H_2O_2-induced senescence. H_2O_2 stress immediately activates $p38^{MAPK}$ which is responsible of ATF-2 activation. Phosphorylated ATF-2 binds to hypophosphorylated pRb, which activates its transcription factor function, and is responsible for the overexpression of TGF-ß1, increasing the secretion of its latent and active protein forms. Active TGF-ß1 is able to bind to its receptors TßRI and TßRII (TGF-ß receptor I and II), leading to their interaction and phosphorylation. Activated TGF-ß1 pathway is responsible for the overexpression of senescence-associated genes as *osteonectin* (*osteo*), *fibronectin* (*fibro*) and *apolipoprotein J* (*apo J*), of morphological change and SA-ßgal activity as demonstrated by using neutralizing antibodies against TGF-ß1 or TßRII

13.7.2 IGF-1R

Several studies have highlighted the role of insulin like growth factor-1 receptor (IGF-1R) in the induction of stress-induced senescence. IGF-1R is a transmembrane tyrosine kinase receptor activated by binding with its ligands IGF-1 and IGF-2, leading to the autophosphorylation of its cytoplasmic domain. This pathway is responsible for the activation of PI3K/Akt, MAPK and mTOR (for a review: Riedemann and Macaulay 2006). Ionizing radiation of human pulmonary artery endothelial cells (HPAEC) induces senescence-associated biomarkers, IGF-1 and IGF-2 overexpression and IGF-1R activation. Treatment of HPAEC cells with an IGF-1R inhibitor (AG1024) protects from ionizing radiation-induced senescence (Panganiban and Day 2013). It was also demonstrated that the presence of a functional IGF-1R receptor was required for the activation of the UVB-induced senescence in normal human keratinocytes (Lewis et al. 2008).

13.8 Stress-Induced Senescence *In Vivo*

It is well established that senescent cells accumulate in tissues with age, as shown by studying SA-ßgal activity and DNA damage (Dimri et al. 1995). These senescent cells could be involved in the functional decline associated with ageing as postulated by Hayflick at the time of the discovery of replicative senescence. Indeed, a link between senescent cells accumulating in tissues and ageing-associated disorders such as cataract and muscular mass loss has been shown in mice by Baker et al. (2011).

How do these senescent cells appear *in vivo* and accumulate with age? Do they appear by telomere-dependent senescence or by another mechanism? By performing a simple calculation of the number of cells that could generate the two first telomerase-negative cells, appearing during the *in vivo* differentiation, based on the number of generations that HDFs can achieve before reaching replicative senescence under physiological low O_2 partial pressure (80 PDs), 2^{80} cells ($>10^{24}$ cells) must be produced before the first telomere-dependent replicatively senescent HDFs appear. Of course, to be correct, this extrapolation should also take account of the cellular turnover and of asymmetric divisions, but should also consider that more than two telomerase-negative cells will be generated by *in vivo* differentiation (Dierick et al. 2003).

In vivo, cells are exposed to a multitude of stresses whose nature depends on their location (pneumocytes facing air pollution and tobacco smoke; melanocytes, keratinocytes and skin HDFs facing UV; enterocytes facing food oxidants), particular conditions (endothelial cells facing inflammation, ischaemia/reperfusion, hypertension, shear stresses or diseases, etc.) and pathologies such as atherosclerosis, diabetes and age-related neurodegenerative diseases (for a review: Toussaint et al. 2002).

Chronic inflammation is associated to ageing and age-related diseases (Chung et al. 2009). This chronic inflammation could increase ROS accumulation and could induce appearance of senescent cells as detected by many studies in which premature appearance of cells presenting certain senescence-associated biomarkers in disease sites, including diseases subjected to chronic inflammation such as venous ulcers (Mendez et al. 1998), arteries subjected from balloon angioplasty (Fenton et al. 2001), emphysema (Muller et al. 2006), chronic hepatitis C and hepatocellular carcinoma (Paradis et al. 2001), prostatic hyperplasia (Choi et al. 2000) and intestinal metaplasia from the stomach (Going et al. 2002).

