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Ageing or cancer: A review

On the role of caretakers and gatekeepers

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ARTICLE INFO

Article history:
Received 12 April 2007
Received in revised form 10 July 2007
Accepted 11 July 2007
Available online 30 August 2007

Keywords:
Ageing
Cancer
Tumour suppression
Antagonistic pleiotropy

ABSTRACT

Ageing is due to the accumulation of damage, which arises because of evolved limitations in mechanisms for maintenance and repair. Accumulated damage may cause genomic instability, which in organisms with renewable tissues may result in cancer. To keep cancer at bay, two different tumour suppression mechanisms evolved: caretakers and gatekeepers. Caretakers protect the genome against mutations, while gatekeepers induce cell death or cell cycle arrest of potentially tumourigenic cells. It has been hypothesised that decreased activity of a caretaker may reduce life span, by increasing cancer risk, while the effects of increased activity of a gatekeeper on cancer risk and life span may be antagonistically pleiotropic. Apoptosis and senescence will promote early-life survival by curtailing the development of cancer, but may eventually limit longevity. This article reviews the evidence for this hypothesis. We conclude that several different findings indeed hint at an important role for gatekeeper mediated processes in ageing and its related pathologies. The relative contribution of apoptosis and senescence in specific age-related pathologies remains to be established.

1. Introduction

Ageing is due to the permanent accumulation of damage to molecules, cells, organs, tissues and the whole organism. Such damage is caused by various intrinsic and extrinsic biological and biochemical stresses. Maintenance mechanisms that counterbalance the accumulation of damage as well as pathways that influence the rate of biological and biochemical stresses are important for the preservation of health and longevity. There is a striking difference in the life spans and onset of ageing between species. The life span of *Caenorabditis elegans* encompasses only a few weeks, and that of *Drosophila melanogaster* only a few months, while mice live several years and humans many decades. One important distinction between these two classes of organisms is that the soma of worms and fruit flies consists (almost exclusively) of postmitotic cells, while the soma of mammals has the capacity for self-renewal. In postmitotic organisms, the accumulation of damage that underlies ageing will lead to a permanent loss of cells which cannot be compensated for. However, organisms with self-renewable tissues can replace damaged and lost cells, thus increasing their possibilities for maintenance, repair and longer lifespan. However, the capacity of cellular self-renewal also brings an enormous danger not experienced by postmitotic organisms: cancer.[1,2] Moreover, because of continuous accumulation of damage, cells might acquire genetic changes that affect cell growth or differentiation, thus becoming at risk of being transformed into a cancer cell during the neoplastic process.[3] Cell growth and differentiation are regulated by complicated interactions between growth promoting and growth inhibiting signals, with growth promoting oncogenes and growth inhibiting tumour suppressor genes being the targets for many genetic changes that increase cancer susceptibility. Tumour suppressor genes can

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0959-8049/$ - see front matter
doi:10.1016/j.ejca.2007.07.011

be separated into two major categories: gatekeepers and caretakers.[4] Caretaker genes protect the genome against damage and mutations, while gatekeeper genes induce cell death or cell cycle arrest of cells that have accumulated damage.

2. DNA repair in ageing and cancer

Caretakers do not directly regulate cell proliferation, but act to prevent genomic instability. It has been hypothesised that increased activity of a caretaker gene increases genome stability and both reduces cancer risk, postpones ageing and increases lifespan. Across species, a positive correlation has been found between cellular stress resistance and species-specific life span.[5] Also within species, mutant models of accelerated or decelerated ageing exhibit a strong correlation between stress resistance and life span, not only on the level of the organism, but also on the level of cells derived from these organisms.[6] However, the exact contribution of DNA repair to the increased stress resistance is difficult to assess experimentally, as reliable assays to measure DNA repair capacity in organisms still need to be developed.

