

Cellular senescence, ageing and disease

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Received: 19 May 2008 / Accepted: 29 July 2008 / Published online: 4 September 2008
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Abstract Cellular senescence is the irreversible growth arrest of individual mitotic cells, which as a consequence display a radically altered phenotype that is thought to impair tissue function and predispose tissues to disease development and/or progression as they gradually accumulate. However, in the past, research into mechanisms of ageing has commonly been researched and treated separately from disease development. This may partly be due to the lack of understanding concerning mechanisms of ageing and the difficulty in implementing what was known into models of disease development. Only in the last 10 years, with increasing knowledge of the senescent phenotype and the ability to detect senescent cells in human tissues, have biologists been able to investigate the relationship between cellular senescence and disease. This review therefore brings together and discusses recent findings which suggest that cellular senescence does contribute to ageing and the development/ progression of disease.

Keywords Ageing · Disease · Cellular senescence · Senescent phenotype

Introduction

Both ageing and disease result in the same outcome: the impairment of normal biological function. It would not, therefore, be a surprise if tissue dysfunction resulting from an ageing mechanism eventually manifested itself as a disease. Therefore, understanding mechanisms of ageing would help understand the processes which govern the development and progression of some diseases. This in turn would lead to the development of new therapeutic methods for disease treatment and, more importantly, prevention.

In the past, the processes governing ageing and disease have often been researched separately. This may partly be due to the mindset that, unlike ageing, diseases are a medical problem, which can be treated, and partly due to the lack of understanding concerning mechanisms of ageing and the difficulty in implementing what was known into models of disease development. However, this has gradually begun to change and there is now an ever-increasing overlap in research between the fields of biology of ageing and that of disease processes.

Overview of replicative senescence

Mitotic tissues consist of cells which have the ability to divide when stimulated. Most mitotic cells (i.e. fibroblasts, endothelial cells, smooth muscle cells, glial cells, astrocytes etc) within tissues are found in a

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reversible growth arrested state known as quiescence. These cells remain quiescent until stimulated to proliferate, usually for the purpose of cellular replacement. How often these cells proliferate is dependent upon how frequently cells become damaged or lost, and this may be connected to the general “wear and tear” of the tissues in which they reside. For example, fibroblasts exposed to environmental UV radiation or endothelial cells in blood vessels exposed to high turbulence in blood flow may be more likely to proliferate to replace lost or damaged cells than less damage-prone tissues. Cells can also become lost or damaged by other mechanisms such as the presence of disease.

The predominant ageing mechanism of mitotic tissues is thought to be due to the gradual accumulation of senescent cells. Senescent cells have undergone an irreversible cell cycle arrest, and display a radically altered phenotype: genetically, morphologically and behaviourally distinct from its growth-competent counterparts. These changes are thought to have a detrimental impact on neighbouring cells, the surrounding extracellular matrix and other structural components, leading to aged tissues, disease, and an increased risk of cancer (Burton et al. 2005, 2007; Campisi 1997a, b).

One mechanism for triggering cellular senescence is the presence of a critically short telomere. Telomeres are regions of highly repetitive DNA at the end of linear chromosomes that are bound by a number of proteins which protect the telomere from being processed as DNA double-strand breaks. Every time a cell divides, the telomeres become progressively shorter due to the inability to replicate DNA at the ends of chromosomes (Joosten et al. 2003). This eventually results in the appearance of a short telomere, which can no longer be protected by telomeric proteins and thus leads to the exposure of DNA ends, a DNA damage response and cellular senescence. This process is commonly known as “replicative senescence”. Oxidative stress and activated oncogenes such as *ras* have also been shown to trigger cellular senescence (Von Zglinicki et al. 2000; Di Micco et al. 2006).

Apoptosis and stem cells

When discussing the impact senescent cells may have on ageing and age-related disease, it is important to take into consideration factors which may result in the

removal and replacement of senescent cells. These include apoptosis and the availability of stem cell reserves. If senescent cells do not persist in tissues as they appear and are instead removed by apoptosis, it would be difficult to see how senescent cells cause prolonged damage. Also, if the removal of senescent cells results in cellular replacement predominantly from stem cell reserves and not from the surrounding somatic tissue, it would also be difficult to see how cell loss could lead to a reduction in the replicative capacity of somatic cells in the surrounding tissue, which would result in the increased appearance of senescent cells.

There are at present no studies that have looked at the survival time of senescent cells *in vivo*. However, a number of *in-vitro* studies have suggested that senescent cells may be resistant to apoptosis (and thus more likely to persist in tissues), at least in fibroblasts where most of the research has been conducted (Wang et al. 1994; Marcotte et al. 2004; Hampel et al. 2005). No difference between the apoptotic potential of senescent human vascular endothelial cells compared with their mitotic counterparts has also been observed (Kalashnik et al. 2000).