The premature appearance of these senescent cells could reinforce the inflammation by their SASP phenotype. This could lead to amplification of the phenomenon, worsen the inflammation and accelerate ageing. This hypothesis was confirmed recently *in vivo* in mice, in which chronic inflammation provoked by knockout of the *nfkb1* subunit of the transcription factor NF-κB induces telomere dysfunction and accelerates ageing (Jurk et al. 2014).

Many studies showed the implication of metals (Cu and Fe) in the development of age-related diseases and more precisely in age-related neurodegenerative diseases. These metals are able to generate ROS through the Fenton and Haber-Weiss reactions (Brewer 2007) and to induce accumulation of DNA damage, by their ability to inhibit a family of DNA glycosylases by oxidation, changing their structure and preventing their binding to downstream repair proteins (Hegde et al. 2011). Interestingly, these metals accumulate in HDFs with RS (Boilan et al. 2013; Killilea et al. 2004). The accumulation of Cu during RS of HDFs was detected by specific fluorescence-probes (CS1) or cytochemistry (rubeanic acid), but the mechanisms and the kinetics of this accumulation is still unknown. Incubation of HDFs at young CPDs with $CuSO_4$ induces senescence, this suggests that a transient increase of copper concentration is sufficient to launch the senescence process.

13.9 SIS in Cancer Treatment

Several studies have shown that in addition to induce apoptosis of cancer cells, some anti-cancer treatments such as chemotherapy and ionizing radiation were also able to induce senescence, generally using lower concentrations than those necessary to provoke apoptosis.

Chemotherapeutic agents able to induce senescence *in vitro* include among others cisplatin (Berndtsson et al. 2007), etoposide (Chiu et al. 2005), doxorubicin (Sliwinska et al. 2009), bleomycin (Aoshiba et al. 2003), vinblastine (Duan et al. 2007), etc (for a review: Bilsland and Keith 2010). An analysis of tumor tissues from patients treated with chemotherapeutic agents show a greater proportion of senescent cells (SA-ßgal positive, $p16^{ink\text{-}4a}$ overexpression) compared to healthy tissues in patients suffering from breast cancer, non-small-cell lung carcinoma or prostate cancer (Coppe et al. 2008; Roberson et al. 2005; te Poele et al. 2002). *In vivo*, first clinical data show that senescence markers ($p16^{ink\text{-}4a}$ overexpression,

VEGFA and MCP1 secretion) are more expressed in T lymphocytes from patients suffering for breast cancer treated with adjuvant chemotherapy (anthracyclin-based) than controls (Sanoff et al. 2014). This therefore accelerates senescence of hematopoietic tissues.

Furthermore ionizing radiation induces senescence in normal and tumor cells *in vitro* (Suzuki and Boothman 2008). So we should be careful about the possible induction of senescence by radiotherapy on the tumor being treated and its (micro)environment.

Induction of senescence-like phenotype in transformed cells and particularly of irreversible cell cycle arrest is seen as an interesting concept in the treatment of cancer. Indeed cancer cells manage to escape cellular senescence. A promising strategy is to successfully redirect cancer cells to cellular senescence by developing senescence-targeted drug. This pro-senescence therapy is presented as a new promising approach in cancer treatment (Nardella et al. 2011). The main pro-senescence strategies are to inhibit the activity of telomerase, to reactivate or stabilize p53, to induce Pten loss induced senescence (PICS) and to inhibit CDKs and MYC (Acosta and Gil 2012).

