

Cellular senescence: immunosurveillance and future immunotherapy

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Abstract

In response to persistent DNA damage, induction into cell senescence promotes an immunogenic program which facilitates immune clearance of these damaged cells. Under physiological conditions, senescent cells can activate both innate and adaptive immune responses, functioning to maintain tissue homeostasis. However, likely owing to ageing of the immune system, a failure to eliminate senescent cells is thought to contribute to their accumulation in tissues with age. As a consequence, senescent cells contribute towards ageing and age-related diseases. Furthermore, induction of cell senescence in immune cells has also been reported, a process which can be both beneficial and detrimental, depending on the biological setting. In addition to activating an immune response, senescent cells have also been reported to promote immunosuppression in pathological contexts. As such, mechanisms associated with senescent cell-immune cell interactions appear complex. In this review, we provide an overview of the research focused on senescence immunosurveillance, including a discussion on the mechanisms by which macrophages may recognise senescent cells. Senescence immunotherapy strategies as an alternative to senolytics for the removal of senescent cells will also be discussed.

Key Words: senescence, macrophages, immunosurveillance, ageing, immunotherapy

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Introduction

Clear definitions concerning the biological differences between “cell ageing” and “cell senescence” are often lacking and not well defined. To those unfamiliar with the subject of cellular senescence, such terminology can lead to confusion. We have attempted to briefly clarify such differences. Cellular ageing results from stochastic processes as a result of gradual accumulating damage over time, whereas cellular senescence is a programmed change in cell state associated with permanent growth inhibition. Aged cells maintain their normal function, albeit at a decreased capacity, whereas senescent cells acquire new functions (Figure 1).

In cultures of actively dividing cells, senescence can be abruptly induced in a matter of days. However, in many tissues and organs which consist primarily of quiescent cells (reversible growth arrest) senescence induction can only occur once cells re-enter the cell cycle. The senescent state is often mediated by a persistent DNA damage response (DDR) (Burton and Faragher, 2015; d’Adda di Fagagna, 2008), induced by stress induced stimuli such as telomere dysfunction, oncogene activation, oxidative stress, cell-cell fusion and chemotherapeutic drugs (Chuprin et al., 2013; Di Micco et al., 2006; Ewald et al., 2010; Toussaint et al., 2002; Victorelli and Passos, 2017). “Induction” of senescence therefore refers to the programmed responses which occur following a DDR triggered by a stress induced stimuli. In addition to permanent growth arrest, the senescent state is accompanied by several additional phenotypic changes which make them distinct from their non-senescent counterparts (Table 1). Although a senescence-like response has been reported in post-mitotic cells such as neurons (Jurk et al., 2012), this review will focus on cell senescence in growth-competent cells.

Probably the most widely researched aspect of the senescent phenotype to date is the altered secretory response, referred to as the senescence-messaging secretome, senescence-associated secretory phenotype or the senescence-associated secretome (Campisi and d’Adda di Fagagna, 2007; Kuilman and Peeper, 2009; Malaquin et al., 2016). Here we refer to this response simply as the “senescent secretome”. The senescent program is often accompanied with transcriptional changes linked to an altered secretome, consisting of pro-inflammatory cytokines, growth factors and proteases which appear to mimic inflammatory wound repair processes (Coppé et al., 2008; Shelton et al., 1999). In fact, senescent cells have been shown to be important in wound healing, tissue plasticity and tissue regeneration (Demaria et al., 2014; Mosteiro et al., 2016; Ritschka et al., 2017), suggesting that such a secretome is likely physiologically important for such processes. However, to date, the senescent secretome has more often been considered from a pathological perspective, with emphasis being placed on the damaging effects of chronic inflammation in age-related diseases and

cancer. In recent years there has been a surge of review articles focused on the role of cell senescence in ageing and disease, a subject beyond the scope of this review (Burton and Krizhanovsky, 2014; Childs et al., 2015; Muñoz-Espín and Serrano, 2014; van Deursen, 2014).

With the understanding that senescent cells can be beneficial in the short term, a process that appears to be regulated by immune processes, the concept of the senescent secretome as pathological can be reframed to instead reflect its immunogenic properties, in other words, a secretome that modulates an immune response. Whether a senescent cell is beneficial or detrimental likely depends on how long senescent cells persist within tissues.

