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Immunosenescence and Senescence

Immunosurveillance: One of the Possible Links

Explaining the Cancer Incidence in Ageing Population

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Additional information is available at the end of the chapter

<http://dx.doi.org/10.5772/55519>

1. Introduction

Since the discovery of cellular senescence significant advances were made to understand its molecular determinants and its physiological role in biological processes such as cancer and aging [1]. Recently, an intimate and complex relationship between senescent cells and the immune system has been highlighted [2]. In addition to their role in senescence immunosurveillance, immune cells display altered functions with age. This process is known as immunosenescence. Although immunosenescence is a slightly different mechanism than cellular senescence, it shares some similarities. This review article briefly describes features, markers, triggers and molecular regulators of cellular senescence and focuses on its role during cancer development. We then introduce immunosenescence and highlight what might be its consequences in cancer development. Finally, taking into account that senescence immunosurveillance is crucial for tumor eradication [2], we provide several hypotheses to explain what could be the impact of immunosenescence on senescence immunosurveillance in a specific cancer context.

2. Cellular senescence

2.1. Historical discovery of cellular senescence

Senescence comes from the Latin word *senex* meaning old age. It was observed approximately half a century ago by Leonard Hayflick while cultivating primary human fibroblast [3, 4]. He observed that primary cells proliferate in culture for approximately 55 population doublings before reaching the “Hayflick limit” which marks the end of their proliferative capacity and

the entry into an irreversible growth arrest state. He proposed a theory “that the finite lifetime of diploid cells strains *in vitro* may be an expression of ageing or senescence at the cellular level”. Since, the term “replicative senescence” has been used to designate this type of cellular senescence but, as we will see, senescence can also be induced in a replicative independent manner in response to various cellular insults. Senescence is not limited to human primary fibroblast as it has been observed in various primary cells [5-8] including immune cells [9] and also takes place in other species such as Mouse [10], Rat [11], Chicken [12], *Caenorhabditis elegans* [13], Zebrafish [14] and Yeast [15].

2.2. Features of cellular senescence

2.2.1. Morphology

Senescent cells lose their original morphology. Larger than their normal counterparts, they also have a much larger flattened cytoplasm that contain many vacuoles and cytoplasmic filaments [16, 17], a bigger nucleus and nucleoli and are sometimes multinucleated [18, 19]. In some cases, senescent cells display an increase in the number of lysosomes and golgi [20-24].

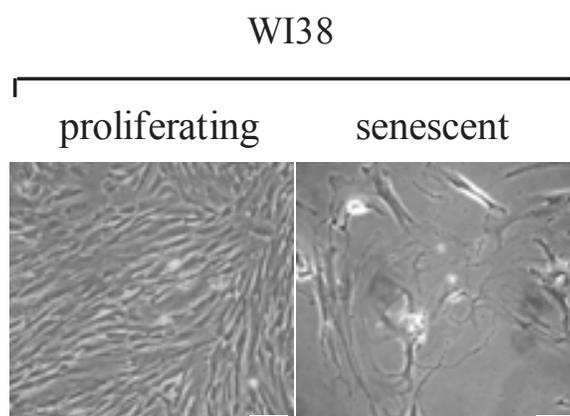


Figure 1. Morphology analysis of primary human fibroblasts (proliferating versus senescent).

2.2.2. Growth arrest

One of the most obvious features of cellular senescence is growth arrest. Indeed, cells are usually blocked in the G1 phase of the cell cycle [25] and in some cases they display (4n) DNA suggesting that cells are either blocked in the late S, G2 or M phases [26, 27]. Cell cycle progression is regulated by cyclin dependent kinases (CDKs) that bind to cyclins [28]. These complexes are regulated by cyclin dependent kinase inhibitors (CKIs) which are essential for the establishment of the senescent growth arrest state [29]. CKIs are divided into two families. The CIP (CDK-interacting protein) and KIP (cyclin-dependent kinase inhibitor protein) [30] and the INK4, for inhibitors of CDK4 [31]. CDK-cyclin complexes favour G1 cell cycle progression by phosphorylating RB family members [32]. RB family proteins are transcriptional

co-factors that interact with and inhibit E2F transcription factors activity required for DNA synthesis. Upon the phosphorylation of RB family members by the CDK-type D and E cyclin complexes, E2F transcription factors are released from their interaction with RB proteins leading to cell cycle progression [33] (Figure 2). The CDK4/6-type D cyclins complexes interact with KIP/CIP inhibitors. However, during cellular senescence, INK4 proteins increase and inhibit CDK4/6-cyclin D formation [32]. This enables the activation of RB family members and the inhibition of E2F transcription factors. Additionally, the bioavailability of KIP and CIP proteins increases and they no longer interact with CDK4/6/Cyclin D complexes. It allows them to interact and inhibit CDK2-type E or A cyclin complexes (Figure 2).

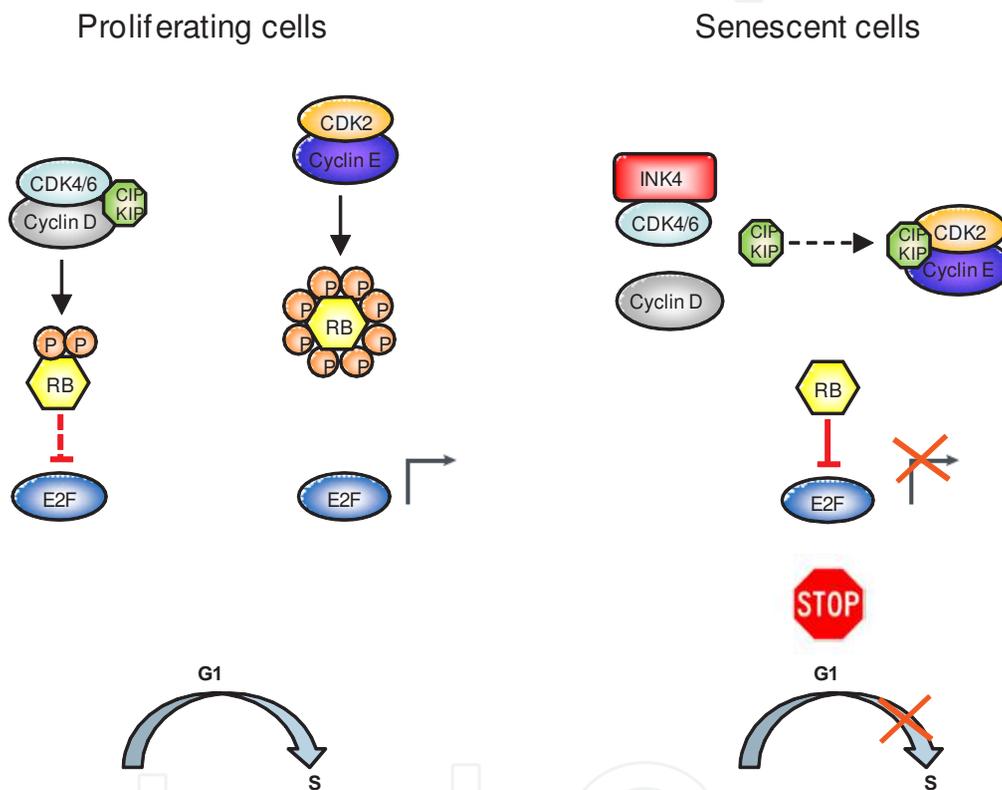


Figure 2. Cell cycle arrest (G1 phase) associated with cellular senescence.

In proliferating cells the CKI levels are low, the CDK/cyclin complexes are functional and RB family members are found hyperphosphorylated leading the E2F family members' activation and G1 progression. In senescent cells, the levels of CKI increase, CDK/Cyclin complexes are inhibited and RB family members are active leading to E2F transcription factors inhibition and G1 cell cycle arrest.

2.2.3. Altered gene expression

Senescent cells also display a specific gene expression signature. A notable example are senescence-associated-secretory-phenotype (SASP) molecules such as interleukin 6 (IL-6), interleukin 8 (IL-8), plasminogen activator inhibitor 1 (PAI-1), insulin growth factor binding

protein 7 (IGBP7), (ECM) degradation enzymes such as collagenase and metalloprotease (MMPs) but also CKIs, CIP and KIP as previously mentioned [34]. Some genes are also found down-regulated during cellular senescence. It is the case for polycomb complex members such as enhancer of zeste homolog 2 (EZH2) and chromobox homolog 7 (CBX7) [5, 35].

2.2.4. Senescence markers

Several markers have been used to specifically identify senescent cells. In addition to an altered cell morphology, growth arrest state and a specific gene expression signature, senescent cells are associated with an increased β -galactosidase activity, detectable at pH 6.0 and known as “senescence-associated-beta-galactosidase (SA- β gal) activity” [36] (Figure 3).

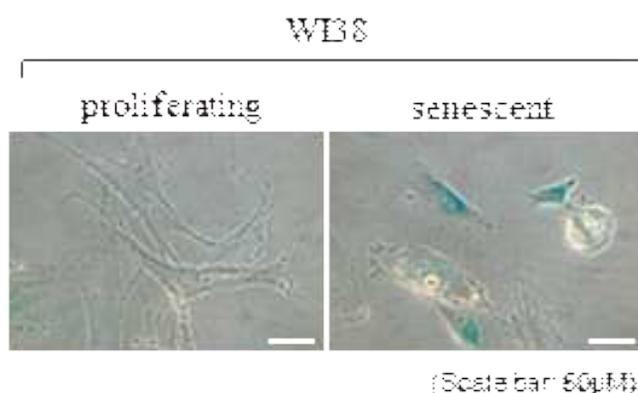


Figure 3. Senescence-associated-beta-galactosidase (SA- β gal) activity detectable at pH6 and yielding a blue colour in senescent cells. Primary human fibroblasts were used for the analysis.