Inactivation of caretaker genes leads to accelerated conversion of a normal cell to a neoplastic cell. Our genome is under the constant threat of acquiring mutations, which can be caused by the intrinsic chemical instability of DNA and by its exposure to genotoxic agents derived from cellular metabolism (such as reactive oxygen species (ROS)), or from the environment.[7] As a first safeguard against the potentially harmful effects of ROS, cells have evolved several antioxidant defence systems. These antioxidant defence systems include enzymatic ROS scavengers such as superoxide dismutase (SOD), catalase and glutathione peroxidase, as well as non-enzymatic low molecular mass ROS scavengers such as ascorbate, pyruvate, flavenoids, carotenoids and glutathione. Despite these defence mechanisms, many lesions of different types will accumulate in our DNA. To deal with these different types of lesions, cells have developed different, partly overlapping DNA repair systems.[7,8] Lesions on one of the two strands of the DNA double helix can be repaired by copying information from the complementary strand. In case such lesions are small and do not distort the DNA double helix, repair is predominantly performed by base excision repair (BER), while more bulky, helix distorting lesions are repaired by nucleotide excision repair (NER). In case of lesions that affect both strands of the DNA double helix, such as DNA double strand breaks (DSBs), the lesion can either be repaired by non-homologous endjoining (NHEJ) or by homologous recombination (HR). In the latter case, the genetic information required for repair is accurately copied via invasion of another intact double stranded DNA template, such as the (identical) sister chromatid. In NHEJ, the two ends of the DSB are religated, with the risk of potentially losing information. However, in case the ends of a DSB are blunt and 5′phosphorylated, repair by NHEJ is accurate as well.[9] Other types of lesions, such as mismatches and crosslinks, are predominantly repaired by other dedicated repair pathways, respectively mismatch repair (MMR) and crosslink repair (CLR). Moreover, DNA repair must be properly coordinated with other aspects of DNA metabolism, such as DNA replication and gene transcription, and with activation of cell cycle checkpoints to ensure sufficient time for proper repair. Components from the DNA damage response machinery that coordinate DNA repair with activation of cell cycle checkpoints, such as ATM and BRCA1/BRCA2, are also classified among the caretakers.[8] The existence of numerous human cancer-prone chromosomal instability syndromes associated with germ line mutations in caretaker genes that, either directly or indirectly, play a role in DNA repair, highlights the importance of caretakers in suppressing neoplastic transformation (Table 1). In all of these syndromes, reduced life span is a direct consequence of increased cancer risk, leading to the hypothesis that, also in a broader sense, inactivation of caretakers may reduce life span, by increasing cancer risk.

3. NER in ageing and cancer

However, inactivation of caretakers does not always lead to a cancer-prone phenotype, as is illustrated by the different clinical phenotypes that are associated with three rare human photosensitive disorders with inborn defects in nucleotide excision repair (NER): Xeroderma pigmentosum (XP), Cockayne syndrome (CS) and Trichothiodystrophy (TTD).[10] The cutaneous hallmarks of XP patients are parchment skin and freckles. XP patients are very sensitive to UV-light and have

Table 1 – Deficiency in caretakers underlying human cancer-prone syndromes

Cancer-prone syndrome	DNA repair systems	Deficient component(s)	Major cancer predisposition
Xeroderma pigmentosum[82]	NER	XPA-XPG	UV-induced skin cancer
Hereditary non-polyposis colorectal cancer (HNPCC)[83–85]	MMR	MSH2, PMS1, PMS2, MLH1	Colorectal cancer
Fanconi anaemia (FA)[86]	CLR	FANCA, B, C, D1, D2, E, F, G, I, J, L, M	Leukaemia
Ligase IV deficiency[87]	NHEJ	Ligase IV	Leukaemia (?)
Nijmegen breakage syndrome (NBS)[88]	DSB repair	NSB	Lymphomas
Bloom syndrome (BS)[89]	HR	Blm	All types
Werner syndrome (WS)[90]	DSB repair?	Wrn	Sarcoma
Rothmund–Thomson syndrome (RTS)[91]	DSB repair?	RecQL4	Sarcoma, Osteosarcoma
Ataxia telangiectasia (AT)[92]	DSB repair	ATM	Lymphomas
Breast (ovarian) cancer[93]	DSB repair and CLR	BRCA1, BRCA2	Breast (ovarian) cancer

Abbreviations DNA repair systems: DSB repair = double strand break repair, CRL = crosslink repair, HR = homologous recombination, MMR = mismatch repair, NHEJ= non-homologous endjoining.