It is not clear to what extent both stem cells and somatic cells play in tissue regeneration, but the functional ability of stem cells appears to become impaired with age (Sharpless and DePinho 2007). Stem cells express telomerase and are unlikely to become senescent in response to telomere shortening (Ruzankina and Brown 2007). However, it may still be possible for stem cells to enter senescence in response to DNA damage (Ruzankina et al. 2008), consequently taking up valuable space in stem cell niches (Lynch 2006), reducing the stem cell pool, leading to an age-related loss in regenerative capacity. Also, if senescent cells persist in tissues, their altered secretome may have detrimental consequences on the local tissue and this may include stem cell niches. Many stem cells lose the capacity for self-renewal when removed from the stem cell niche, suggesting that the local environment plays a crucial role in determining stem cell behaviour (Boyle et al. 2007). Senescent cells may alter the environment of stem cell niches, thus altering stem cell behaviour, leading to functional decline.

Biological impact of the senescent phenotype

Common features of the senescent phenotype, which can potentially be detrimental to the tissues in which

they reside, are the up-regulation of growth factors, extracellular matrix (ECM)-degrading proteins and pro-inflammatory cytokines (Coppe et al. 2006; West et al. 1989; Kletsas et al. 2004). Why senescent cells adopt this phenotype is currently unknown. One possibility is that the senescent cell secretes cytokines to attract immune cells to its location (for its removal), secretes matrix degrading proteins to allow the immune cells access and secrete growth factors to stimulate surrounding cells to proliferate once the cell has been removed. This process may be effective in young organisms, but may gradually decline with age. Since immune cells are also governed by the ageing process, the removal of senescent cells may gradually become impaired. This would lead to the accumulation of senescent cells in tissues, causing detrimental alterations to the structure and consequently the function of those tissues.

Senescent cells are often observed to up-regulate matrix metalloproteinases (MMPs), enzymes capable of degrading proteins such as collagen and elastin which make up the extracellular matrix (Sandeman et al. 2001; Campisi 2005). Since the ECM is important for providing support and anchorage for cells, separating different tissues and regulating intercellular communication, its degradation by MMPs is likely to impact all areas of ECM function. MMP activity is normally inhibited by TIMPs (tissue inhibitor of metalloproteinases), but research suggests that these inhibitors themselves are down-regulated at senescence, thereby further contributing to matrix degradation (Hornebeck 2003). MMP secretion by senescent cells has been suggested to play a role in the progression of disease such as in the pathogenesis of coronary heart disease (CAD) (Nanni et al. 2007), and implicated in the progression of osteoporosis, since MMPs play important roles in bone resorption (Logar et al. 2007). Price et al. (2002) has also shown that the secretion of MMPs by senescent chondrocytes may contribute to the development or progression of osteoarthritis.

Senescent cells also secrete many cytokines which, due to their diverse function, could have multiple consequences on the ageing of tissues and the development/progression of disease (Ginaldi et al. 2005; Payne 2006; Salvioli et al. 2006; Sue and Griffin 2006; Libby 2006). These secreted proteins may not just impact on local tissue but also tissues found throughout the organism. The presence of cytokines can alter cell

functions by up-regulating or down-regulating several genes and their transcription factors, resulting in the production of other cytokines and an increase in the number of surface receptors for other molecules (Gallin 1999). The ability of cytokines to reach many tissues and have such diverse consequences on cell function suggests that only a small fraction of senescent cells may need to be present for there to be any significant impact on tissue impairment or disease development/progression.

Vascular smooth muscle cells that have become senescent due to the activation of Ras have been shown to drastically increase the expression of pro-inflammatory cytokines (Minamino et al. 2003). IL1 α was shown to be up-regulated 11-fold, IL1 β 50-fold, IL-6 12-fold and IL-8 77-fold. With such dramatic changes, it was suggested that this proinflammatory phenotype may contribute to the progression of atherosclerosis. Senescent T cells *in vivo* have also been shown to produce high levels of two cytokines, IL6 and TNF α (Effros 2004a, b). Interestingly, the up-regulation of TNF α by T-cells in the bone marrow has been implicated as a causal mechanism in bone loss (Roggia et al. 2001).

Replicative senescence of human hepatic stellate cells (a major cell type involved in liver fibrosis) in culture also display a higher expression of inflammation genes (Schnabl et al. 2003). Interleukin-8 is among the cytokines up-regulated in senescent stellate cells (SC), which correlates with increase expression observed with disease activity in human alcoholic liver fibrosis (Sheron et al. 1993). Interleukin-6 is a known fibrogenic cytokine, which was also shown to be up-regulated in SC senescent cultures. In normal conditions, chronic tissue damage results in the activation of SC characterised by proliferation, motility, contractility and synthesis of ECM (Gutiérrez-Ru et al. 2002). Since SC are stimulated to proliferate in response to tissue damage, the replicative capacity of these cells will be reduced and the accumulation of senescent cells accelerated. This activation of SC in response to tissue damage is regulated by cytokines and growth factors. Therefore, unregulated secretion of pro-inflammatory cytokines and growth factors from senescent SC within the liver may cause further damage. Trak-Smayra et al. (2004) has also shown that replicative senescence does have a significant impact in the progression of fibrosis in hepatitis C virus recurrence after liver transplantation. The presence of senescent hepatocytes in liver

grafts was assessed by SA- β -Gal staining, with a total of 34 of the 103 biopsies analysed within the first year of follow-up displayed SA- β -Gal-positive cells.