At a chromatin level, specific facultative heterochromatin structures associated with senescent cells were discovered and termed senescence-associated-heterochromatin-foci (SAHF) [37]. These structures regulated by the Retinoblastoma gene (RB) are involved in E2F target genes repression and maintain the cell cycle arrest. They contain markers of heterochromatin such as hypo-acetylated histones, methylated histones (H3K9Me) and the presence of heterochromatin protein 1 (HP1). The histone variant macroH2A, and HMGA a non histone protein, have been identified as crucial regulators in SAHF formation [38] (Figure 4).

2.3. Replicative senescence

Although replicative senescence was first observed in 1961, it took more than 30 years to gain insights into the molecular regulators underlying this process. We now know that it is largely due to telomere lengths and structures. Telomeres are protective structures that cap the end of all eukaryotic chromosomes. They are long double stranded DNA sequences composed of TTAGGG repeats, oriented 5'-to-3' towards the end of the chromosome (Figure 5) [39]. Telomeres contain a complex composed of six proteins known as the shelterin complex. It comprises telomeric repeat binding factor 1 (TRF1), telomeric repeat binding factor 2 (TRF2), transcriptional repressor/activator protein (RAP1), TRF1-interacting nuclear factor 2 (TIN2),

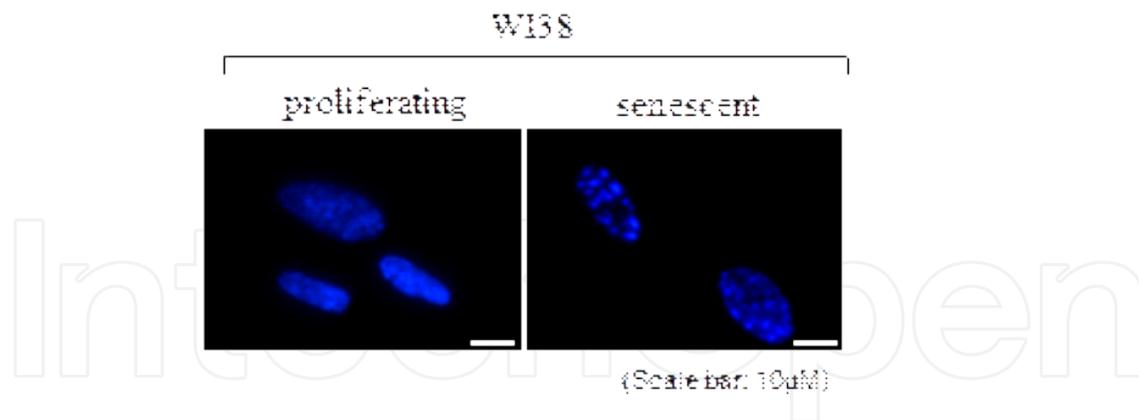


Figure 4. Senescence-associated-heterochromatin-foci (SAHF) of normal and senescent primary human fibroblasts. Dots representing SAHF are visible in senescent cells.

TIN2-interacting protein (TPP1) and protection of telomeres 1 (POT1) (Figure 5). The last two components, TPP1 and POT1 regulate the access of the telomeric substrate for the telomerase [40]. Telomere lengths are maintained by telomerase, a ribonucleoprotein complex that includes a RNA template (known as TERC) and the reverse transcriptase catalytic subunit (TERT) (Figure 5) [40]. Telomerase activity is mainly dictated by the TERT expression, as TERC seems to be ubiquitously transcribed [41]. In 1990, it was noticed that the telomeres length decreased during serially passage human primary fibroblasts and it was suggested that telomere size might be responsible for replicative senescence [42]. Eight years later, it was functionally demonstrated that re-introducing telomerase expression in normal primary cells led to elongated telomeres, lifespan extension and abrogation of replicative senescence [43, 44]. Since then, telomerase re-expression alone [45] or in combination with other alterations [46] has been associated with the immortalisation of various human cells types. Although telomere size is a critical trigger for replicative senescence, telomere structure is also a main determinant [47, 48]. In conclusion, dysfunctional telomeres both short or with an altered structure trigger replicative senescence.

2.4. Premature senescence

2.4.1. Oncogene-induced senescence (OIS)

In 1997, it was observed that in response to an oncogenic form of Ras (H-Ras^{G12V}) primary cells entered a premature senescence state [50]. Oncogene-induced senescence (OIS) is not restricted to Ras but can be extended to most of the MAPK pathway actors (Raf, Mek) and other oncogenic pathways. In accordance, the loss of bona fide tumour suppressors genes that restrain the activity of oncogenic pathways, such as phosphatase and tensin homolog (PTEN), Retinoblastoma (Rb), von Hippel-Lindau tumor suppressor (VHL) and neurofibromin 1 (NF1), has also been associated with premature senescence [10]. OIS is suggested to be a replicative senescence independent mechanism as cells expressing the catalytic subunit of the telomerase (TERT) still undergo OIS [51]. Whereas senescence in ageing tissues had been observed since 1995 [36], the

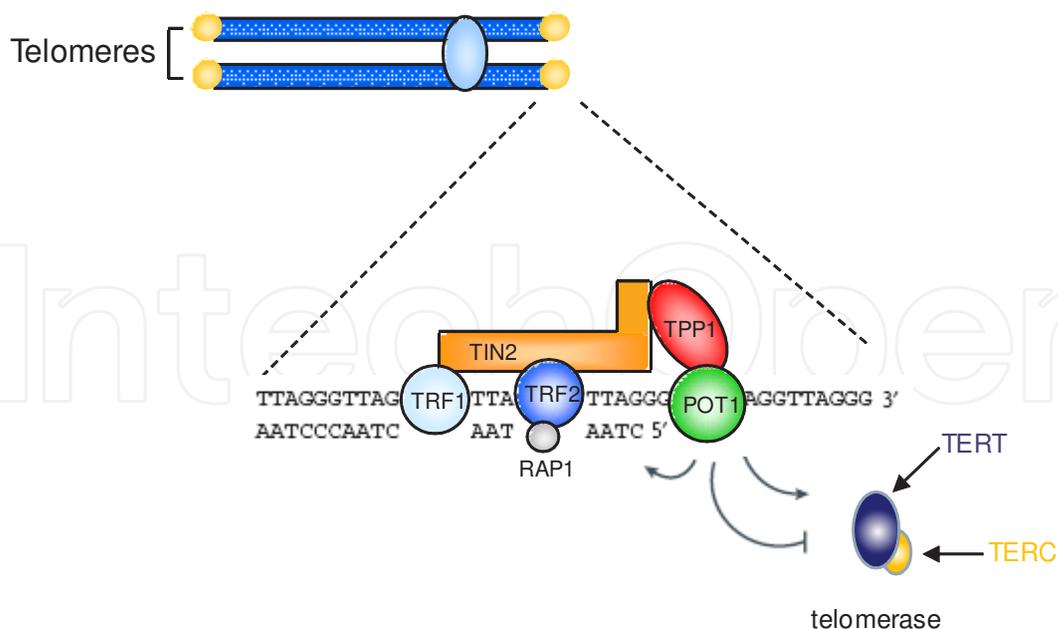


Figure 5. The telomeres and the telomerase. Telomeres, TTAGGG repeats, oriented 5' to 3' towards the end of the chromosome are regulated by six proteins that make up the shelterin complex. The telomeres lengths are maintained by the telomerase, a ribonucleoprotein complex composed of a RNA template (TERC) and the reverse transcriptase catalytic subunit (TERT). Adapted from [49].

demonstration of OIS in a physiological setting was demonstrated ten years later using various mouse model expressing a hyper-active oncogene form or a deleted tumor suppressor [10].

2.4.2. Oncogene-inactivation-induced senescence (OIIS)

Cellular senescence is not limited to primary cells but can also be triggered in cancer cells [52]. Cancer cells develop oncogene addiction, a term to describe a cell dependence on an oncogenic pathway to maintain its tumoral properties [53]. Various groups have been trying to identify these oncogene addictions using synthetic lethal screening [53, 54]. Interestingly, targeting oncogene addiction can result in cellular senescence. For example the inhibition of CDK4, a cyclin dependant kinase involved in cell cycle progression, in K-Ras^{G12V} driven non small cell lung carcinoma is associated with cellular senescence induction [55]. Moreover, the inhibition of several embryonic factors known to exert oncogenic properties (T-box 2 (TBX2), twist homolog 1/2 (TWIST 1/2)) can also result in cellular senescence [56, 57]. Finally c-Myc inhibition, a bHLH-LZ transcription factor involved in several tumoral processes such as proliferation, angiogenesis and cell metabolism [58], can leads to senescence induction in cancer cell lines and also in c-Myc transgenic driven lymphomas or osteosarcomas mouse models [59].

2.4.3. Stress-induced premature senescence (SIPS)

Stresses are major determinants of cellular senescence [21]. Even if replicative senescence, OIS and OIIS can also generate similar stresses and could be classified in the stress-induced cellular senescence category, we decided to write a specific section for SIPS. Oxygen is one of the major

determinants of stress-induced-premature-senescence (SIPS). Oxygen singlet (O_2) is not toxic for cells however O_2 consumption leads to reactive oxygen species (ROS) [21]. ROS can be classified into two groups. The first group which includes reactive species such as superoxide and hydroxyl radicals is composed of molecules with free radical containing one or more unpaired electrons in their outer molecular orbitals. The second group which includes hydrogen peroxide, ozone, peroxynitrate and hydroxide is composed of non-radical ROS that remain chemically reactive and can be converted to radical ROS [60].