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References

Aan GJ, Hairi HA, Makpol S, Rahman MA, Karsani SA (2013) Differences in protein changes between stress-induced premature senescence and replicative senescence states. Electrophoresis 34(15):2209–2217. doi:10.1002/elps.201300086

Acosta JC, Gil J (2012) Senescence: a new weapon for cancer therapy. Trends Cell Biol 22(4):211–219. doi:10.1016/j.tcb.2011.11.006

Ahmed S, Passos JF, Birket MJ, Beckmann T, Brings S, Peters H, Birch-Machin MA, von Zglinicki T, Saretzki G (2008) Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress. J Cell Sci 121(Pt 7):1046–1053. doi:10.1242/jcs.019372

Alcorta DA, Xiong Y, Phelps D, Hannon G, Beach D, Barrett JC (1996) Involvement of the cyclin-dependent kinase inhibitor p16 (INK4a) in replicative senescence of normal human fibroblasts. Proc Natl Acad Sci U S A 93(24):13742–13747

Alekseenko LL, Zemelko VI, Domnina AP, Lyublinskaya OG, Zenin VV, Pugovkina NA, Kozhukharova IV, Borodkina AV, Grinchuk TM, Fridlyanskaya II, Nikolsky NN (2014) Sublethal heat shock induces premature senescence rather than apoptosis in human mesenchymal stem cells. Cell Stress Chaperones 19(3):355–366. doi:10.1007/s12192-013-0463-6

Aoshiba K, Tsuji T, Nagai A (2003) Bleomycin induces cellular senescence in alveolar epithelial cells. Eur Respir J 22(3):436–443

Baker DJ, Wijshake T, Tchkonia T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM (2011) Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. Nature 479(7372):232–236. doi:10.1038/nature10600

Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S, Nelson PS (2006) The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. Cancer Res 66(2):794–802. doi:10.1158/0008-5472.CAN-05-1716

Bayreuther K, Rodemann HP, Hommel R, Dittmann K, Albiez M, Francz PI (1988) Human skin fibroblasts in vitro differentiate along a terminal cell lineage. Proc Natl Acad Sci U S A 85(14):5112–5116

Berndtsson M, Hagg M, Panaretakis T, Havelka AM, Shoshan MC, Linder S (2007) Acute apoptosis by cisplatin requires induction of reactive oxygen species but is not associated with damage to nuclear DNA. Int J Cancer 120(1):175–180. doi:10.1002/ijc.22132

Bilsland A, Keith W (2010) Mining cellular senescence for drug targets. In: Sedivy JM, Adams PD (eds) Cellular senescence and tumor suppression. Springer, New York, pp 235–265

Boilan E, Winant V, Dumortier E, Piret JP, Bonfitto F, Osiewacz HD, Debacq-Chainiaux F, Toussaint O (2013) Role of p38MAPK and oxidative stress in copper-induced senescence. Age (Dordr) 35(6):2255–2271. doi:10.1007/s11357-013-9521-3

Borlon C, Chretien A, Debacq-Chainiaux F, Toussaint O (2007) Transient increased extracellular release of H2O2 during establishment of UVB-induced premature senescence. Ann N Y Acad Sci 1119:72–77. doi:10.1196/annals.1404.002

Brewer GJ (2007) Iron and copper toxicity in diseases of aging, particularly atherosclerosis and Alzheimer's disease. Exp Biol Med (Maywood) 232(2):323–335

Campisi J, d'Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 8(9):729–740. doi:10.1038/nrm2233

Campisi J, Dimri GP, Hara E (1996) Control of replicative senescence. In: Rowe J, Rowe J, Schneider E (eds) Handbook of the biology of aging. Academic Press, San Diego, pp 121–149

Chen Q, Ames BN (1994) Senescence-like growth arrest induced by hydrogen peroxide in human diploid fibroblast F65 cells. Proc Natl Acad Sci U S A 91(10):4130–4134

Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN (1998) Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. Biochem J 332(Pt 1):43–50

Chen QM, Prowse KR, Tu VC, Purdom S, Linskens MH (2001) Uncoupling the senescent phenotype from telomere shortening in hydrogen peroxide-treated fibroblasts. Exp Cell Res 265(2):294–303. doi:10.1006/excr.2001.5182

Chen J, Brodsky SV, Goligorsky DM, Hampel DJ, Li H, Gross SS, Goligorsky MS (2002) Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. Circ Res 90(12):1290–1298