This pro-inflammatory phenotype may partly be due to the up-regulation of intercellular adhesion molecule-1 (ICAM-1), a molecule known to be involved in inflammatory response and is over-expressed in senescent cells and aged tissues (Gorgoulis et al. 2005).

Abnormal secretion of some growth factors has also been shown to be another characteristic of the senescent phenotype. Coppe et al. (2006), for example, found that vascular endothelial growth factor (VEGF) secretion is elevated in human senescent fibroblast cultures. Since growth factors are capable of stimulating cellular proliferation, it has been suggested that while initially cellular senescence may be a mechanism to suppress tumourigenesis early in life, it may promote cancer in aged organisms (Campisi 1997a, b). Human senescent fibroblasts have been shown to stimulate premalignant and malignant, but not normal epithelial cells to proliferate in culture and form tumours in mice (Krtolica et al. 2001; Krtolica and Campisi 2002). Bavik et al. (2006) sought to characterise the molecular alterations that occur during prostate fibroblast senescence to identify factors which may be capable of promoting the proliferation and, potentially, the neoplastic progression of prostate epithelium. Fibroblast growth factor 7 (FGF7), hepatocyte growth factor and amphiregulin (AREG) were found to be elevated in the extracellular environment of senescent prostate fibroblasts. Direct co-culture and conditioned medium from senescent prostate fibroblasts stimulated epithelial cell proliferation threefold and twofold, respectively. These results suggest that senescent cells may contribute to the progression of prostate neoplasia by altering the prostate microenvironment.

Some of the changes observed during cellular senescence are likely to be cell-type specific. For example, in senescent vascular endothelial cells, nitric oxide synthase (eNOS) activity has been found to be decreased (Matsushita et al. 2001; Minamino et al. 2002). Since nitric oxide (NO) is important in regulating vascular function, a decline in its production may have detrimental consequences. A reduction in NO production by eNOS, for example, has been suggested to be a significant risk factor for cardiovascular disease (Cannon 1998). Also, eNOS activity has been shown to be present in astrocytes (Wienchen and Casagrande 1999; Lin et al. 2007), but the impact

of cellular senescence (if any) on eNOS activity on this cell type is currently lacking.

Currently unpublished work on the transcriptional analysis of human vascular smooth muscle cells has also demonstrated cell-type specific alterations (Burton and co-workers). A 24-fold decrease in the expression of matrix Gla protein (an inhibitor of calcification) and over a fourfold increase in bone morphogenic protein 2 (a promoter of calcification) in senescent vascular smooth muscle cells was observed. A comparison of these changes with the transcriptional profiles of other senescent cell types show that these changes appear to be specific to senescent vascular smooth muscle cells. Thus, these results suggest that senescent vascular smooth muscle cells may play a role in the age-related hardening and stiffening of the arteries due to increases in calcification. Stiffening of the arteries is the major cause of high blood pressure in older people, which in turn is a leading risk factor for stroke, coronary artery disease, heart attack, and heart failure (O'Rourke and Hashimoto 2007).

Another known change associated with cellular senescence is the reduced ability to migrate (Schneider and Mitsui 1976; Sandeman et al. 2000; Reed et al. 2000; Ruiz-Torres et al. 2003). This impaired ability to migrate may be related to changes which occur to the cytoskeleton during cellular senescence (Nishio and Inoue 2005). Actin is an important component of the cytoskeleton required for cellular migration. However, in senescent fibroblasts, for example, it has been shown that vimentin is produced in place of actin, which is down-regulated (Nishio and Inoue 2005). This migration deficit has important implications during wound healing since cells are stimulated to migrate into the wound, proliferate and construct the new ECM. Since senescent cells tend to secrete proteins which degrade the matrix, wound repair would be further impaired. An alternative explanation for the decline in the ability of senescent cells to migrate is the loss or impairment of the migratory response to stimuli such as growth factors (Matsuda et al. 1992; Cavallaro et al. 2000).

The majority of the research into the senescent phenotype has been carried out on fibroblasts with little or no understanding of the senescent phenotype of other cell types, especially those cell types linked to age-related pathology. A detailed understanding of the senescent phenotype of cells linked to age-related pathology may provide an alternative perceptive for

disease development/progression which could be used to develop new approaches in treatment and prevention.