Evidences demonstrating that ROS can trigger cellular senescence are now common. For example, cultivating primary human cells in 3% O_2 levels which is closer to the physiological conditions (normal physiological conditions vary from 1-2% in some parts of the brain, skin, heart and kidney to 14% in the lungs) [61], allowed cells to undergo 20 supplemental population doublings before reaching replicative senescence [62]. Conversely, raising the oxygen levels over 20% or exposing cells to sublethal doses of ROS such as H_2O_2 led to a premature senescence like state [63, 64].

SIPS is not restrained to oxidative stress. It can be induced in response to additional inadequate culturing conditions such as abnormal growth factors, the absence of neighbour cells and extracellular matrix components and inadequate concentrations of nutrients, [45, 65, 66]. Other physical, chemical and cellular stressors such as mitomycin C and ionizing radiation can also trigger SIPS [67].

2.5. Pathways regulating cellular senescence

2.5.1. The *INK4B/ARF/INK4A*

The *INK4B/ARF/INK4A* locus encodes three tumour suppressor genes that play critical regulatory roles in cellular senescence [31]. Two of these tumour suppressors, $p15^{INK4B}$ and $p16^{INK4A}$, are cyclin dependent kinase inhibitors (CKIs) that trigger cell cycle arrest by inhibiting CDK4 and CDK6 complexes [34]. ARF, the third gene encoded by the locus, is a critical regulator of the p53 tumour suppressor pathway [68]. ARF and $p16^{INK4A}$ share common exons but are encoded in alternative reading frames leading to the production of unique proteins [31].

Polycomb repressive group complexes (PRCs) play a crucial role in the regulation of the locus [69]. The polycomb family is composed of two repressive complexes PRC1 and PRC2. PRC2 establishes the repressive mark leading to the recruitment of the maintenance polycomb complex PRC1. Various polycomb proteins such as CBX7, CBX8, TBX2, TBX3 and Bmi1 have been functionally implicated in the repression of cellular senescence by inhibiting the *INK4B/ARF/INK4A* locus [5, 70-72]. To counteract the repressive action of the polycomb complexes, several mechanisms are activated during cellular senescence. These include the removal or inhibition of polycomb complexes by MAPKAP [73], by the chromatin remodeling SWI/SNF complex [74] and/or the activation of the *INK4B/ARF/INK4A* locus by JMJD3 [75, 76] (Figure 6).

Additional regulators of the locus include the stress activated p38MAPKinase. Activated in response to various stresses such as high levels of ROS, dysfunctional telomeres and OIS, it regulates cellular senescence in various contexts [77]. It can activate $p16^{INK4A}$, $p15^{INK4B}$ and ARF

[78]. Although the locus products are mainly regulated by epigenetic mechanism, post-translational modifications can also play a role in their regulation. For example, ARF stability is regulated by the E3 ubiquitin ligase TRIP12/ULF [68] and TGF- β stabilises p15^{INK4B} [79]. p16^{INK4A} is activated in response to UV treatment through the inhibition of a SKP2 related degradation [80].

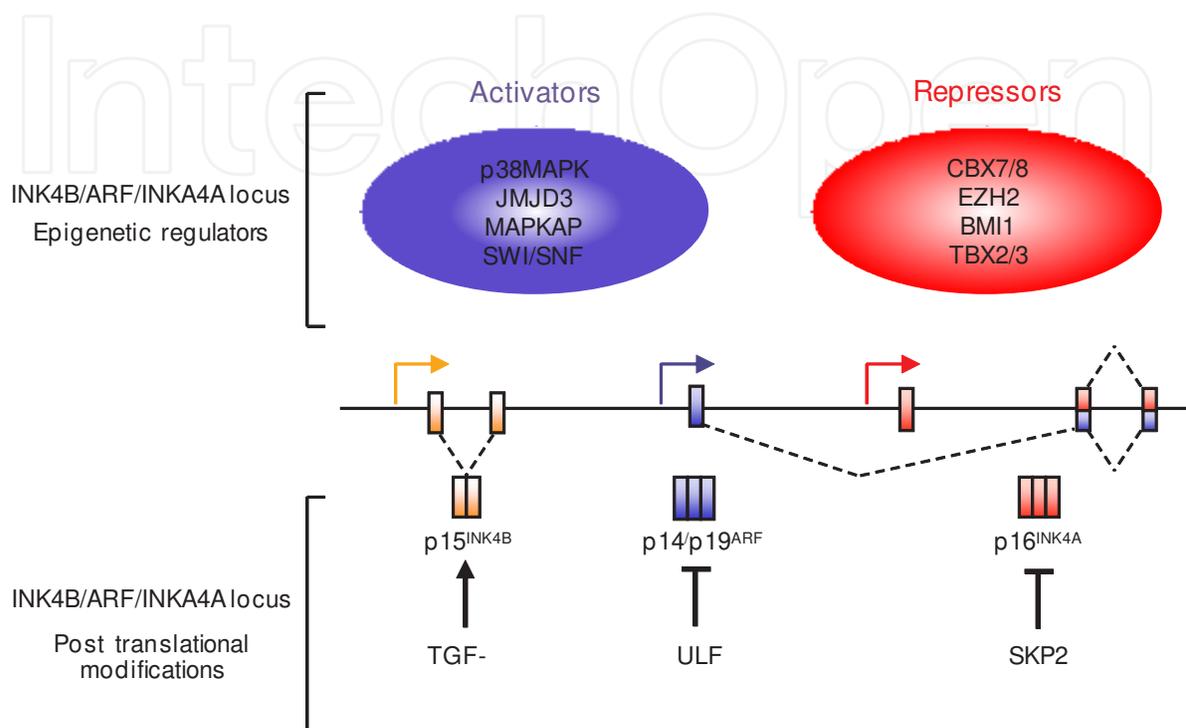


Figure 6. The INK4B/ARF/INK4A locus in cellular senescence.

The locus is mostly regulated by epigenetic mechanism. It involves repressors such as the polycomb proteins (CBX7/8, EZH2, BMI1 and TBX2/3) and activators including histone demethylases (JMJD3), protein kinases (p38MAPK, MAPKAP) and chromatin remodeling complexes (SWI/SNF). Post translational modifications also regulate the locus products.

2.5.2. The DNA damage/p53 pathway

Activated in response to stimuli that trigger a DDR such as dysfunctional telomeres, OIS, ionising radiation and ROS, the p53 pathway is critical in the regulation of senescence [81] (Figure 7).

The activation of a DNA damage response consists in the recruitment of DNA damage sensors at the sites of damage. Various DNA damage sites associated with cellular senescence have been described. These include telomere-dysfunction-induced-foci (TIFs) [82], senescence-associated DNA damage foci (SDF) [83] and DNA segments with chromatin alterations reinforcing senescence (DNA-SCARS) [84].

The DNA damage pathway is activated following the activation of two large protein kinases ataxia-telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR). Once recruited at the DNA damage sites, they phosphorylate and activate the histone variant H2AX [85]. The molecular events involved in single stranded breaks (SSBs) and double stranded breaks (DSBs) then differ and will not be described herein. The activation of the DDR kinases cascade, involving DNA damage mediators and diffusible kinases, results in the phosphorylation and activation of p53 which in turn activates p21^{CIP1}, one of its transcriptional targets to regulate growth arrest and cellular senescence [81]. The functional role of DDR in cellular senescence has been demonstrated using various functional approaches. Inactivating DDR proteins as well as p53 and its transcriptional target p21^{CIP1} is sufficient to abrogate cellular senescence in various settings [81]. The p53 pathway can also be activated in a DDR independent mechanism. For example, ARF plays a crucial role in the activation of p53. Activated in response to oncogenic stimulations, it activates p53 by sequestering the E3 ubiquitin protein ligase mouse double minute 2 (MDM2 or HDM2 in humans), an inhibitor of p53 [86] (Figure 7).

The DNA damage/p53 pathway is activated in response to dysfunctional telomeres, oncogenic or oxidative stress, ionizing radiations and cytotoxic drugs. A DNA damage response (DDR) is elicited leading to the activation of local apical kinases, DNA-damage mediators, diffusible kinases and ultimately p53 and p21^{CIP1}. The p53 pathway can also be activated by ARF following an oncogenic stress. Adapted from [81].

2.5.3. *Reactive oxygen species (ROS)*

ROS are critical regulators of OIS [87]. In accordance, ROS regulated proteins such as seladin-1 (modulators of peroxiredoxines, a class of antioxidants) have also been involved in OIS [88]. Enzymes that generate ROS such as 5-lipoxygenase (5LO) mediate Ras induced senescence [89]. ROS are not only involved in Ras induced senescence. Akt was recently identified as a major determinant of various types of cellular senescence by modulating oxygen consumption and down-regulating ROS scavengers [90]. ROS can also mediate replicative senescence. For example, large amount of ROS produced by dysfunctional mitochondria can modulate telomere length and replicative senescence [91]. In accordance, antioxidant proteins can negatively regulate cellular senescence. The extracellular superoxide dismutase (SOD) increases the lifespan of primary cells [92] and over-expressing the antioxidant enzyme catalase to the mitochondria increase mice lifespan [93].

2.5.4. *Small non coding RNAs*

miRNAs are small (approximately 23 nucleotides) RNAs that play a crucial role in gene regulation [94]. miRNAs bind 3'UTR and sometimes 5'UTR of the mRNA to modulate their translation and/or stability. The regulatory role of miRNAs during cellular senescence has recently been addressed [95]. Expression profiling studies indicate an altered expression among various miRNAs during cellular senescence [95]. Functionally, several miRNAs have been identified as repressor [96] or activators of cellular senescence [97] in normal but also cancer cells [98].