Table 2 – The NER components which are affected in different NER syndromes

NER syndrome	Mutated NER component(s)
Xeroderma pigmentosum (XP)	XPA-XPG
Cockayne syndrome (CS)	CSA, CSB
Combined Xeroderma pigmentosum/ Cockayne syndrome (XP/CS)	XPB, XPD, XPG
Trichothiodystrophy (TTD)	TTDA, XPB, XPD

a 1000-fold increased risk of developing skin cancer. However, in CS and TTD patients, no predisposition to cancer is observed. CS patients display severe neurodevelopmental defects, including a short stature and mental retardation, in combination with striking features of premature ageing, such as hearing loss, retinopathy, and cataracts. TTD patients share many symptoms of CS patients, but with the additional hallmarks of brittle hair, nails and scaly skin. The phenotypes associated with these three disorders display a high degree of heterogeneity, and combinations also occur. The differences in phenotype between the three disorders can be explained because these syndromes are caused by different mutations in the components that play a role during the NER process (Table 2).

During the NER process, about 30 components participate in the following successive steps: recognition of damage, opening of the DNA double helix around the lesion, excision of a patch of the damaged strand of approximately 24–32 nucleotides including the injury, and filling of the resulting gap by DNA repair synthesis followed by strand ligation.[11] Most of these components are shared between the two different NER pathways, global genome nucleotide excision repair (GG-NER) and transcription coupled repair (TCR), except those implicated in the initial damage recognition step, which is performed by XPC/HRAD23B in GG-NER.[12] In TCR, the lesion is detected during transcription, by the elongating RNA polymerase II. The stalled polymerase must be displaced to make the injury accessible for repair, which requires the specific components CSA and CSB. Mutations in CSA and CSB causing CS specifically affect TCR, which ensures that genes that are being transcribed are repaired with higher priority and more efficiently than the rest of the genome, which is not used by the cell. Besides being shared between GG-NER and TCR, some components are also shared between NER and other processes. XPB and XPD form part of the larger complex TFIIH that plays a role in the opening of the DNA helix around the lesion to facilitate access of the repair machinery to the lesion. The TFIIH complex plays a similar role in opening of the DNA double helix during transcription to facilitate access of the transcription machinery to the gene promoter. Mutations in XPB or XPD that affect their role in repair will result in XP, while mutations that affect their role in transcription may result either in CS (in combination with XP), or (in case the stability of the TFIIH complex is affected) in TTD.[10]

Why would mutations that specifically affect TCR or transcription result in premature ageing phenotypes, while mutations that affect GG-NER will result in cancer predisposition? This could be explained because TCR is dedicated to removing cytotoxic lesions, while GG-NER is dedicated to removing premutagenic lesions.[13,14] Cytotoxic lesions interfere with DNA metabolic processes such as transcription, which will trigger a stress response. If transcription is not restored within a certain time frame, cells may undergo apoptosis and cell death in a p53-dependent and independent manner.[13,14] The mechanisms by which apoptosis and cell death may be induced include a shift in the balance between the levels of pro-apoptotic factors compared to anti-apoptotic factors, aberrant accumulation of proteins in the nucleus, accumulation of p53 at mitochondria and complications during replication. To counteract apoptosis, cytotoxic transcription blocking lesions are removed by TCR. GG-NER is important for the removal of premutagenic lesions, which do not directly interfere with DNA metabolic processes, but may result in mutations if not removed before replication. If GG-NER is not efficient enough, cells will display deficits in the removal of premutagenic lesions, and carcinogenesis is promoted. On the other hand, if TCR is not efficient enough, excess apoptosis is promoted, leading to ageing. Different lines of evidence suggest a role for gatekeepers mechanisms that include activation of ATR and suppression of the GH/IGF-1 axis in the premature ageing phenotypes without cancer predisposition that are observed in CS and TTD.[12] The work discussed above has lead to the hypothesis that also in the population at large, gatekeeper factors which determine the decision between the induction of cell fates such as proliferation, apoptosis or stimulation of the DNA damage response, may play a critical role in both ageing and cancer.