Cellular senescence and disease

Cellular senescence is unlikely to contribute solely to the development and/or progression of age-related diseases, but also other diseases or tissue dysfunction unrelated to age. Some age-related diseases may progress as a result of gradual accumulation of senescent cells, while other diseases, unrelated to age, may progress at a faster rate due to factors which accelerate the formation of cellular senescence. The underlying mechanism of both is the same, but the rate at which they progress differ.

Another important point to mention is that although senescent cells are generated within mitotic tissues, their impact is not likely limited to that tissue type. Tissues are made up of a mixture of mitotic cells, post-mitotic cells and long-lived proteins. The predominant ageing mechanism for each of these components is likely to be different, but since all these components interact with each other, a change in one will have a direct impact on another.

Le Maitre et al. (2007) has provided strong evidence to suggest that intervertebral disc degeneration, a major cause of low-back pain, may be due to accelerated cellular senescence. Cells isolated from normal and degenerate human tissue were assessed for mean telomere length, senescence-associated β -galactosidase (SA- β -Gal) staining [for determining the senescent fraction (Dimri et al. 1995)], and replicative potential. Mean telomere length decreased with age in cells from non-degenerate tissue and also decreased with progressive stages of degeneration. SA- β -Gal staining was not observed in non-degenerative patients, unlike cells from degenerative discs, which did exhibit 10–12% SA- β -Gal staining and a decrease in replicative potential. However, the factors which may have led to accelerated senescence in this instance were not discussed. There are three possible reasons why cellular senescence was accelerated in this instance: (1) unknown factors resulted in the damage and removal of cells, resulting in cell turnover for replacement, (2) oxidative stress could have been involved, resulting in stress induced premature senescence (SIPS), or (3) telomeres in these cells for some unknown reason started off shorter than normal, meaning less cell turnover is required for the appearance of senescent cells.

Paradis et al. (2001) have also shown a correlation between disease states and the presence of senescent cells *in vivo*. SA- β -Gal staining was used to detect senescent hepatocytes in normal liver, liver with chronic hepatitis C and hepatocellular carcinoma (HCC). They found senescent cells present in three of 15 (20%) normal livers tested, 16 of 32 (50%) in livers with chronic hepatitis C and in six of ten (60%) livers with HCC. The presence of senescent cells in normal livers was found to be associated with old age. This increase in the senescent fraction of liver with chronic hepatitis is probably due to accelerated senescence as a result of the disease, but their presence may contribute further to the progression of liver damage. Interestingly, the presence of senescent cells in non-tumoural tissues was strongly correlated with the presence of HCC in the surrounding liver. The presence of senescent cells in non-tumoural tissues could stimulate premalignant and malignant cells to proliferate, demonstrating not only that the ageing of one tissue can have a direct impact on another, but also that senescent cells may contribute to carcinogenesis.

Choi et al. (2000) looked at cellular senescence in human benign prostatic hyperplasia (BPH) specimens. BPH is a disease associated with an abnormal growth of the adult prostate that begins mid to late life. Results from this study found that 40% of the analysed samples showed positive staining for SA- β -Gal and only in the epithelial cells. A high prostate weight (>55 g) was found to correlate strongly with the expression of SA- β -Gal. Prostates weighing less than 55 g tended to lack senescent epithelial cells. It was suggested that the accumulation of senescent epithelial cells may play a role in the development of prostatic enlargement associated with BPH. The accumulation of senescent cells in this case is likely to be a consequence of the disease, but may lead further to its progression. The enlargement of the prostate may be the result of unregulated stimulated proliferation, increasing cell turnover and consequently the appearance of senescent cells. This would explain why a stronger expression of SA- β -Gal is detected in prostates weighing more than 55 g, since they have undergone more cellular divisions.

During the pathogenesis of type-2 diabetes, insulin resistance causes compensatory proliferation of pancreatic beta cells. This compensatory proliferation might accelerate cellular senescence, contributing further to the progression of diabetes. To investigate

this, Sone and Kagawa (2005) used nutrient-induced diabetic mice to analyse beta cells for SA- β -Gal and the proliferation marker Ki67. At 4 months, the proliferation of beta cells was 2.2-fold higher than in the control group. At 12 months, the frequency of Ki67 decreased to one-third that of the control and SA- β -Gal-positive cells increased to 4.7-fold that of the control group. This increase in the senescent beta-cell fraction correlated with insufficient insulin release, suggesting cellular senescence may contribute to diet-induced diabetes. In this instance, it is difficult to determine whether cellular senescence is the cause or the consequence of insulin resistance. It later appears to be a contributor, but whether it is also the initiating factor is unknown.

Muller et al. (2006) found there to be an increase in the number of senescent primary lung fibroblasts in patients with emphysema compared with normal controls (an average of 4% of cells from control patients stained positive for SA- β -Gal compared with an average of 16% in patients with emphysema). It is possible that long-term exposure to tobacco smoke accelerates the formation of senescent cells, which subsequently may lead to loss of elasticity of the lung tissue, destruction of structures supporting the alveoli, and destruction of capillaries feeding the alveoli observed with emphysema. Tsuji et al. (2004) has shown, for example, that cigarette smoke induces senescence in alveolar epithelial cells.