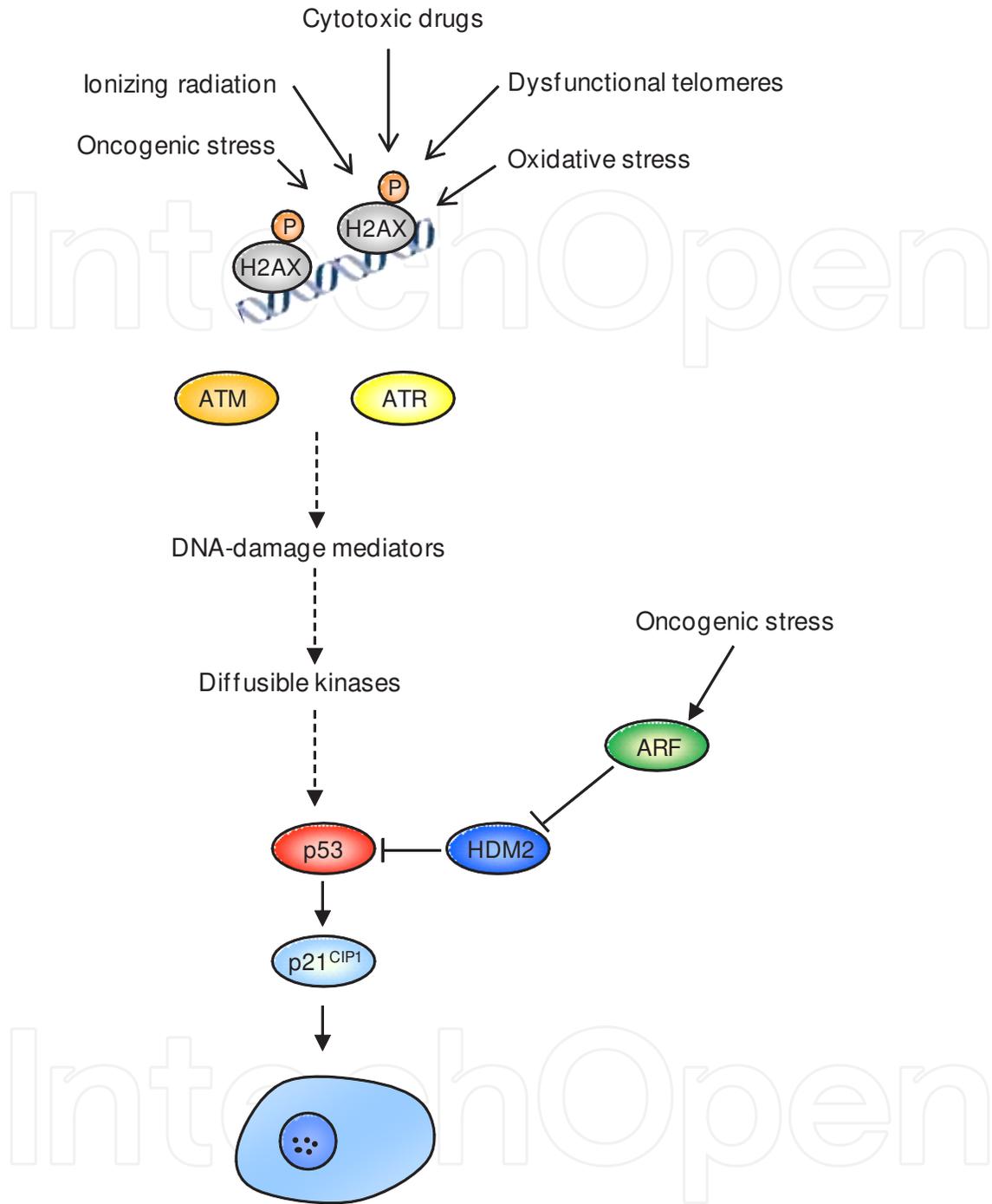


Figure 7. The DNA damage/p53 pathway in cellular senescence.

2.5.5. The autophagic pathway

Autophagy is a recycling process mediated by autophagosomes, which are double membrane vesicles that engulf cytoplasmic contents and then fuse and deliver their content to lysosomes. Lysosomes contain hydrolases that digest the material which ultimately leads to a breakdown of the vesicles and their constituents [99].

Senescent cells display an increase in autophagic vacuoles, a gradual shift from the proteasome pathway to autophagy for polyubiquitinated protein degradation [100] and an upregulation of autophagy regulators such as the ATG related genes (ULK1 and ULK3) [101]. Modulating critical components of the autophagic process can regulate cellular senescence. Inhibition of ATG proteins (ATG-5 and ATG-7) is sufficient to induce an escape from cellular senescence [101]. Conversely, over-expressing ATG target genes such as of ULK3 reduces cell growth [101].

2.6. Cellular senescence and cancer: The tumour point of view

Ever since the discovery and the term replicative senescence introduced by Hayflick in 1965 [3], it has been proposed that senescence can block tumour progression. Primary normal cells are said to be “mortal”, and in contrast, cancer cell lines are immortal. Therefore, an escape from replicative senescence is a critical stage that has to be overcome during tumour progression. To physiologically demonstrate this hypothesis, mouse models knockout for the RNA component of the telomerase (*TERC*^{-/-}) were used. The replicative senescence triggered in response to dysfunctional telomeres in these mice was shown to limit tumour progression and lead to tumour regression [102, 103]. In accordance with these results, late generation *TERC*^{-/-} deficient mice have been shown to be resistant to multistage skin carcinogenesis [104] and are more resistant to tumour formation in a subset of cancer mouse models [105, 106]. Oncogene-induced senescence (OIS) has also been identified as a failsafe program *in vivo*, and this, in response to a physiological aberrant oncogenic activation [10]. Additional inactivation of tumour suppressor genes regulating cellular senescence leads to progression towards malignant stages and full blown tumours [10]. In accordance, premalignant tumours display high levels of senescence whereas it is absent in later stages of tumorigenesis [10]. Finally, cellular senescence is not only a barrier against early stages of tumor progression. It can be reactivated in established tumors leading to tumor eradication [52].

In response to various cellular stresses such as dysfunctional telomeres, oncogene activation, oxidative stress and cytotoxic stresses normal cells acquire various alterations. Cellular senescence is activated to restrain neoplastic expansion. The inactivation of critical tumor suppressor genes (*p53*, *p16^{INK4}*, *ARF*...) leads to cellular senescence escape and progression towards more aggressive tumors. Cellular senescence can be reactivated in tumors in response to the activation of tumor suppressors or oncogene inhibition. This reactivation leads to tumor regression [52].

3. Immunosenescence

3.1. Features of immunosenescence

Cellular senescence can also be detected in immune cells [107]. Immunosenescence is a slightly different process than cellular senescence. Indeed, immunosenescence refers to cellular hypoproliferation in response to mitogenic or antigen stimulation and is often observed in ageing immune cells. In contrast, cellular senescence is associated with a permanent cell cycle arrest underlined with specific molecular markers. Immunosenescence affects both adaptive

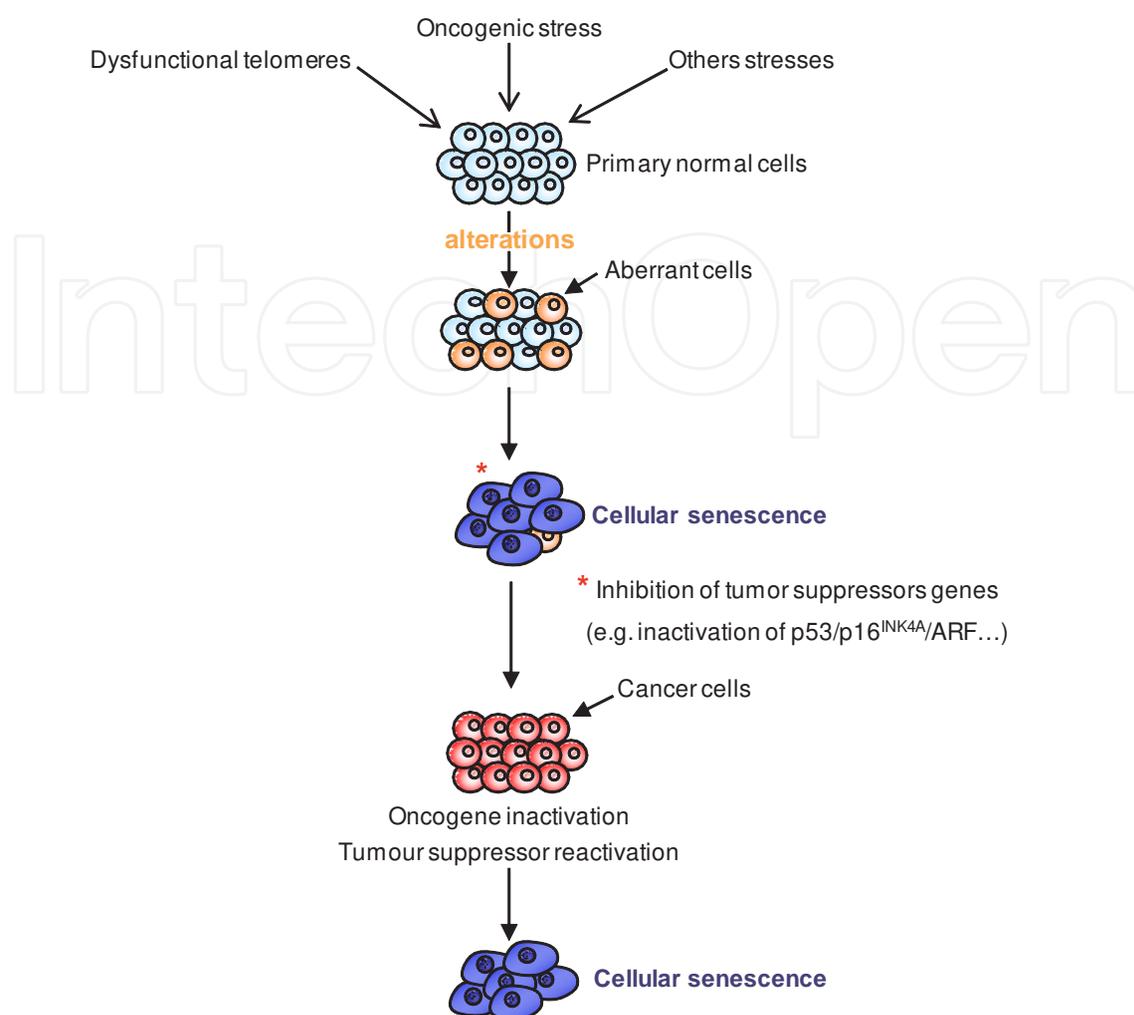


Figure 8. Cellular senescence acts as a tumor suppressive mechanism.