4. P53 in cancer and ageing

Within the category gatekeepers, the *TP53* gene is the most well known. Human *TP53* encodes a 53-kd multifunctional transcription factor that, in response to a variety of intracellular stress signals, regulates the expression of genes involved in cell cycle control, apoptosis, DNA repair, and angiogenesis.[15] The nature and intensity of the stress signals, and the cellular context determine which response occurs. In general, it seems likely that high dosages of stress will lead to apoptosis, while more moderate levels of stress may induce cell cycle arrest. This strongly depends on the type of stress and on cell type though, as some cell types are more apoptotic prone and others are more prone to cellular senescence.[16] The gatekeeper gene *TP53* is the most frequently mutated gene in human cancer. About half of all human cancers display structural alterations in one or both *TP53* alleles.[17] A typical example of a (familial) cancer-prone clinical syndrome that is associated with *TP53* mutations in the germ line is the Li-Fraumeni syndrome.[18] Several lines of evidence show that increasing gatekeeper activity may be antagonistically pleiotropic in that, although it diminishes cancer risk, it may at the same time accelerate age-related pathologies (Box 1).

Modulating p53 activity in mice has hinted at an intimate link between cancer resistance and ageing. Loss of p53 results in increased genomic instability and predisposes cells to becoming cancerous. Indeed, p53 knockout mice ($p53^{-/-}$ and $p53^{+/-}$) develop normally but die early due to cancers.[19] Interestingly however, two of the $p53^{+/-}$ mice that managed to

evade tumours lived far longer than any of their wild type ($p53^{+/+}$) counterparts.[20] However, elevated p53 activity does not lead to a long, cancer-free life. Mutant p53 ($p53^{+/m}$) mice that have constitutively activated p53 show greatly reduced cancer incidence but faster ageing; their lifespan is shortened and is accompanied by accelerated age-related reduction in mass and cellularity of various tissues.[21] The premature ageing phenotype of the $p53^{+/m}$ mice has been reproduced in another mouse model engineered to overexpress the short isoform of p53.[22] However, yet another mouse model in which an extra copy of wild-type p53 was inserted in the mouse genome resulted in increased p53 activity in response to DNA damage and improved tumour suppression, but did not enhance ageing phenotypes.[23] The absence of an overt premature ageing phenotype in these mice may be attributed to the non-constitutive expression of p53, as compared to the constitutive expression of p53 in the $p53^{+/m}$ mice. However, it cannot be excluded that minor premature ageing features in the mouse model of the Serrano group have been overlooked or depend on the inbred mouse strain. The premature age related pathologies of the $p53^{+/m}$ mice with constitutive expression of p53 may be caused by increased p53-mediated induction of cellular senescence and apoptosis. How exactly would increased induction of apoptosis and/or senescence contribute to (premature) age related pathologies?

5. Apoptosis in cancer and ageing

Apoptosis is the well-characterised process of programmed cell death, eliminating cells in a tightly controlled way without damaging neighbouring cells as in necrosis. Apoptosis is an evolutionarily highly conserved mechanism, seen in all multicellular organisms ranging from *C. elegans* to humans.[24] In mammalian cells, apoptosis can be activated via either an intrinsic or extrinsic pathway (Fig. 1).[25] The extrinsic pathway is initiated by signals coming from outside the cell, which activate death receptors such as Fas on the cell membrane. Activated death receptors then trigger activity of caspase 8, which activates the downstream caspase 3 and cleaves tBid, which eventually leads to release of cytochrome C from mitochondria.[25] Released cytochrome C not only causes a disruption of the mitochondrial membrane potential, but is also an important factor for further activation of the caspase cascade. The caspases act as key engines of cellular destruction by cleaving and activating diverse cellular substrates, which in their turn mediate hallmarks of apoptosis such as genomic DNA fragmentation and breakdown of the cytoskeleton. The intrinsic pathway can be initiated by many cellular stressors, including damaged DNA or reactive oxygen species (ROS), which increase the posttranslational activity of p53.[26] Activated p53 enhances the expression of many pro-apoptotic genes, including *Bax*.[27] The pro-apoptotic proteins encoded by these genes locate to the mitochondria where they stimulate cytochrome C release, where after apoptosis continues as described for the extrinsic pathway. Intrinsic apoptosis can also be triggered in a p53 independent fashion.