Chiu CC, Li CH, Ung MW, Fuh TS, Chen WL, Fang K (2005) Etoposide (VP-16) elicits apoptosis following prolonged G2-M cell arrest in p53-mutated human non-small cell lung cancer cells. Cancer Lett 223(2):249–258. doi:10.1016/j.canlet.2004.10.049

Choi J, Shendrik I, Peacocke M, Peehl D, Buttyan R, Ikeguchi EF, Katz AE, Benson MC (2000) Expression of senescence-associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia. Urology 56(1):160–166

Chung HY, Cesari M, Anton S, Marzetti E, Giovannini S, Seo AY, Carter C, Yu BP, Leeuwenburgh C (2009) Molecular inflammation: underpinnings of aging and age-related diseases. Ageing Res Rev 8(1):18–30. doi:10.1016/j.arr.2008.07.002

Coppe JP, Patil CK, Rodier F, Sun Y, Munoz DP, Goldstein J, Nelson PS, Desprez PY, Campisi J (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6(12):2853–2868. doi:10.1371/journal.pbio.0060301

d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP (2003) A DNA damage checkpoint response in telomere-initiated senescence. Nature 426(6963):194–198. doi:10.1038/nature02118

de Magalhaes JP, Chainiaux F, Remacle J, Toussaint O (2002) Stress-induced premature senescence in BJ and hTERT-BJ1 human foreskin fibroblasts. FEBS Lett 523(1–3): 157–162

Debacq-Chainiaux F, Borlon C, Pascal T, Royer V, Eliaers F, Ninane N, Carrard G, Friguet B, de Longueville F, Boffe S, Remacle J, Toussaint O (2005) Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-beta1 signaling pathway. J Cell Sci 118(Pt 4):743–758. doi:10.1242/jcs.01651

Debacq-Chainiaux F, Pascal T, Boilan E, Bastin C, Bauwens E, Toussaint O (2008) Screening of senescence-associated genes with specific DNA array reveals the role of IGFBP-3 in premature senescence of human diploid fibroblasts. Free Radic Biol Med 44(10):1817–1832. doi:10.1016/j.freeradbiomed.2008.02.001

Debacq-Chainiaux F, Boilan E, Dedessus Le Moutier J, Weemaels G, Toussaint O (2010) p38(MAPK) in the senescence of human and murine fibroblasts. Adv Exp Med Biol 694:126–137

Dierick JF, Kalume DE, Wenders F, Salmon M, Dieu M, Raes M, Roepstorff P, Toussaint O (2002) Identification of 30 protein species involved in replicative senescence and stress-induced premature senescence. FEBS Lett 531(3):499–504

Dierick JF, Frippiat C, Salmon M, Chainiaux F, Toussaint O (2003) Stress, cells and tissue aging. In: Rattan SIS (ed) Modulating aging and longevity. Kluwer Academic Publishers, Dordrecht/Boston, pp 101–125

Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O et al (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc Natl Acad Sci U S A 92(20):9363–9367

Dings J, Meixensberger J, Jager A, Roosen K (1998) Clinical experience with 118 brain tissue oxygen partial pressure catheter probes. Neurosurgery 43(5):1082–1095

Duan L, Sterba K, Kolomeichuk S, Kim H, Brown PH, Chambers TC (2007) Inducible overexpression of c-Jun in MCF7 cells causes resistance to vinblastine via inhibition of drug-induced apoptosis and senescence at a step subsequent to mitotic arrest. Biochem Pharmacol 73(4):481–490. doi:10.1016/j.bcp.2006.10.026

Dumont P, Balbeur L, Remacle J, Toussaint O (2000a) Appearance of biomarkers of in vitro ageing after successive stimulation of WI-38 fibroblasts with IL-1alpha and TNF-alpha: senescence associated beta-galactosidase activity and morphotype transition. J Anat 197(Pt 4):529–537

Dumont P, Burton M, Chen QM, Gonos ES, Frippiat C, Mazarati JB, Eliaers F, Remacle J, Toussaint O (2000b) Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast. Free Radic Biol Med 28(3):361–373