(T and B Lymphocytes) and innate immune cells (Natural killer cells (NK), Natural killer T cells (NKT), Macrophages, Neutrophils, Monocytes and Dendritic cells) [108, 109]. To date, the molecular determinants of immunosenescence have not been very well described. However, similarities with senescence in non immune cells are found. For example, immunosenescence of T cells is regulated by p16^{INK4A} and p21^{CIP1} [110, 111]. Differentiated T cells have shorter telomeres and an inactive telomerase [107]. Functionally, the ectopic telomerase expression maintains telomere size and delays immunosenescence [107]. However, immunosenescence also differs from cellular senescence. For example, inhibiting TNF- α receptor 1 partially reverses T cell immunosenescence in what is suggested to be a caspase-3 dependent mechanism [112]. Specific immunosenescence markers also exist. For example, cell surface markers such as CD27 and CD28 are lost in differentiated senescent CD4⁺ and CD8⁺ T cells and the killer cell lectin-like receptor subfamily G member 1 (KLRG1) is also known as a cell surface marker of immunosenescence [107]. Premature immunosenescence has not been characterized so far but one might expect that similar mechanism could be involved in premature senescence and premature immunosenescence. It could be interesting to assess whether stresses such as

high ROS levels, chemical or physical cellular stressors can trigger premature senescence. Additionally, it would be interesting to determine if the autophagic pathway and miRNAs are also implicated in various immunosenescence settings.

3.2. The triggers of immunosenescence

Various factors could explain immunosenescence. Thymic involution might play a role. It consists of shrinkage of the thymus with age and results in a decrease output of naïve T lymphocytes [113]. Altered immune signaling also seems to play a role. For example, in the elderly, the composition of lipid rafts is altered and this is associated with a decreased activity of some signaling pathways [114]. Above all, chronic antigen stimulation (e.g. chronic infections, tumor antigens) is the major trigger of immunosenescence. Cytomegalovirus (CMV) but also Epstein-Barr Virus (EBV) infection, to a lesser extent, are determinants of immunosenescence [115]. In accordance, the number of CD8+ cells expressing T cell receptor (TCR) specific CMV antigens in the ageing population increases. This has no effect in the number of T cell in the periphery however the expansion of CMV specific T cells results in a decrease in T cell repertoire [115]. In innate immune cells, an alteration of various receptors activity (e.g. Toll like receptor (TLR)) and in the number of some circulating innate immune cells seem to favor innate immunosenescence [109].

3.3. The consequences of immunosenescence

Immunosenescence is associated with decreased immune functions [109]. Neutrophils, the first innate immune cell to be at the site of pathogen entry, display a decreased chemotaxis and in free radical production and a decreased intracellular killing. Monocytes and macrophages both exhibit a decreased in phagocytosis and NK cells a decreased cytotoxicity. Dendritic cells display decreased phagocytosis, chemotaxis but also a reduced ability to activate the adaptive immune cells via antigen presentation [109]. Additionally to not being efficiently activated by the innate immune cells, adaptive immune cells are also affected by immunosenescence (particularly the T cell compartment). An inhibition of interleukine-2 (IL-2) production, increased DNA damage, telomere shortening but also a decrease in the number of naïve T cell (both CD4+ and CD8+) and an increase in the number of memory T cell leading to a weaker immunosurveillance are observed [115].

4. Immunosenescence and cancer

4.1. Tumors evade the immune system

Escape from immunosurveillance is now considered a cancer hallmark [116]. Immunosurveillance is a term used to define how immune cells identify and destroy abnormal antigen upon recognition [117]. This immunological process required for clearing tumors is often altered during cancer progression giving place to cancer favoring processes such as immunoselection, immunosubversion and ultimately immunosuppression [115, 117]. During cancer develop-

ment, a complex crosstalk between the tumor and the immune microenvironment takes place. Premalignant tumors send stress signals to the immune cells activating both innate and adaptative responses. In this context of immunosurveillance, the immune response is highly functional and eliminates surrounding tumors. However, tumor cells activate various mechanisms that allow immunosurveillance escape. These include the secretion of various cytokines, mechanisms to induce T cell apoptosis, anergy of naïve T cells, the activation of myeloid suppressor cells, the physical interaction of tumor cells with T cells, the suppression of NKT cell activities and downregulation of cell surface markers [115]. Immunosurveillance escape leads to a state of “equilibrium” between the tumor and the immune system, a process known as immunoselection. It is named as such because aggressive tumor cells that are capable of co-existing or suppressing the immune system are selected [115]. These cells eventually increase in number and secrete various immunosuppressive molecules (e.g. prostaglandin E2 (PGE2), nitric oxides) [117]. The immune system is therefore critical in suppressing tumor growth and its alteration is required from tumor progression. Interestingly, virtually of all the immune cells involved in immunosurveillance are known to be altered with age [115].

4.2. Cancer arises with age: A role for immunosenescence?

Cancer is a pathology associated with age [118]. Indeed, cancer incidence and prevalence increase with age suggesting an important relationship between the two biological processes [118]. During life, an organism is doomed to accumulate mutations favoring cancer development. The triggers of such mutations include environmental factors such as carcinogens, UV lights, viruses and free radicals among others [116]. On the other hand, cancer can no longer be regarded as a cell autonomous process. Recent advances demonstrate that the microenvironment plays a crucial role in modulating cancer development [116]. Although the role of the immune system in cancer development in ageing population is currently uncertain, it is clear that the immune system is altered with age [115]. As previously mentioned, the immune system is critical for clearing tumors, at least in early stages of tumorigenesis. Additionally, we have seen that immunosenescence is associated with profound immune alterations leading to a decrease in various immune cells activity. The activation of the adaptative immune response by dendritic cells is crucial for the activation of T-cell. Interestingly in the elders, immunosenescence of dendritic cells which impacts various co-receptors leads to a weakened T cell response [115]. The alteration of TLRs could also favor cancer development in ageing populations. Indeed, TLRs are crucial in the activation of innate immune cells. Their alterations leads to a decreased phagocytosis of innate immune cells and renders them less capable of destroying tumors. Ageing populations also have an increase amount of immune suppressive cells such as T regulatory cells that inhibit innate immune cells activity through direct cell-cell interaction. Myeloid derived suppressor cells (MDSC) are composed of a heterogeneous population of innate immune cells that can suppress the activation of CD4⁺ and CD8⁺ T cells. Interestingly, MDSC are activated by various anti-inflammatory signals (e.g IL-10, TGF- β) that are found increased in the elderly. Additionally, IDO, an immunosuppressive molecule that inhibits T cells responses, is also found up-regulated in ageing populations. Changes in the immune system that could favor cancer development are not limited to the innate immune cells. With ageing there is a gradual shift of Th1 towards Th2 in the T cells repertoire and this

leads to an alteration in the activity of both naïve and cytotoxic lymphocytes. In summary, various immunosuppressive mechanisms are found up-regulated in the elderly [115]. Considering the crucial role of the immune system in cancer eradication, it suggests that immunosenescence could play a role in cancers associated with age.

5. Immunosenescence and senescence immunosurveillance

5.1. Senescence immunosurveillance

An intimate relationship between cellular senescence and the immune system has recently been identified [2]. Using various mouse models, it was demonstrated that the reactivation of p53 in established tumours was associated with cellular senescence and tumour regression [119, 120]. However, as senescent cells do not die of apoptosis and can persist in some tissue for many years [121], the fate of senescent cells remained uncertain especially in a cancer context. Since the discovery of a secretory phenotype associated with cellular senescence (SASP), it has been suggested that senescent cells can initiate cross-talks with the microenvironment [2]. It was therefore hypothesised that senescent cells could be cleared by the immune system. In accordance, it was demonstrated in a mouse model of liver carcinoma that the activation of an innate immune response (NK cells macrophage and neutrophils) was responsible for the clearance of senescent tumours [120]. In liver cancer, senescence immunosurveillance thus seems to be required to clear senescent tumors. Interestingly, senescence immunosurveillance is not limited to liver cancer as it was also required for the complete remission of lymphoma and leukemia mouse models [122]. In these models, the adaptative immune response and more specifically CD4⁺ T cells were necessary for tumour eradication and also for senescence induction suggesting a complex interplay between senescent cells and the immune system [2]. Such interplay has been tackled in a mouse model of lymphomagenesis [123]. In a model of OIS *in vivo* resulting in apoptosis, macrophage were attracted and required to engulf apoptotic reminders. Both cellular senescence and apoptosis were shown to act together via the innate immune response to inhibit tumour progression. In turn, activated macrophages secreted various cytokines such as TGF- β to induce cellular senescence of malignant cells [123]. These results clearly demonstrate a crucial role of immunosurveillance in clearing senescent tumors and amplifying senescence induction. To reinforce the role of senescence immunosurveillance as a barrier against cancer, the inactivation of CD4⁺ T cell following OIS of hepatocytes in mice led to tumor progression [124]. The role CD4⁺ T cell in preventing tumor progression in this mouse model was dependent upon the activation of monocytes and macrophages [124]. What is surprising is that the inhibition of cellular senescence did not activate the immune system suggesting that during cancer development a functional senescence response might be required for an efficient immunosurveillance and tumor suppression [125]. It is therefore tempting to suggest that cellular senescence plays a significant role during immunosuppression [126]. It will also be interesting to demonstrate if senescence immunosurveillance also takes place in other organs during cancer development and whether it is involved in tumor suppression.