Apoptosis kills and eliminates potential cancer cells. However, apoptosis may also lead to the exhaustion of the number of division-competent stem cells. Such a quantitative loss of stem cells might be an important contributor to ageing. Over time, when cell loss starts to exceed cell renewal, impaired physiological functioning and ageing might result. An illustrative example of this is the greying of hair, which was found to follow loss of melanocyte stem cells and melanocytes in mice.[28] In rodents, an enhanced age-related apoptotic potential was found for diverse cell types, albeit with a few exceptions.[29] Most studies detected an upregulation of the expression of pro-apoptotic genes in ageing tissues, and a down regulation of the expression of anti-apoptotic genes.[30–33] Likewise, in a variety of human tissues an increased incidence of apoptosis was observed during ageing or age-related pathologies, which was linked to increased expression of pro-apoptotic genes and/or decreased expression of anti-apoptotic genes.[34] One of the human organ systems for which changes in cellular apoptotic potential have most convincingly been linked to ageing is the immune system.[35] During human ageing, lymphocytes become increasingly sensitive to apoptosis and increase expression of pro-apoptotic proteins including death receptors and Bax, while decreasing expression of Bcl-2.[36] It is tempting to speculate that a decreased proliferative potential of the immune system, caused by apoptosis, is responsible for the declined immune function observed in elderly. Apoptosis has also been linked to other age-related pathologies, such as Alzheimer's disease,[29] myocardiopathy,[29] and sarcopenia.[37]

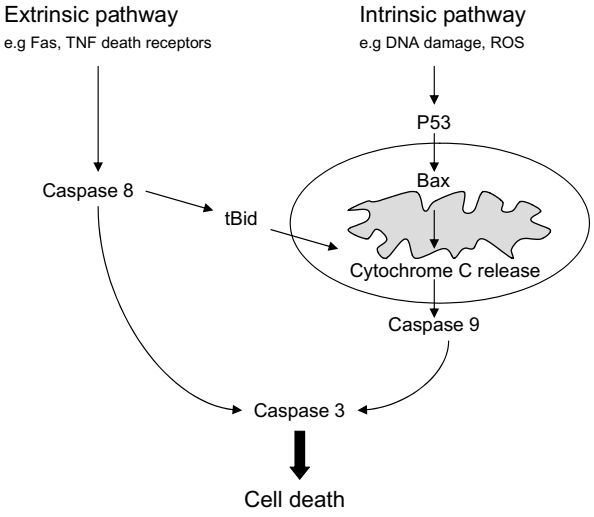

Fig. 1 – Apoptosis can be induced via either signals from outside the cell, such as TNF-alfa and Fas ligand, or from signals inside the cell, such as damaged DNA or reactive oxygen species (ROS). Crucial in the intrinsic pathway are the pro- (e.g Bax) and anti-apoptotic (e.g BCL2, BCL-xl) members of the BCL2-family, who regulate the release of cytochrome c from the mitochondrial membrane. Both pathways activate the same effector-caspases that execute the final common pathway of apoptosis. The structure shown represents a mitochondrion.

6. Senescence in cancer and ageing

Cellular senescence involves a permanent arrest of the cell cycle, preferentially in the G1 phase.[38] Cellular senescence

can be induced by a wide variety of stressors, including UV light, oxidative stress, γ-irradiation, overexpression of oncogenes and demethylation of DNA. These stresses are signalled, via several communicating pathways (including the mitogen-activated protein kinase (MAPK) pathway and the Insulin/IGF-1/phosphatidylinositol kinase (PI3K) pathway), to the p53-p21 and the p16-Rb pathways (Fig. 2). MDM2 (murine double minute) inhibits p53, both by direct binding and by mediating its proteasomal degradation.[39] Upon cellular stress and damage to DNA, the inhibition of p53 by MDM2 is relieved and p53 activity is increased. Oncogenic stress, such as overexpression of *Ras* and *Myc*, increases p53 activity by increasing the levels of $p14^{ARF}$, which binds and sequesters MDM2.[40,41] When p53 activity is increased, it activates the down stream cyclin kinase inhibitor (CKI) $p21^{WAF1/CIP1}$[42,43] and other members of the CIP-KIP family of cyclin-dependent kinase inhibitors.[44,45] Activation of the CKI $p16^{INK4A}$ seems as important as p53 in inducing cellular senescence.[46] The p16 protein binds to Cdk4 and Cdk6, blocking interaction of these Cdk's with D-cyclins and inhibiting cell cycle progression.[47] In mitotically active cells, the retinoblastoma protein (Rb) is inactivated through phosphorylation by Cdk4 and 6. However, when these Cdk's are inactivated by p16, Rb becomes hypophosphorelated and its inhibition is relieved.[48,49] Active Rb inhibits cell cycle progression by binding to certain members of the E2F family of transcription factors, known to regulate S-phase gene transcription.[50] There is extensive crosstalk between and overlap in the pathways that regulate senescene and apoptosis.[51,52]