Dumont P, Royer V, Pascal T, Dierick JF, Chainiaux F, Frippiat C, de Magalhaes JP, Eliaers F, Remacle J, Toussaint O (2001) Growth kinetics rather than stress accelerate telomere shortening in cultures of human diploid fibroblasts in oxidative stress-induced premature senescence. FEBS Lett 502(3):109–112

Fenton M, Barker S, Kurz DJ, Erusalimsky JD (2001) Cellular senescence after single and repeated balloon catheter denudations of rabbit carotid arteries. Arterioscler Thromb Vasc Biol 21(2):220–226

Freund A, Patil CK, Campisi J (2011) p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 30(8):1536–1548. doi:10.1038/emboj.2011.69

Freund A, Laberge RM, Demaria M, Campisi J (2012) Lamin B1 loss is a senescence-associated biomarker. Mol Biol Cell 23(11):2066–2075. doi:10.1091/mbc.E11-10-0884

Frippiat C, Chen QM, Zdanov S, Magalhaes JP, Remacle J, Toussaint O (2001) Subcytotoxic H2O2 stress triggers a release of transforming growth factor-beta 1, which induces biomarkers of cellular senescence of human diploid fibroblasts. J Biol Chem 276(4):2531–2537. doi:10.1074/jbc.M006809200

Frippiat C, Dewelle J, Remacle J, Toussaint O (2002) Signal transduction in H2O2-induced senescence-like phenotype in human diploid fibroblasts. Free Radic Biol Med 33(10):1334–1346

Going JJ, Stuart RC, Downie M, Fletcher-Monaghan AJ, Keith WN (2002) 'Senescence-associated' beta-galactosidase activity in the upper gastrointestinal tract. J Pathol 196(4):394–400. doi:10.1002/path.1059

Gorbunova V, Seluanov A, Pereira-Smith OM (2002) Expression of human telomerase (hTERT) does not prevent stress-induced senescence in normal human fibroblasts but protects the cells from stress-induced apoptosis and necrosis. J Biol Chem 277(41):38540–38549. doi:10.1074/jbc.M202671200

Gorgoulis VG, Halazonetis TD (2010) Oncogene-induced senescence: the bright and dark side of the response. Curr Opin Cell Biol 22(6):816–827. doi:10.1016/j.ceb.2010.07.013

Harley CB, Futcher AB, Greider CW (1990) Telomeres shorten during ageing of human fibroblasts. Nature 345(6274):458–460. doi:10.1038/345458a0

Hayflick L, Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25:585–621

Hegde ML, Hegde PM, Rao KS, Mitra S (2011) Oxidative genome damage and its repair in neurodegenerative diseases: function of transition metals as a double-edged sword. J Alzheimers Dis 24(Suppl 2):183–198. doi:10.3233/JAD-2011-110281

Hornsby PJ (2010) Stress-induced senescence. In: Sedivy JM, Adams PD (eds) Cellular senescence and tumor suppression. Springer, New York, pp 85–106

Igarashi K, Sakimoto I, Kataoka K, Ohta K, Miura M (2007) Radiation-induced senescence-like phenotype in proliferating and plateau-phase vascular endothelial cells. Exp Cell Res 313(15):3326–3336. doi:10.1016/j.yexcr.2007.06.001

Jurk D, Wilson C, Passos JF, Oakley F, Correia-Melo C, Greaves L, Saretzki G, Fox C, Lawless C, Anderson R, Hewitt G, Pender SL, Fullard N, Nelson G, Mann J, van de Sluis B, Mann DA, von Zglinicki T (2014) Chronic inflammation induces telomere dysfunction and accelerates ageing in mice. Nat Commun 2:4172. doi:10.1038/ncomms5172

Killilea DW, Wong SL, Cahaya HS, Atamna H, Ames BN (2004) Iron accumulation during cellular senescence. Ann N Y Acad Sci 1019:365–367. doi:10.1196/annals.1297.063