5.2. Defects in senescence immunosurveillance: A role for immunosenescence?

It appears that a functional immune system is required for efficient senescence clearance and tumor suppression. As immunosenescence affects normal immune homeostasis and senescent cells increase with age [118], it is tempting to speculate that in the elderly a defect in senescence immunosurveillance could contribute to the increased cancer incidence. In line with this hypothesis is the fact that all the immune cells involved in senescence immunosurveillance seem to be altered with age. However, to date no functional experiments have been carried to test such hypothesis. Mouse models of ageing recapitulating immunosenescence in which cellular senescence is induced could be of great interest to determine this possibility.

6. Conclusion

Immunosenescence is a field that holds great promises. To date, some of the molecular determinants of immunosenescence are still to be discovered. Identifying such mechanism could shed light on this cellular process and might help us to find ways to counteract such mechanism. Additionally, assessing the role of immunosenescence during cancer development in ageing population and more specifically its role during senescence immunosurveillance in the elderly could shed light on the mechanism associated with cancer.

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References

- [1] Collado M, Blasco MA, Serrano M. Cellular senescence in cancer and aging. *Cell*. 2007 Jul 27;130(2):223-33.
- [2] Serrano M. Cancer: final act of senescence. *Nature*. 2011 Nov 24;479(7374):481-2.
- [3] Hayflick L. The Limited in Vitro Lifetime of Human Diploid Cell Strains. *Exp Cell Res*. 1965 Mar;37:614-36.
- [4] Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. *Exp Cell Res*. 1961 Dec;25:585-621.

- [5] Gil J, Bernard D, Martinez D, Beach D. Polycomb CBX7 has a unifying role in cellular lifespan. *Nat Cell Biol.* 2004 Jan;6(1):67-72.
- [6] Rheinwald JG, Green H. Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells. *Cell.* 1975 Nov;6(3):331-43.
- [7] Romanov SR, Kozakiewicz BK, Holst CR, Stampfer MR, Haupt LM, Tlsty TD. Normal human mammary epithelial cells spontaneously escape senescence and acquire genomic changes. *Nature.* 2001 Feb 1;409(6820):633-7.
- [8] Tassin J, Malaise E, Courtois Y. Human lens cells have an in vitro proliferative capacity inversely proportional to the donor age. *Exp Cell Res.* 1979 Oct 15;123(2):388-92.
- [9] Tice RR, Schneider EL, Kram D, Thorne P. Cytokinetic analysis of the impaired proliferative response of peripheral lymphocytes from aged humans to phytohemagglutinin. *J Exp Med.* 1979 May 1;149(5):1029-41.
- [10] Collado M, Serrano M. Senescence in tumours: evidence from mice and humans. *Nat Rev Cancer.* 2010 Jan;10(1):51-7.
- [11] Melk A, Kittikowit W, Sandhu I, Halloran KM, Grimm P, Schmidt BM, et al. Cell senescence in rat kidneys in vivo increases with growth and age despite lack of telomere shortening. *Kidney Int.* 2003 Jun;63(6):2134-43.
- [12] Kim TW, Kim HJ, Lee C, Kim HY, Baek SH, Kim JH, et al. Identification of replicative senescence-associated genes in human umbilical vein endothelial cells by an annealing control primer system. *Exp Gerontol.* 2008 Apr;43(4):286-95.
- [13] Burks DJ, Font de Mora J, Schubert M, Withers DJ, Myers MG, Towery HH, et al. IRS-2 pathways integrate female reproduction and energy homeostasis. *Nature.* 2000 Sep 21;407(6802):377-82.
- [14] Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, Murphey RD, et al. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. *Curr Biol.* 2005 Feb 8;15(3):249-54.
- [15] Lundblad V, Szostak JW. A mutant with a defect in telomere elongation leads to senescence in yeast. *Cell.* 1989 May 19;57(4):633-43.
- [16] Comings DE, Okada TA. Electron microscopy of human fibroblasts in tissue culture during logarithmic and confluent stages of growth. *Exp Cell Res.* 1970 Aug;61(2):295-301.
- [17] Lipetz J, Cristofalo VJ. Ultrastructural changes accompanying the aging of human diploid cells in culture. *J Ultrastruct Res.* 1972 Apr;39(1):43-56.
- [18] Cristofalo VJ, Kritchevsky D. Cell size and nucleic acid content in the diploid human cell line WI-38 during aging. *Med Exp Int J Exp Med.* 1969;19(6):313-20.

- [19] Mitsui Y, Schneider EL. Increased nuclear sizes in senescent human diploid fibroblast cultures. *Exp Cell Res.* 1976 Jun;100(1):147-52.
- [20] Brunk U, Ericsson JL, Ponten J, Westermark B. Residual bodies and "aging" in cultured human glia cells. Effect of entrance into phase 3 and prolonged periods of confluence. *Exp Cell Res.* 1973 Apr;79(1):1-14.
- [21] Cristofalo VJ, Lorenzini A, Allen RG, Torres C, Tresini M. Replicative senescence: a critical review. *Mech Ageing Dev.* 2004 Oct-Nov;125(10-11):827-48.
- [22] Gerland LM, Peyrol S, Lallemand C, Branche R, Magaud JP, Ffrench M. Association of increased autophagic inclusions labeled for beta-galactosidase with fibroblastic aging. *Exp Gerontol.* 2003 Aug;38(8):887-95.
- [23] Kurz DJ, Decary S, Hong Y, Erusalimsky JD. Senescence-associated (beta)-galactosidase reflects an increase in lysosomal mass during replicative ageing of human endothelial cells. *J Cell Sci.* 2000 Oct;113 (Pt 20):3613-22.
- [24] Robbins E, Levine EM, Eagle H. Morphologic changes accompanying senescence of cultured human diploid cells. *J Exp Med.* 1970 Jun 1;131(6):1211-22.
- [25] Di Leonardo A, Linke SP, Clarkin K, Wahl GM. DNA damage triggers a prolonged p53-dependent G1 arrest and long-term induction of Cip1 in normal human fibroblasts. *Genes Dev.* 1994 Nov 1;8(21):2540-51.
- [26] Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, et al. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature.* 2006 Nov 30;444(7119):638-42.
- [27] Wada T, Joza N, Cheng HY, Sasaki T, Kozieradzki I, Bachmaier K, et al. MKK7 couples stress signalling to G2/M cell-cycle progression and cellular senescence. *Nat Cell Biol.* 2004 Mar;6(3):215-26.
- [28] Malumbres M, Barbacid M. Mammalian cyclin-dependent kinases. *Trends Biochem Sci.* 2005 Nov;30(11):630-41.
- [29] Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol.* 2007 Sep;8(9):729-40.
- [30] Malumbres M, Barbacid M. Cell cycle, CDKs and cancer: a changing paradigm. *Nat Rev Cancer.* 2009 Mar;9(3):153-66.
- [31] Kim WY, Sharpless NE. The regulation of INK4/ARF in cancer and aging. *Cell.* 2006 Oct 20;127(2):265-75.
- [32] Gil J, Peters G. Regulation of the INK4b-ARF-INK4a tumour suppressor locus: all for one or one for all. *Nat Rev Mol Cell Biol.* 2006 Sep;7(9):667-77.
- [33] Burkhardt DL, Sage J. Cellular mechanisms of tumour suppression by the retinoblastoma gene. *Nat Rev Cancer.* 2008 Sep;8(9):671-82.

- [34] Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. *Genes Dev.* 2010 Nov 15;24(22):2463-79.
- [35] Bracken AP, Kleine-Kohlbrecher D, Dietrich N, Pasini D, Gargiulo G, Beekman C, et al. The Polycomb group proteins bind throughout the INK4A-ARF locus and are dissociated in senescent cells. *Genes Dev.* 2007 Mar 1;21(5):525-30.
- [36] Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A.* 1995 Sep 26;92(20):9363-7.
- [37] Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, et al. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell.* 2003 Jun 13;113(6):703-16.
- [38] Zhang R, Chen W, Adams PD. Molecular dissection of formation of senescence-associated heterochromatin foci. *Mol Cell Biol.* 2007 Mar;27(6):2343-58.
- [39] Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet.* 2005 Aug;6(8):611-22.
- [40] Verdun RE, Karlseder J. Replication and protection of telomeres. *Nature.* 2007 Jun 21;447(7147):924-31.
- [41] Cech TR. Beginning to understand the end of the chromosome. *Cell.* 2004 Jan 23;116(2):273-9.
- [42] Harley CB, Futcher AB, Greider CW. Telomeres shorten during ageing of human fibroblasts. *Nature.* 1990 May 31;345(6274):458-60.
- [43] Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, et al. Extension of life-span by introduction of telomerase into normal human cells. *Science.* 1998 Jan 16;279(5349):349-52.
- [44] Vaziri H, Benchimol S. Reconstitution of telomerase activity in normal human cells leads to elongation of telomeres and extended replicative life span. *Curr Biol.* 1998 Feb 26;8(5):279-82.
- [45] Ramirez RD, Morales CP, Herbert BS, Rohde JM, Passons C, Shay JW, et al. Putative telomere-independent mechanisms of replicative aging reflect inadequate growth conditions. *Genes Dev.* 2001 Feb 15;15(4):398-403.
- [46] Kiyono T, Foster SA, Koop JI, McDougall JK, Galloway DA, Klingelhutz AJ. Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature.* 1998 Nov 5;396(6706):84-8.
- [47] Karlseder J, Hoke K, Mirzoeva OK, Bakkenist C, Kastan MB, Petrini JH, et al. The telomeric protein TRF2 binds the ATM kinase and can inhibit the ATM-dependent DNA damage response. *PLoS Biol.* 2004 Aug;2(8):E240.