Sis et al. (2007) used senescent-associated p16 increases instead of SA- β -Gal to detect senescent cells in kidneys with glomerular disease (GD). GDs include many conditions, which fall into two major categories: glomerulonephritis describes the inflammation of the membrane tissue in the kidney that serves as a filter, separating wastes and extra fluid from the blood; glomerulosclerosis is the scarring or hardening of the tiny blood vessels within the kidney. This study found an increased expression of the nuclear p16 in samples with GD compared with normal. Independently, older age and interstitial inflammation was associated with increased expression of nuclear p16. Since senescent cells adopt a pro-inflammatory phenotype, their presence may be a contributing factor in inflammation observed in glomerulonephritis.

All these examples demonstrate the presence of senescent cells not only in tissues but also, more importantly, in disease states. The appearance of senescent cells may occur gradually through general

cell loss and replacement or much quicker by mechanisms which accelerate cellular senescence, such as the presence of disease. This suggests that if no injuries occurred as a consequence of disease, environmental factors or by normal biological/ mechanical wear and tear, ageing of mitotic tissues and the appearance of disease would be greatly reduced.

In review, senescent cells within tissues may contribute to the ageing process and disease development/progression by:

1. Cellular dysfunction: inability to function properly
2. Altering the behaviour of neighbouring cells
3. Degradation of structural components such as the extracellular matrix
4. Reducing the pool of growth-competent mitotic cells
5. Stimulating cancer formation

Future considerations

Understanding how ageing mechanisms cause alterations to tissues and understanding the consequences of those alterations brings us one step closer to developing new therapeutic ways of treating and, more importantly, preventing the appearance of age-related impairment and disease. In regard to cellular senescence, an in-depth understanding of the senescent phenotype of all mitotic cell-types is required so we can better assess the potential consequences of their appearance.

To combat the problem of senescent cells in tissues, ongoing and future research should concentrate on the following three strategies: (1) prevention, (2) removal and (3) replacement.

Telomerase therapy is aimed at preventing the appearance of senescent cells by elongating the telomeres of somatic cells (Shawi and Autexier 2008). The idea is to develop strategies for transiently turning on telomerase in cells. This results in longer telomeres, a higher replicative capacity and a reduced chance of a cell becoming senescent through replication. However, not all cells appear to senesce as a result of telomere shortening. Some cells have been shown to senesce independently of telomere shortening (Itahana et al. 2003,) and as such would be unaffected by the elongation of telomeres by telomerase (Kiyono et al. 1998). Also, cells can become senescent as a result of

DNA damage (Robles and Adami, 1998, Chen et al. 1998), meaning even those cells with elongated telomeres can become senescent. It is not known to what degree DNA damage plays in the formation of senescent cells in vivo.

Even if telomerase therapy is made possible, there will still be a fraction of senescent cells present in tissues. As such, strategies should also focus on direct removal of senescent cells from tissues. Two potential approaches include the development of drugs which specifically target and destroy senescent cells, and the use of the body's own immune system to target and remove senescent cells.

At present, a drug which specifically targets senescent cells is unavailable. However, drugs which are currently being developed (Gillies and Frechet 2005; Alexis et al. 2008) to target specific cancer cells could one day be adapted to target senescent cells. They would recognise senescent specific cell-surface markers, bind to them and induce apoptosis, thus removing the cell.

If senescent cells appear in tissues as a result of failure of an aged immune system to remove them, then rejuvenation of the aged immune system would be greatly beneficial. An understanding of the biology which leads to the functional decline in the immune system is thus essential for the development of rejuvenation medicine. One area of focus should be on age-related changes associated with tumour-antigen capture and presentation. In brief, dendritic cells capture and process tumour-specific antigens, they begin to mature and migrate to the lymph nodes where they interact with cytotoxic T-lymphocytes (CTLs), presenting the tumour information and causing the CTLs to become activated and proliferate, targeting tumour cell removal (Chan and Housseau 2008). If senescent cells are indeed removed by the immune system, the mechanism is probably similar to that of tumour cell removal. Although not discussed in detail here, there are a number of stages during antigen capture and presentation that could become altered with age and thus in need of further investigation (Shurin et al. 2007).

1. Dendritic cells did not recognise the target cell
2. The dendritic cell did not display the target cell specific marker
3. Lymphocytes were not activated in response to dendritic cells

Finally, the third strategy is the replacement of cells. If telomerase therapy is not possible, the removal of senescent cells from tissues will only stimulate the proliferation of surrounding cells for its replacement, reducing the replicative capacity of that tissue and increasing the appearance of more senescent cells. However, tissues may also consist of a population of stem cells involved in cell replacement (Li and Neaves 2006), but it is not known to what extent these stem cells or the surrounding somatic cell population plays in tissue regeneration. Stem cells may also be affected by ageing mechanisms and as a result may become functionally impaired (Sharpless and DePinho 2007). As such, the addition of stem cell populations into tissues (Smart and Riley 2008) after the removal of senescent cells is thus another strategy in need of research.