Senescent cells adapt a characteristic enlarged morphology and display diverse senescence-specific markers, including enhanced expression of senescence-associated β-galactosidase[53] and of p16.[54] Unlike with apoptosis, senescent cells do not vanish. In fact, some senescent cell types, including fibroblasts, become resistant to the induction of apoptosis,[55,56] which may explain why these cells accumulate in ageing organisms.[54,57,58] Because senescent cells do not proliferate, these may contribute quantitatively to loss of tissue cellularity.[59] However, senescent cells also show striking differences in gene expression that are not related to the cell cycle arrest,[60] such as the (enhanced) expression of various cytokines associated with the inflammatory response.[61,62] This reflects a qualitative rather than quantitative change in cells, which could have a major impact on tissue integrity and contribute to ageing. Especially the pro-inflammatory phenotype of senescent cells might create a hazardous micro-environment for neighbouring cells, actually predisposing for the creation of cancer cells.[2] In addition, the disturbance of cell-type specific functions by cellular senescence might further negatively influence tissue integrity. For example, senescent fibroblasts have been found to increase the production of collagenase, actually promoting the breakdown of the extra-cellular matrix they made earlier in life. Indeed, the addition of senescent fibroblasts to a human artificial skin model has been found to increase fragility and blistering.[61]

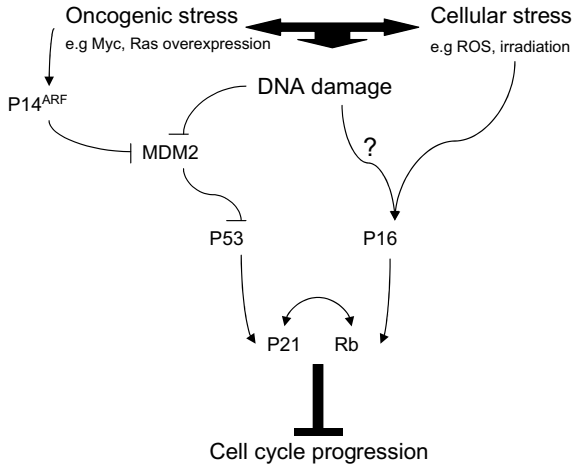

Fig. 2 – Cellular senescence can be induced via several sorts of stress, which increase expression or posttranscriptional activity of the tumour supressors p53 or p16. p53 activates p21 and other members of the CIP-KIP family of cyclin-dependent kinase inhibitors. Rb inhibits the E2F family of transcription factors. Both pathways lead to cell cycle arrest.

The changed microenvironment caused by senescent cells, even if few in number, might also have an effect on stem cells. It is possible that a changed microenvironment disturbs the normal function of stem cells in tissue renewal, either by inhibiting proliferation or by over-stimulating it in a cancerous way. For example, leukaemia, a form of cancer which significantly increases with advancing age, was consistently found to have a haematopoietic stem cell origin.[63] Moreover, when establishing a shared circulatory system between young and old mice it was found that the proliferative and regenerative capacity of aged muscle stem cells from old mice was restored by exposure to serum from young mice, suggesting that the age-related decline of progenitor cell activity can be modulated by systemic factors that change with age.[64] In addition to resident stem cells, circulating stem cells might also be affected by changing the environments within tissues. Perhaps circulating stem cells could no longer correctly home to the tissues where they are needed in the ageing soma. However, impaired stem cell functioning during ageing might also reflect qualitative changes within stem cells themselves.[65] Diverse types of stem cells have been found to become increasingly susceptible to either apoptosis or cellular senescence during human and murine ageing.[28,66]