- [48] Stewart SA, Ben-Porath I, Carey VJ, O'Connor BF, Hahn WC, Weinberg RA. Erosion of the telomeric single-strand overhang at replicative senescence. *Nat Genet.* 2003 Apr;33(4):492-6.
- [49] Deng Q, Liao R, Wu BL, Sun P. High intensity ras signaling induces premature senescence by activating p38 pathway in primary human fibroblasts. *J Biol Chem.* 2004 Jan 9;279(2):1050-9.
- [50] Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell.* 1997 Mar 7;88(5):593-602.
- [51] Wei S, Wei S, Sedivy JM. Expression of catalytically active telomerase does not prevent premature senescence caused by overexpression of oncogenic Ha-Ras in normal human fibroblasts. *Cancer Res.* 1999 Apr 1;59(7):1539-43.
- [52] Nardella C, Clohessy JG, Alimonti A, Pandolfi PP. Pro-senescence therapy for cancer treatment. *Nat Rev Cancer.* 2011 11(7):503-11.
- [53] Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. *Cell.* 2009 Mar 6;136(5):823-37.
- [54] Barbie DA, Tamayo P, Boehm JS, Kim SY, Moody SE, Dunn IF, et al. Systematic RNA interference reveals that oncogenic KRAS-driven cancers require TBK1. *Nature.* 2009 Nov 5;462(7269):108-12.
- [55] Puyol M, Martin A, Dubus P, Mulero F, Pizcueta P, Khan G, et al. A synthetic lethal interaction between K-Ras oncogenes and Cdk4 unveils a therapeutic strategy for non-small cell lung carcinoma. *Cancer Cell.* 2010 Jul 13;18(1):63-73.
- [56] Ansieau S, Bastid J, Doreau A, Morel AP, Bouchet BP, Thomas C, et al. Induction of EMT by twist proteins as a collateral effect of tumor-promoting inactivation of premature senescence. *Cancer Cell.* 2008 Jul 8;14(1):79-89.
- [57] Vance KW, Carreira S, Brosch G, Goding CR. Tbx2 is overexpressed and plays an important role in maintaining proliferation and suppression of senescence in melanomas. *Cancer Res.* 2005 Mar 15;65(6):2260-8.
- [58] Pelengaris S, Khan M, Evan G. c-MYC: more than just a matter of life and death. *Nat Rev Cancer.* 2002 Oct;2(10):764-76.
- [59] Wu CH, van Riggelen J, Yetil A, Fan AC, Bachireddy P, Felsher DW. Cellular senescence is an important mechanism of tumor regression upon c-Myc inactivation. *Proc Natl Acad Sci U S A.* 2007 Aug 7;104(32):13028-33.
- [60] Trachootham D, Alexandre J, Huang P. Targeting cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat Rev Drug Discov.* 2009 Jul;8(7):579-91.

- [61] Vaupel P, Kallinowski F, Okunieff P. Blood flow, oxygen and nutrient supply, and metabolic microenvironment of human tumors: a review. *Cancer Res.* 1989 Dec 1;49(23):6449-65.
- [62] Chen Q, Fischer A, Reagan JD, Yan LJ, Ames BN. Oxidative DNA damage and senescence of human diploid fibroblast cells. *Proc Natl Acad Sci U S A.* 1995 May 9;92(10):4337-41.
- [63] Alaluf S, Muir-Howie H, Hu HL, Evans A, Green MR. Atmospheric oxygen accelerates the induction of a post-mitotic phenotype in human dermal fibroblasts: the key protective role of glutathione. *Differentiation.* 2000 Oct;66(2-3):147-55.
- [64] Horikoshi T, Balin AK, Carter DM. Effects of oxygen tension on the growth and pigmentation of normal human melanocytes. *J Invest Dermatol.* 1991 Jun;96(6):841-4.
- [65] Bennett DC, Medrano EE. Molecular regulation of melanocyte senescence. *Pigment Cell Res.* 2002 Aug;15(4):242-50.
- [66] Loo DT, Fuquay JL, Rawson CL, Barnes DW. Extended culture of mouse embryo cells without senescence: inhibition by serum. *Science.* 1987 Apr 10;236(4798):200-2.
- [67] Toussaint O, Dumont P, Remacle J, Dierick JF, Pascal T, Fripiat C, et al. Stress-induced premature senescence or stress-induced senescence-like phenotype: one in vivo reality, two possible definitions? *ScientificWorldJournal.* 2002 Jan 29;2:230-47.
- [68] Collado M, Serrano M. The TRIP from UFL to ARF. *Cancer Cell.* 2010 Apr 13;17(4):317-8.
- [69] Popov N, Gil J. Epigenetic regulation of the INK4b-ARF-INK4a locus: in sickness and in health. *Epigenetics.* 2010 Nov-Dec;5(8):685-90.
- [70] Brummelkamp TR, Kortlever RM, Lingbeek M, Trettel F, MacDonald ME, van Lohuizen M, et al. TBX-3, the gene mutated in Ulnar-Mammary Syndrome, is a negative regulator of p19ARF and inhibits senescence. *J Biol Chem.* 2002 Feb 22;277(8):6567-72.
- [71] Jacobs JJ, Keblusek P, Robanus-Maandag E, Kristel P, Lingbeek M, Nederlof PM, et al. Senescence bypass screen identifies TBX2, which represses Cdkn2a (p19(ARF)) and is amplified in a subset of human breast cancers. *Nat Genet.* 2000 Nov;26(3):291-9.
- [72] Jacobs JJ, Kieboom K, Marino S, DePinho RA, van Lohuizen M. The oncogene and Polycomb-group gene bmi-1 regulates cell proliferation and senescence through the ink4a locus. *Nature.* 1999 Jan 14;397(6715):164-8.
- [73] Voncken JW, Niessen H, Neufeld B, Rennefahrt U, Dahlmans V, Kubben N, et al. MAPKAP kinase 3pK phosphorylates and regulates chromatin association of the polycomb group protein Bmi1. *J Biol Chem.* 2005 Feb 18;280(7):5178-87.

- [74] Kia SK, Gorski MM, Giannakopoulos S, Verrijzer CP. SWI/SNF mediates polycomb eviction and epigenetic reprogramming of the INK4b-ARF-INK4a locus. *Mol Cell Biol*. 2008 May;28(10):3457-64.
- [75] Agger K, Cloos PA, Rudkjaer L, Williams K, Andersen G, Christensen J, et al. The H3K27me3 demethylase JMJD3 contributes to the activation of the INK4A-ARF locus in response to oncogene- and stress-induced senescence. *Genes Dev*. 2009 May 15;23(10):1171-6.
- [76] Barradas M, Anderton E, Acosta JC, Li S, Banito A, Rodriguez-Niedenfuhr M, et al. Histone demethylase JMJD3 contributes to epigenetic control of INK4a/ARF by oncogenic RAS. *Genes Dev*. 2009 May 15;23(10):1177-82.
- [77] Wagner EF, Nebreda AR. Signal integration by JNK and p38 MAPK pathways in cancer development. *Nat Rev Cancer*. 2009 Aug;9(8):537-49.
- [78] Wong ES, Le Guezennec X, Demidov ON, Marshall NT, Wang ST, Krishnamurthy J, et al. p38MAPK controls expression of multiple cell cycle inhibitors and islet proliferation with advancing age. *Dev Cell*. 2009 Jul;17(1):142-9.
- [79] Sandhu C, Garbe J, Bhattacharya N, Daksis J, Pan CH, Yaswen P, et al. Transforming growth factor beta stabilizes p15INK4B protein, increases p15INK4B-cdk4 complexes, and inhibits cyclin D1-cdk4 association in human mammary epithelial cells. *Mol Cell Biol*. 1997 May;17(5):2458-67.
- [80] Al-Khalaf HH, Hendrayani SF, Aboussekhra A. The atr protein kinase controls UV-dependent upregulation of p16INK4A through inhibition of Skp2-related polyubiquitination/degradation. *Mol Cancer Res*. Mar;9(3):311-9.
- [81] d'Adda di Fagagna F. Living on a break: cellular senescence as a DNA-damage response. *Nat Rev Cancer*. 2008 Jul;8(7):512-22.
- [82] Takai H, Smogorzewska A, de Lange T. DNA damage foci at dysfunctional telomeres. *Curr Biol*. 2003 Sep 2;13(17):1549-56.
- [83] d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, et al. A DNA damage checkpoint response in telomere-initiated senescence. *Nature*. 2003 Nov 13;426(6963):194-8.
- [84] Rodier F, Coppe JP, Patil CK, Hoeijmakers WA, Munoz DP, Raza SR, et al. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol*. 2009 Aug;11(8):973-9.
- [85] Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, et al. Genomic instability in mice lacking histone H2AX. *Science*. 2002 May 3;296(5569):922-7.
- [86] Weber JD, Taylor LJ, Roussel MF, Sherr CJ, Bar-Sagi D. Nucleolar Arf sequesters Mdm2 and activates p53. *Nat Cell Biol*. 1999 May;1(1):20-6.