Strategies involving the prevention, removal and replacement of senescent cells is at its infancy. It is only through a deeper understanding of the biology of ageing and the ability to translate such knowledge into practical therapeutic applications that we will begin to see inevitable benefits in regenerative medicine.

References

- Alexis F, Rhee JW, Richie JP, Radovic-Moreno AF, Langer R, Farokhzad OC (2008) New frontiers in nanotechnology for cancer treatment Urologic Oncology. Semin Original Invest 26:74–85
- Bavik C, Coleman I, Dean JP, Knudsen B, Plymate S, Nelson PS (2006) The gene expression program of prostate fibroblast senescence modulates neoplastic epithelial cell proliferation through paracrine mechanisms. Cancer Res 66:794–802
- Boyle M, Wong C, Rocha M, Jones DL (2007) Decline in self-renewal factors contributes to aging of the stem cell niche in the *Drosophila* testis. Cell Stem Cell 1:470–478
- Burton DGA, Allen MC, Bird JLE, Faragher RGA (2005) Bridging the gap: ageing, pharmacokinetics and pharmacodynamics. J Pharm Pharmacol 57:671–679
- Burton DGA, Sheerin A, Ostler EL, Smith K, Giles PJ, Lowe J et al (2007) Cyclin D1 over-expression permits the reproducible detection of senescent human vascular smooth muscle cells. Ann N Y Acad Sci 1119:20–31
- Campisi J (1997a) Aging and cancer: the double-edged sword of replicative senescence. J Am Geriatr Soc 45:482–488
- Campisi J (1997b) The biology of replicative senescence. Eur J Cancer 33:703–709
- Campisi J (2005) Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. Cell 120:513–522
- Cannon RO 3rd (1998) Role of nitric oxide in cardiovascular disease: focus on the endothelium. Clin Chem 44:1809–1819