Kim KS, Kim JE, Choi KJ, Bae S, Kim DH (2014) Characterization of DNA damage-induced cellular senescence by ionizing radiation in endothelial cells. Int J Radiat Biol 90(1):71–80. doi:10.3109/09553002.2014.859763

Kurz DJ, Decary S, Hong Y, Erusalimsky JD (2000) Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. J Cell Sci 113(Pt 20):3613–3622

Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD (2004) Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. J Cell Sci 117(Pt 11):2417–2426. doi:10.1242/jcs.01097

Lewis DA, Yi Q, Travers JB, Spandau DF (2008) UVB-induced senescence in human keratinocytes requires a functional insulin-like growth factor-1 receptor and p53. Mol Biol Cell 19(4):1346–1353. doi:10.1091/mbc.E07-10-1041

Lopez-Otin C, Blasco MA, Partridge L, Serrano M, Kroemer G (2013) The hallmarks of aging. Cell 153(6):1194–1217. doi:10.1016/j.cell.2013.05.039

Ma W, Wlaschek M, Hommel C, Schneider LA, Scharffetter-Kochanek K (2002) Psoralen plus UVA (PUVA) induced premature senescence as a model for stress-induced premature senescence. Exp Gerontol 37(10–11):1197–1201

Maejima Y, Adachi S, Ito H, Hirao K, Isobe M (2008) Induction of premature senescence in cardiomyocytes by doxorubicin as a novel mechanism of myocardial damage. Aging Cell 7(2):125–136. doi:10.1111/j.1474-9726.2007.00358.x

Matos L, Gouveia A, Almeida H (2012) Copper ability to induce premature senescence in human fibroblasts. Age (Dordr) 34(4):783–794. doi:10.1007/s11357-011-9276-7

Medrano EE, Im S, Yang F, Abdel-Malek ZA (1995) Ultraviolet B light induces G1 arrest in human melanocytes by prolonged inhibition of retinoblastoma protein phosphorylation associated with long-term expression of the p21Waf-1/SDI-1/Cip-1 protein. Cancer Res 55(18): 4047–4052

Mendez MV, Stanley A, Phillips T, Murphy M, Menzoian JO, Park HY (1998) Fibroblasts cultured from distal lower extremities in patients with venous reflux display cellular characteristics of senescence. J Vasc Surg 28(6):1040–1050

Muller KC, Welker L, Paasch K, Feindt B, Erpenbeck VJ, Hohlfeld JM, Krug N, Nakashima M, Branscheid D, Magnussen H, Jorres RA, Holz O (2006) Lung fibroblasts from patients with emphysema show markers of senescence in vitro. Respir Res 7:32. doi:10.1186/1465-9921-7-32

Nardella C, Clohessy JG, Alimonti A, Pandolfi PP (2011) Pro-senescence therapy for cancer treatment. Nat Rev Cancer 11(7):503–511. doi:10.1038/nrc3057

Packer L, Fuehr K (1977) Low oxygen concentration extends the lifespan of cultured human diploid cells. Nature 267(5610):423–425

Palm W, de Lange T (2008) How shelterin protects mammalian telomeres. Annu Rev Genet 42:301–334. doi:10.1146/annurev.genet.41.110306.130350

Panganiban RA, Day RM (2013) Inhibition of IGF-1R prevents ionizing radiation-induced primary endothelial cell senescence. PLoS One 8(10):e78589. doi:10.1371/journal.pone.0078589

Panganiban RA, Mungunsukh O, Day RM (2013) X-irradiation induces ER stress, apoptosis, and senescence in pulmonary artery endothelial cells. Int J Radiat Biol 89(8):656–667. doi:10.3109/09553002.2012.711502

Paradis V, Youssef N, Dargere D, Ba N, Bonvoust F, Deschatrette J, Bedossa P (2001) Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. Hum Pathol 32(3):327–332. doi:10.1053/hupa.2001.22747

Pascal T, Debacq-Chainiaux F, Chretien A, Bastin C, Dabee AF, Bertholet V, Remacle J, Toussaint O (2005) Comparison of replicative senescence and stress-induced premature senescence combining differential display and low-density DNA arrays. FEBS Lett 579(17):3651–3659. doi:10.1016/j.febslet.2005.05.056