- [87] Lee AC, Fenster BE, Ito H, Takeda K, Bae NS, Hirai T, et al. Ras proteins induce senescence by altering the intracellular levels of reactive oxygen species. *J Biol Chem.* 1999 Mar 19;274(12):7936-40.
- [88] Wu C, Miloslavskaya I, Demontis S, Maestro R, Galaktionov K. Regulation of cellular response to oncogenic and oxidative stress by Seladin-1. *Nature.* 2004 Dec 2;432(7017):640-5.
- [89] Catalano A, Rodilossi S, Caprari P, Coppola V, Procopio A. 5-Lipoxygenase regulates senescence-like growth arrest by promoting ROS-dependent p53 activation. *Embo J.* 2005 Jan 12;24(1):170-9.
- [90] Nogueira V, Park Y, Chen CC, Xu PZ, Chen ML, Tonic I, et al. Akt determines replicative senescence and oxidative or oncogenic premature senescence and sensitizes cells to oxidative apoptosis. *Cancer Cell.* 2008 Dec 9;14(6):458-70.
- [91] Passos JF, Saretzki G, Ahmed S, Nelson G, Richter T, Peters H, et al. Mitochondrial dysfunction accounts for the stochastic heterogeneity in telomere-dependent senescence. *PLoS Biol.* 2007 May;5(5):e110.
- [92] Serra V, von Zglinicki T, Lorenz M, Saretzki G. Extracellular superoxide dismutase is a major antioxidant in human fibroblasts and slows telomere shortening. *J Biol Chem.* 2003 Feb 28;278(9):6824-30.
- [93] Schriner SE, Linford NJ, Martin GM, Treuting P, Ogburn CE, Emond M, et al. Extension of murine life span by overexpression of catalase targeted to mitochondria. *Science.* 2005 Jun 24;308(5730):1909-11.
- [94] Bartel DP. MicroRNAs: target recognition and regulatory functions. *Cell.* 2009 Jan 23;136(2):215-33.
- [95] Liu FJ, Wen T, Liu L. MicroRNAs as a novel cellular senescence regulator. *Ageing Res Rev.* 2011 Jun 13.
- [96] Voorhoeve PM, le Sage C, Schrier M, Gillis AJ, Stoop H, Nagel R, et al. A genetic screen implicates miRNA-372 and miRNA-373 as oncogenes in testicular germ cell tumors. *Cell.* 2006 Mar 24;124(6):1169-81.
- [97] He L, He X, Lim LP, de Stanchina E, Xuan Z, Liang Y, et al. A microRNA component of the p53 tumour suppressor network. *Nature.* 2007 Jun 28;447(7148):1130-4.
- [98] Xu D, Takeshita F, Hino Y, Fukunaga S, Kudo Y, Tamaki A, et al. miR-22 represses cancer progression by inducing cellular senescence. *J Cell Biol.* 2011 Apr 18;193(2):409-24.
- [99] Levine B, Kroemer G. Autophagy in the pathogenesis of disease. *Cell.* 2008 Jan 11;132(1):27-42.

- [100] Gamerding M, Hajieva P, Kaya AM, Wolfrum U, Hartl FU, Behl C. Protein quality control during aging involves recruitment of the macroautophagy pathway by BAG3. *Embo J*. 2009 Apr 8;28(7):889-901.
- [101] Young AR, Narita M, Ferreira M, Kirschner K, Sadaie M, Darot JF, et al. Autophagy mediates the mitotic senescence transition. *Genes Dev*. 2009 Apr 1;23(7):798-803.
- [102] Cosme-Blanco W, Shen MF, Lazar AJ, Pathak S, Lozano G, Multani AS, et al. Telomere dysfunction suppresses spontaneous tumorigenesis in vivo by initiating p53-dependent cellular senescence. *EMBO Rep*. 2007 May;8(5):497-503.
- [103] Feldser DM, Greider CW. Short telomeres limit tumor progression in vivo by inducing senescence. *Cancer Cell*. 2007 May;11(5):461-9.
- [104] Gonzalez-Suarez E, Samper E, Flores JM, Blasco MA. Telomerase-deficient mice with short telomeres are resistant to skin tumorigenesis. *Nat Genet*. 2000 Sep;26(1):114-7.
- [105] Greenberg RA, Chin L, Femino A, Lee KH, Gottlieb GJ, Singer RH, et al. Short dysfunctional telomeres impair tumorigenesis in the INK4a(delta2/3) cancer-prone mouse. *Cell*. 1999 May 14;97(4):515-25.
- [106] Siegl-Cachedenier I, Munoz P, Flores JM, Klatt P, Blasco MA. Deficient mismatch repair improves organismal fitness and survival of mice with dysfunctional telomeres. *Genes Dev*. 2007 Sep 1;21(17):2234-47.
- [107] Akbar AN, Henson SM. Are senescence and exhaustion intertwined or unrelated processes that compromise immunity? *Nat Rev Immunol*. 2011 Apr;11(4):289-95.
- [108] Caruso C, Buffa S, Candore G, Colonna-Romano G, Dunn-Walters D, Kipling D, et al. Mechanisms of immunosenescence. *Immun Ageing*. 2009;6:10.
- [109] Panda A, Arjona A, Sapey E, Bai F, Fikrig E, Montgomery RR, et al. Human innate immunosenescence: causes and consequences for immunity in old age. *Trends Immunol*. 2009 Jul;30(7):325-33.
- [110] Liu Y, Johnson SM, Fedoriw Y, Rogers AB, Yuan H, Krishnamurthy J, et al. Expression of p16(INK4a) prevents cancer and promotes aging in lymphocytes. *Blood*. 2011 Mar 24;117(12):3257-67.
- [111] Menzel O, Migliaccio M, Goldstein DR, Dahoun S, Delorenzi M, Rufer N. Mechanisms regulating the proliferative potential of human CD8+ T lymphocytes overexpressing telomerase. *J Immunol*. 2006 Sep 15;177(6):3657-68.
- [112] Parish ST, Wu JE, Effros RB. Modulation of T lymphocyte replicative senescence via TNF- α inhibition: role of caspase-3. *J Immunol*. 2009 Apr 1;182(7):4237-43.
- [113] Lynch HE, Goldberg GL, Chidgey A, Van den Brink MR, Boyd R, Sempowski GD. Thymic involution and immune reconstitution. *Trends Immunol*. 2009 Jul;30(7):366-73.

- [114] Larbi A, Dupuis G, Khalil A, Douziech N, Fortin C, Fulop T, Jr. Differential role of lipid rafts in the functions of CD4+ and CD8+ human T lymphocytes with aging. *Cell Signal*. 2006 Jul;18(7):1017-30.
- [115] Fulop T, Kotb R, Fortin CF, Pawelec G, de Angelis F, Larbi A. Potential role of immunosenescence in cancer development. *Ann N Y Acad Sci*. 2010 Jun;1197:158-65.
- [116] Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011 Mar 4;144(5):646-74.
- [117] Zitvogel L, Tesniere A, Kroemer G. Cancer despite immunosurveillance: immunoselection and immunosubversion. *Nat Rev Immunol*. 2006 Oct;6(10):715-27.
- [118] Campisi J. Cancer and ageing: rival demons? *Nat Rev Cancer*. 2003 May;3(5):339-49.
- [119] Ventura A, Kirsch DG, McLaughlin ME, Tuveson DA, Grimm J, Lintault L, et al. Restoration of p53 function leads to tumour regression in vivo. *Nature*. 2007 Feb 8;445(7128):661-5.
- [120] Xue W, Zender L, Miething C, Dickins RA, Hernando E, Krizhanovsky V, et al. Senescence and tumour clearance is triggered by p53 restoration in murine liver carcinomas. *Nature*. 2007 Feb 8;445(7128):656-60.
- [121] Michaloglou C, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, et al. BRAF600-associated senescence-like cell cycle arrest of human naevi. *Nature*. 2005 Aug 4;436(7051):720-4.
- [122] Rakhra K, Bachireddy P, Zabuawala T, Zeiser R, Xu L, Kopelman A, et al. CD4(+) T cells contribute to the remodeling of the microenvironment required for sustained tumor regression upon oncogene inactivation. *Cancer Cell*. 2010 Nov 16;18(5):485-98.
- [123] Reimann M, Lee S, Loddenkemper C, Dorr JR, Tabor V, Aichele P, et al. Tumor stroma-derived TGF-beta limits myc-driven lymphomagenesis via Suv39h1-dependent senescence. *Cancer Cell*. 2010 Mar 16;17(3):262-72.
- [124] Kang TW, Yevsa T, Woller N, Hoenicke L, Wuestefeld T, Dauch D, et al. Senescence surveillance of pre-malignant hepatocytes limits liver cancer development. *Nature*. 2011 Nov 24;479(7374):547-51.
- [125] Hoenicke L, Zender L. Immune surveillance of senescent cells--biological significance in cancer- and non-cancer pathologies. *Carcinogenesis*. 2011 Nov;33(6):1123-6.
- [126] Schreiber RD, Old LJ, Smyth MJ. Cancer immunoediting: integrating immunity's roles in cancer suppression and promotion. *Science*. 2011 Mar 25;331(6024):1565-70.