- Cavallaro U, Castelli V, Ugo DM, Soria MR (2000) Phenotypic alterations in senescent large-vessel and microvascular endothelial cells. *Mol Cell Biol Res Commun* 4:117–121
- Chan CW, Housseau F (2008) The ‘kiss of death’ by dendritic cells to cancer cells. *Cell Death Differ* 15:58–69
- Chen QM, Bartholomew JC, Campisi J, Acosta M, Reagan JD, Ames BN (1998) Molecular analysis of H2O2-induced senescent-like growth arrest in normal human fibroblasts: p53 and Rb control G1 arrest but not cell replication. *Biochem J* 332(Pt 1):43–50
- Choi J, Shendrik I, Peacocke M, Peehl D, Buttyan R, Ikeguchi EF et al (2000) Expression of senescence-associated beta-galactosidase in enlarged prostates from men with benign prostatic hyperplasia. *Urology* 56:160–166
- Coppe JP, Kauser K, Campisi J, Beausejour CM (2006) Secretion of vascular endothelial growth factor by primary human fibroblasts at senescence. *J Biol Chem* 281:29568–29574
- Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C et al (2006) Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. *Nature* 444:638–642
- Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C et al (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci USA* 92:9363–9367
- Effros RB (2004a) Replicative senescence of CD8 T cells: effect on human ageing. *Exp Gerontol* 39:517–524
- Effros RB (2004b) T cell replicative senescence: pleiotropic effects on human aging. *Ann NY Acad Sci* 1019:123–126
- Gallin JSR (1999) Inflammation: basic principles and clinical correlates, 3rd edn. Lippincott William and Wilkins, Philadelphia
- Gillies ER, Fréchet JMJ (2005) Dendrimers and dendritic polymers in drug delivery. *Drug Discov Today* 10:35–43
- Ginaldi L, Benedetto MC, De Martinis M (2005) Osteoporosis, inflammation and ageing. *Immun Ageing* 2:14
- Gorgoulis VG, Pratsinis H, Zacharatos P, Demoliou C, Sigala F, Asimacopoulos PJ et al (2005) p53-dependent ICAM-1 overexpression in senescent human cells identified in atherosclerotic lesions. *Lab Invest* 85:502–511
- Gutierrez-Ruiz MC, Robles-Diaz G, Kershenovich D (2002) Emerging concepts in inflammation and fibrosis. *Arch Med Res* 33:595–599
- Hampel B, Wagner M, Teis D, Zwerschke W, Huber LA, Jansen-Durr P (2005) Apoptosis resistance of senescent human fibroblasts is correlated with the absence of nuclear IGFBP-3. *Aging Cell* 4:325–330
- Hornebeck W (2003) Down-regulation of tissue inhibitor of matrix metalloproteinase-1 (TIMP-1) in aged human skin contributes to matrix degradation and impaired cell growth and survival. *Pathol Biol (Paris)* 51:569–573
- Itahana K, Zou Y, Itahana Y, Martinez JL, Beausejour C, Jacobs JJ et al (2003) Control of the replicative life span of human fibroblasts by p16 and the polycomb protein Bmi-1. *Mol Cell Biol* 23:389–401
- Joosten SA, van Ham V, Nolan CE, Borrias MC, Jardine AG, Shiels PG et al (2003) Telomere shortening and cellular senescence in a model of chronic renal allograft rejection. *Am J Pathol* 162:1305–1312
- Kalashnik L, Bridgeman CJ, King AR, Francis SE, Mikhailovsky S, Wallis C et al (2000) A cell kinetic analysis of human umbilical vein endothelial cells. *Mech Ageing Dev* 120:23–32
- Kiyono T, Foster SA, Koop JJ, McDougall JK, Galloway DA, Klingelhutz AJ (1998) Both Rb/p16INK4a inactivation and telomerase activity are required to immortalize human epithelial cells. *Nature* 396:84–88
- Kletsas D, Pratsinis H, Mariatos G, Zacharatos P, Gorgoulis VG (2004) The proinflammatory phenotype of senescent cells: the p53-mediated ICAM-1 expression. *Ann N Y Acad Sci* 1019:330–332
- Krtolica A, Campisi J (2002) Cancer and aging: a model for the cancer promoting effects of the aging stroma. *Int J Biochem Cell Biol* 34:1401–1414
- Krtolica A, Parrinello S, Lockett S, Desprez PY, Campisi J (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc Natl Acad Sci USA* 98:12072–12077
- Le Maitre CL, Freemont AJ, Hoyland JA (2007) Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. *Arthritis Res Ther* 9:R45
- Li L, Neaves WB (2006) Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 66:4553–4557
- Libby P (2006) Inflammation and cardiovascular disease mechanisms. *Am J Clin Nutr* 83(Suppl):456S–460S
- Lin LH, Taktakishvili O, Talman WT (2007) Identification and localization of cell types that express endothelial and neuronal nitric oxide synthase in the rat nucleus tractus solitarius. *Brain Res* 1171:42–51
- Logar DB, Komadina R, Prezelj J, Ostanek B, Trost Z, Marc J (2007) Expression of bone resorption genes in osteoarthritis and in osteoporosis. *J Bone Miner Metab* 25:219–225
- Lynch MD (2006) How does cellular senescence prevent cancer. *DNA Cell Biol* 25:69–78
- Marcotte R, Lacelle C, Wang E (2004) Senescent fibroblasts resist apoptosis by downregulating caspase-3. *Mech Ageing Dev* 125:777–783
- Matsuda T, Okamura K, Sato Y, Morimoto A, Ono M, Kohno K et al (1992) Decreased response to epidermal growth factor during cellular senescence in cultured human microvascular endothelial cells. *J Cell Physiol* 150:510–516
- Matsushita H, Chang E, Glassford AJ, Cooke JP, Chiu CP, Tsao PS (2001) eNOS activity is reduced in senescent human endothelial cells: Preservation by hTERT immortalization. *Circ Res* 89:793–798
- Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I (2002) Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation* 105:1541–1544
- Minamino T, Yoshida T, Tateno K, Miyauchi H, Zou Y, Toko H et al (2003) Ras induces vascular smooth muscle cell senescence and inflammation in human atherosclerosis. *Circulation* 108:2264–2269
- Muller KC, Welker L, Paasch K, Feindt B, Erpenbeck VJ, Hohlfield JM et al (2006) Lung fibroblasts from patients with emphysema show markers of senescence in vitro. *Respir Res* 7:32
- Nanni S, Melandri G, Hanemaaijer R, Cervi V, Tomasi L, Altissimi A et al (2007) Matrix metalloproteinases in premature coronary atherosclerosis: influence of inhibitors,