Piegari E, De Angelis A, Cappetta D, Russo R, Esposito G, Costantino S, Graiani G, Frati C, Prezioso L, Berrino L, Urbanek K, Quaini F, Rossi F (2013) Doxorubicin induces senescence and impairs function of human cardiac progenitor cells. Basic Res Cardiol 108(2):334. doi:10.1007/s00395-013-0334-4

Ressler S, Bartkova J, Niederegger H, Bartek J, Scharffetter-Kochanek K, Jansen-Durr P, Wlaschek M (2006) p16INK4A is a robust in vivo biomarker of cellular aging in human skin. Aging Cell 5(5):379–389. doi:10.1111/j.1474-9726.2006.00231.x

Riedemann J, Macaulay VM (2006) IGF1R signalling and its inhibition. Endocr Relat Cancer 13(Suppl 1):S33–S43. doi:10.1677/erc.1.01280

Roberson RS, Kussick SJ, Vallieres E, Chen SY, Wu DY (2005) Escape from therapy-induced accelerated cellular senescence in p53-null lung cancer cells and in human lung cancers. Cancer Res 65(7):2795–2803. doi:10.1158/0008-5472.CAN-04-1270

Robles SJ, Adami GR (1998) Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. Oncogene 16(9):1113–1123. doi:10.1038/sj.onc.1201862

Rodemann HP (1989) Differential degradation of intracellular proteins in human skin fibroblasts of mitotic and mitomycin-C (MMC)-induced postmitotic differentiation states in vitro. Differentiation 42(1):37–43

Rodemann HP, Bayreuther K, Francz PI, Dittmann K, Albiez M (1989a) Selective enrichment and biochemical characterization of seven human skin fibroblasts cell types in vitro. Exp Cell Res 180(1):84–93

Rodemann HP, Bayreuther K, Pfleiderer G (1989b) The differentiation of normal and transformed human fibroblasts in vitro is influenced by electromagnetic fields. Exp Cell Res 182(2):610–621

Rudolf E, Rezacova K, Cervinka M (2014) Activation of p38 and changes in mitochondria accompany autophagy to premature senescence-like phenotype switch upon chronic exposure to selenite in colon fibroblasts. Toxicol Lett 231(1):29–37. doi:10.1016/j.toxlet.2014.09.006

Sanoff HK, Deal AM, Krishnamurthy J, Torrice C, Dillon P, Sorrentino J, Ibrahim JG, Jolly TA, Williams G, Carey LA, Drobish A, Gordon BB, Alston S, Hurria A, Kleinhans K, Rudolph KL,