7. The human *TP53* codon 72 polymorphism in apoptosis and senescence

A subtle hint as to the relative contributions of apoptosis and senescence to human ageing comes from studies on a common codon 72 polymorphism in human *TP53*, which encodes either Arginine (*Arg*) or Proline (*Pro*).[67] The *TP53* codon 72 polymorphism was shown to influence the apoptotic potential of human cells. Both in p53-inducible cell lines[68–70] and in normal diploid fibroblasts,[71] it was found that the *Arg* variant had a significantly higher apoptotic potential than the *Pro* variant. Recently, it was shown that stressed fibroblasts containing the *Pro/Pro* genotype showed signifi-

cantly higher expression of the senescence-associated cell cycle regulator $p21^{WAF1}$ and β-galactosidase compared to fibroblasts with the *Arg/Arg* genotype.[72] Earlier reports showed that the *TP53* codon 72 *Pro* variant, when stably transfected in two different human cell lines, induced a higher level of G1 arrest than the *Arg* variant.[69,70] These findings suggest that the *Arg/Arg* genotype of the *TP53* codon 72 polymorphism underlies a more apoptotic prone cellular response, while the *Pro/Pro* genotype associates with an increased senescence response. In line with these data, we found that, in comparison to *Arg/Arg* fibroblasts, *Pro/Pro* fibroblasts exhibited a significantly higher dose-dependent increase in the percentages of β-galactosidase positive, micronucleated and nuclear abnormal cells at 3 days after X-irradiation (unpublished results). These data are in line with the epidemiological data from the same cohort of elderly, from which these fibroblasts were derived.[73] In the Leiden 85-plus Study, *Pro/Pro* subjects were shown to have a significantly increased survival compared to the *Arg/Arg* subjects, despite an increased proportional mortality from cancer.[73] In contrast, proportional mortality in the category 'other causes' was significantly decreased for *Pro/Pro* subjects. Remarkably, within the category 'other causes', *Pro/Pro* subjects died less often than *Arg/Arg* subjects from general exhaustion and frailty, a human phenotype reminiscent of that described for the $p53^{+/m}$ mice.[21] Proportional mortality from cancer was significantly higher in *Pro/Pro* subjects, but not accompanied by a different cancer spectrum, although it is noteworthy that one *Pro/Pro* subject died of a cause not found in the other genotype groups, namely polycythemia vera, a rare clonal disorder arising in multipotent haematopoietic progenitor cells (unpublished results). The increased old age survival of *Pro/Pro* subjects, compared to the *Arg/Arg* subjects, might be speculated to derive from their decreased cellular apoptotic potential resulting in a lower age-related loss of cellularity. The increased proportional mortality from cancer in the *Pro/Pro* subjects might be speculated to derive from a reduced apoptotic potential and increased senescence. It has been argued that cellular senescence is a double-edged sword in that it evolved to suppress cancer early in life, but may actually contribute to tumour formation later in life as senescent cells accumulate to sufficiently large numbers to influence tissue functioning.[1,2] Using a formal meta-analysis of the published literature we showed that carriers of the *TP53* codon 72 *Pro/Pro* genotype have a significantly increased cancer risk compared to *Arg/Arg* carriers.[73] This disadvantage of *TP53* codon 72 *Pro/Pro* on (life long) cancer risk may be balanced by the advantage it confers in the last stages of life, resulting in the similar *Pro* allele frequencies found in the Italian population in three different age groups: young people (aged 20–65 years), old people (aged 66–99 years) and centenarians (aged 100 years and over).[74] However, such a cross sectional comparison of allele frequencies between groups of individuals of different ages and/or different geographic origins between (or within) studies, always holds the risk of cryptic population stratification, as the populations that are being compared may differ with respect to genetic background en/or exposure to environmental factors.