- inflammation, and genetic polymorphisms. *Transl Res* 149:137–144
- Nishio K, Inoue A (2005) Senescence-associated alterations of cytoskeleton: extraordinary production of vimentin that anchors cytoplasmic p53 in senescent human fibroblasts. *Histochem Cell Biol* 123:263–273
- O'Rourke MF, Hashimoto J (2007) Mechanical factors in arterial aging: a clinical perspective. *J Am Collf Cardiol* 50:1–13
- Paradis V, Youssef N, Dargere D, Ba N, Bonvoust F, Deschatrette J et al (2001) Replicative senescence in normal liver, chronic hepatitis C, and hepatocellular carcinomas. *Hum Pathol* 32:327–332
- Payne GP (2006) Effect of inflammation on the aging microcirculation: impact on skeletal muscle blood flow control. *Microcirculation* 13:343–352
- Price JS, Waters JG, Darrah C, Pennington C, Edwards DR, Donell ST et al (2002) The role of chondrocyte senescence in osteoarthritis. *Aging Cell* 1:57–65
- Reed MJ, Corsa AC, Kudravi SA, McCormick RS, Arthur WT (2000) A deficit in collagenase activity contributes to impaired migration of aged microvascular endothelial cells. *J Cell Biochem* 77:116–126
- Robles SJ, Adami GR (1998) Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. *Oncogene* 16:1113–1123
- Roggia C, Gao Y, Cenci S, Weitzmann MN, Toraldo G, Isaia G et al (2001) Up-regulation of TNF-producing T cells in the bone marrow: a key mechanism by which estrogen deficiency induces bone loss in vivo. *Proc Natl Acad Sci USA* 98:13960–13965
- Ruiz-Torres A, Lozano R, Melón J, Carraro R (2003) Age-dependent decline of in vitro migration (basal and stimulated by IGF-1 or insulin) of human vascular smooth muscle cells. *J Gerontol A Biol Sci Med Sci* 58:B1074–B1077
- Ruzankina Y, Brown EJ (2007) Relationships between stem cell exhaustion, tumour suppression and ageing. *Br J Cancer* 97:1189–1193
- Ruzankina Y, Asare A, Brown EJ (2008) Replicative stress, stem cells and aging. *Mech Ageing Dev* 129:460–466
- Salvioli S, Capri M, Valensin S, Tieri P, Monti D, Ottaviani E, Franceschi C (2006) Inflamm-aging, cytokines and aging: state of the art, new hypotheses on the role of mitochondria and new perspectives from systems biology. *Curr Pharm Des* 12:3161–3171
- Sandeman SR, Allen MC, Liu C, Faragher RG, Lloyd AW (2000) Human keratocyte migration into collagen gels declines with in vitro ageing. *Mech Ageing Dev* 119:149–157
- Sandeman SR, Faragher RG, Allen MC, Liu C, Lloyd AW (2001) Does MMP-2 expression and secretion change with increasing serial passage of keratocytes in culture. *Mech Ageing Dev* 122:157–167
- Schnabl B, Purbeck CA, Choi YH, Hagedorn CH, Brenner D (2003) Replicative senescence of activated human hepatic stellate cells is accompanied by a pronounced inflammatory but less fibrogenic phenotype. *Hepatology* 37:653–664
- Schneider EL, Mitsui Y (1976) The relationship between in vitro cellular aging and in vivo human age. *Proc Natl Acad Sci USA* 73:3584–3588
- Sharpless NE, DePinho RA (2007) How stem cells age and why this makes us grow old. *Nat Rev Mol Cell Biol* 8:703–713
- Shawi M, Autexier C (2008) Telomerase, senescence and ageing. *Mech Ageing Dev* 129:3–10
- Sheron N, Bird G, Koskinas J, Portmann B, Ceska M, Lindley I et al (1993) Circulating and tissue levels of the neutrophil chemotaxin interleukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration. *Hepatology* 18:41–46
- Shurin MS, Shurin GV, Chatta GS (2007) Aging and the dendritic cell system: Implications for cancer. *Crit Rev Oncol Hematol* 64:90–105
- Sis B, Tasanarong A, Khoshjou F, Dadras F, Solez K, Halloran PF (2007) Accelerated expression of senescence associated cell cycle inhibitor p16INK4A in kidneys with glomerular disease. *Kidney Int* 71:218–226
- Smart S, Riley PR (2008) The stem cell movement. *Circ Res* 102:1155–1168
- Sone H, Kagawa Y (2005) Pancreatic beta cell senescence contributes to the pathogenesis of type 2 diabetes in high-fat diet-induced diabetic mice. *Diabetologia* 48:58–67
- Sue W, Griffin T (2006) Inflammation and neurodegenerative diseases. *Am J Clin Nutr* 83(Suppl):470S–474S
- Trak-Smayra V, Contreras J, Dondero F, Durand F, Dubois S, Sommacale D et al (2004) Role of replicative senescence in the progression of fibrosis in hepatitis C virus (HCV) recurrence after liver transplantation. *Transplantation* 77:1755–1760
- Tsuiji T, Aoshiba K, Nagai A (2004) Cigarette smoke induces senescence in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 31:643–649
- von Zglinicki T, Pilger R, Sitté N (2000) Accumulation of single-strand breaks is the major cause of telomere shortening in human fibroblasts. *Free Radic Biol Med* 28:64–74
- Wang E, Lee MJ, Pandey S (1994) Control of fibroblast senescence and activation of programmed cell death. *J Cell Biochem* 54:432–439
- West MD, Pereira-Smith OM, Smith JR (1989) Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. *Exp Cell Res* 184:138–147
- Wiencken AE, Casagrande VA (1999) Endothelial nitric oxide synthetase (eNOS) in astrocytes: another source of nitric oxide in neocortex. *Glia* 26:280–290