Sharpless NE, Muss HB (2014) Effect of cytotoxic chemotherapy on markers of molecular age in patients with breast cancer. J Natl Cancer Inst 106(4):dju057
Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88(5):593–602
Sherr CJ, Roberts JM (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev 13(12):1501–1512
Sliwinska MA, Mosieniak G, Wolanin K, Babik A, Piwocka K, Magalska A, Szczepanowska J, Fronk J, Sikora E (2009) Induction of senescence with doxorubicin leads to increased genomic instability of HCT116 cells. Mech Ageing Dev 130(1–2):24–32. doi:10.1016/j.mad.2008.04.011
Stucker M, Struk A, Altmeyer P, Herde M, Baumgartl H, Lubbers DW (2002) The cutaneous uptake of atmospheric oxygen contributes significantly to the oxygen supply of human dermis and epidermis. J Physiol 538(Pt 3):985–994
Suzuki M, Boothman DA (2008) Stress-induced premature senescence (SIPS) – influence of SIPS on radiotherapy. J Radiat Res 49(2):105–112
Suzuki K, Mori I, Nakayama Y, Miyakoda M, Kodama S, Watanabe M (2001) Radiation-induced senescence-like growth arrest requires TP53 function but not telomere shortening. Radiat Res 155(1 Pt 2):248–253
Takai H, Smogorzewska A, de Lange T (2003) DNA damage foci at dysfunctional telomeres. Curr Biol 13(17):1549–1556
te Poele RH, Okorokov AL, Jardine L, Cummings J, Joel SP (2002) DNA damage is able to induce senescence in tumor cells in vitro and in vivo. Cancer Res 62(6):1876–1883
Toussaint O, Raes M, Remacle J (1991) Aging as a multi-step process characterized by a lowering of entropy production leading the cell to a sequence of defined stages. Mech Ageing Dev 61(1):45–64
Toussaint O, Medrano EE, von Zglinicki T (2000) Cellular and molecular mechanisms of stress-induced premature senescence (SIPS) of human diploid fibroblasts and melanocytes. Exp Gerontol 35(8):927–945
Toussaint O, Remacle J, Dierick JF, Pascal T, Frippiat C, Zdanov S, Magalhaes JP, Royer V, Chainiaux F (2002) From the Hayflick mosaic to the mosaics of ageing. Role of stress-induced premature senescence in human ageing. Int J Biochem Cell Biol 34(11):1415–1429
Toussaint O, Weemaels G, Debacq-Chainiaux F, Scharffetter-Kochanek K, Wlaschek M (2011) Artefactual effects of oxygen on cell culture models of cellular senescence and stem cell biology. J Cell Physiol 226(2):315–321. doi:10.1002/jcp.22416
Vedsted P, Blangsted AK, Sogaard K, Orizio C, Sjogaard G (2006) Muscle tissue oxygenation, pressure, electrical, and mechanical responses during dynamic and static voluntary contractions. Eur J Appl Physiol 96(2):165–177. doi:10.1007/s00421-004-1216-0
Villalobos LA, Uryga A, Romacho T, Leivas A, Sanchez-Ferrer CF, Erusalimsky JD, Peiro C (2014) Visfatin/Nampt induces telomere damage and senescence in human endothelial cells. Int J Cardiol 175(3):573–575. doi:10.1016/j.ijcard.2014.05.028
von Zglinicki T, Saretzki G, Docke W, Lotze C (1995) Mild hyperoxia shortens telomeres and inhibits proliferation of fibroblasts: a model for senescence? Exp Cell Res 220(1):186–193. doi:10.1006/excr.1995.1305
von Zglinicki T, Pilger R, Sitte N (2000) Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. Free Radic Biol Med 28(1):64–74
Wang W, Chen JX, Liao R, Deng Q, Zhou JJ, Huang S, Sun P (2002) Sequential activation of the MEK-extracellular signal-regulated kinase and MKK3/6-p38 mitogen-activated protein kinase pathways mediates oncogenic ras-induced premature senescence. Mol Cell Biol 22(10):3389–3403
Wei S, Sedivy JM (1999) Expression of catalytically active telomerase does not prevent premature senescence caused by overexpression of oncogenic Ha-Ras in normal human fibroblasts. Cancer Res 59(7):1539–1543
Xu D, Neville R, Finkel T (2000) Homocysteine accelerates endothelial cell senescence. FEBS Lett 470(1):20–24

Yoon G, Kim HJ, Yoon YS, Cho H, Lim IK, Lee JH (2002) Iron chelation-induced senescence-like growth arrest in hepatocyte cell lines: association of transforming growth factor beta1 (TGF-beta1)-mediated p27Kip1 expression. Biochem J 366(Pt 2):613–621. doi:10.1042/BJ20011445

Zdanov S, Debacq-Chainiaux F, Remacle J, Toussaint O (2006a) Identification of p38MAPK-dependent genes with changed transcript abundance in H2O2-induced premature senescence of IMR-90 hTERT human fibroblasts. FEBS Lett 580(27):6455–6463. doi:10.1016/j.febslet.2006.10.064

Zdanov S, Remacle J, Toussaint O (2006b) Establishment of H2O2-induced premature senescence in human fibroblasts concomitant with increased cellular production of H2O2. Ann N Y Acad Sci 1067:210–216. doi:10.1196/annals.1354.025