8. The (relative) contribution of apoptosis and senescence in ageing

Besides subtle hints, not much is known about how apoptosis and cellular senescence relate to each other in the ageing process. Moreover, the relative contribution of apoptosis and senescence may vary among tissues. So far, apoptosis is mainly linked with degenerative diseases and ageing in tissues predominantly composed of post-mitotic cells, including most notably the brain, peripheral nervous system and skeletal and cardiac muscle.[75] Cellular senescence on the other hand, is also linked to ageing in tissues containing regularly dividing cells, such as fibroblasts in the dermis. However, the real incidences of apoptosis and cellular senescence in diverse tissues are unknown. Although there are marked differences in the alignment of different cell types to apoptosis and senescence, all cell types have the potential to undergo both. Moreover, senescence-associated increases in p16 have also been demonstrated in postmitotic tissues such as the brain[76–78] and cardiac muscle.[79,80] Possibly, this senescence-specific increase in p16 originates from stem cells, interspersed between the postmitotic cells. The loss of functioning stem cells would lead to loss of the capacity for self-renewal of these tissues, giving an alternative explanation for loss of cellularity in post-mitotic tissues. However, stem cells are not specifically aligned to senescence, as apoptosis can just as well eliminate functional stem cells. While senescence has been reported to be dominant in haematopoietic stem cells,[66] apoptosis has been reported to be dominant in stressed melanocyte stem cells,[28] and murine chondrocytes[81] have been reported to be equally prone to senescence and to apoptosis during ageing *in vivo*. The disparity continues in the premature ageing mouse models. Overactive p53 was found to significantly decrease overall cellularity and the lifespan of mice, but in one mouse model, cells were more prone to undergo apoptosis,[21] while in the other mouse model, cells rather became senescent when stressed in vitro.[22] Therefore, based on current findings, no conclusions can yet be drawn as to the relative contributions of apoptosis and senescence to ageing.

9. Conclusions

The damage that accumulates with ageing may cause genomic instability and cellular dysfunction, which in organisms with renewable tissues can result in cancer. To keep cancer at bay, two fundamentally different tumour suppression mechanisms evolved: caretakers and gatekeepers. Caretaker genes protect the genome against damage and mutations, while gatekeeper genes induce cell death and cell cycle arrest. Although caretakers may contribute to both a reduced cancer risk and increased life span, the effects of gatekeepers on these two processes may be antagonistically pleiotropic. Although apoptosis and senescence protect individuals from cancer early in life, these may promote ageing phenotypes, including late life cancer, in older individuals. Here, we presented several lines of evidence that support this hypothesis, including studies on model organisms and (rodent and human) cells. Moreover, we present evidence for the existence of a similar trade-off in humans, based on

epidemiological data. We conclude that these lines all hint at an important role for gatekeeper mediated processes in ageing and its related pathologies. Direct evidence for the (relative) contribution of apoptosis and senescence in specific human age-related pathologies, including the identification of the key mediators/components, is currently lacking. This represents, in our opinion, an important and challenging area for future research, which will be of direct relevance for understanding and treating human diseases. With an increasingly aged population, many more patients will present with cancer in old age and will suffer from co-existing morbidity.[95] The DNA-damaging drugs used to treat cancer might activate gatekeeper mechanisms which may accelerate other age-related disorders. This is a testable hypothesis, and the prospect alone underscores the need for medics to be involved in studying the basic mechanisms of ageing, next to the assessment of older patients with cancer.[96]

Box 1 Antagonistic pleiotropy

- One of the evolutionary theories explaining ageing is the theory of 'antagonistic pleiotropy' formulated by Williams in 1957.[94] Williams build on ideas first put forward by Medawar, Haldane and others, stating that the force of natural selection declines with age. Negative selection against mutations causing harmful effects late in life fails because, in the wild, most organisms do not live long enough to experience the harmful effects of such mutations. Thus, the negative effects of ageing are not a selected trait, but rather the consequence of alleles fixed in evolution by their reproductive advantage early in life, with harmful effects in the post reproductive period, a process Williams called antagonistic pleiotropy. According to this theory, the somatic decline associated with ageing would be an inevitable late-life by-product of adaptations that increase fitness early in life. In line with this reasoning, the cellular responses apoptosis and cellular senescence may have antagonistically pleiotropic effects on cancer and life span, as these protect individuals from cancer early in life, but may promote ageing phenotypes, including late life cancer, in older individuals.

Conflict of interest statement

None declared.

Acknowledgements

This work was supported by a grant from the Centre for Medical Systems Biology (CMSB), which is a centre of excellence approved by the Netherlands Genomics Initiative/Netherlands Organization for Scientific Research (NWO) and by an Innovative Orientated Research (IOP) grant from the Dutch Ministry of Economic Affairs (Grant No. IGE010114).

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