Whilst senescent cells secrete factors known to function in the activation, migration, adhesion and differentiation of immune cells in other systems, much more research is needed to investigate the impact of the senescent secretome on specific immune cell functions. Some studies have reported a correlation between the presence of secretory factors associated with cell senescence and immune cell infiltration/localisation within tissues (Kang et al., 2011; Krizhanovsky et al., 2008a; Xue et al., 2007). An overview of factors associated with an immunogenic senescent secretome and their response within different immune cell types has been reviewed previously (Sagiv and Krizhanovsky, 2013).

The senescent program: mechanisms initiating an immune response

In the majority of instances, induction of the senescence program is initiated by activation of a DDR (d'Adda di Fagagna, 2008). Such a DDR is known to function in the activation of innate immunity and in the development and function of adaptive immunity (Nakad and Schumacher, 2016; Xu, 2006). A DDR during induction of cell senescence appears to be important for inducing an altered secretome capable of attracting and activating immune cells. As later discussed, a DDR has also been shown to be important for upregulating the expression of NKG2D ligands found on senescent cells, enabling their recognition by immune cells (Sagiv et al., 2016).

DNA damage can occur within chromatin packed nuclear DNA leading to activation of transcription factors and other regulators of cell secretion. These include, but are not limited to p38MAPK, NFκB, HMGB1, BRD4, mTOR and GATA4 (Chien et al., 2011; Davalos et al., 2013; Freund et al., 2011; Herranz et al., 2015; Kang et al., 2015; Tasdemir et al., 2016). In addition to nuclear DNA, increased damage to the nucleotide pool has also been shown to induce cell senescence (Rai et al., 2009) and may also involve activation of an immune response. Interestingly, it also appears that DNA damage during induction of cell senescence leads to accumulation of damaged DNA in cytoplasmic foci which

promotes a senescent secretome. Studies have shown that activation of cyclic GMP-AMP synthase (cGAS), a cytosolic DNA sensor, in senescent cells triggers stimulator of interferon genes (STING) which play an important role in innate immunity (Dou et al., 2017; Glück et al., 2017; Ng et al., 2017; Yang et al., 2017). Mackenzie et al investigated the mechanisms by which cellular DNA gains access to the cytoplasm since it is normally compartmentalised within the nucleus to prevent autoimmunity (Mackenzie et al., 2017). It was reported that cGAS localises to micronuclei arising from genomic instability and that breakdown of the micronuclei envelope exposes self-DNA to the cytosol.

Mechanisms other than a DDR cannot be ruled out. For example, treatment of tumour cells with cyclin-dependent kinase 4 and 6 (CDK4/6) inhibitors were shown to not only induce cell cycle arrest but to also promote anti-tumour immunity (Goel et al., 2017). CDK4/6 inhibition activated the expression of endogenous retroviral elements which stimulated the production of type III interferons leading to enhanced tumour antigen presentation. Rather than activating a DDR, CDK4/6 inhibitors in the context of tumour biology may enhance the susceptibility of such cells to immune checkpoint blockades. However, it should be noted that whilst such cells underwent cell cycle arrest, it does not mean that such cells are senescent and instead may have undergone a state of quiescence (Yoshida and Diehl, 2015).

From a therapeutic perspective, a more in depth understanding of the initial molecular mechanisms involved in activating an immune response is required. Such knowledge may one day be utilised to therapeutically enhance immune clearance of senescent cells as a strategy to alleviate and prevent age-related diseases.

Senescent cells activate an innate immune response

Scientific research focused on immune surveillance of senescent cells is an emerging field. It has now been approximately ten years since the first evidence for such a response was reported. In that study, Xue et al demonstrated that reactivation of p53 in p53-deficient tumours promoted induction of a cellular senescence program (Xue et al., 2007). This was associated with activation of an innate immune system as evident by increased expression of transcripts specific to natural killer (NK) cells, macrophages and neutrophils. This immune activation consequently resulted in tumour clearance, suggesting that the senescence program functions to prevent cancer development. It also suggested that induction of cell senescence rather than cell death may prove a more effective strategy for targeting cancers.

The following year, Krizhanovsky et al reported another benefit of immune clearance of senescent cells: the resolution of liver fibrosis following damage (Krizhanovsky et al., 2008b). Immune cells such as NK cells, macrophages and neutrophils were found to be in proximity to senescent cells in fibrotic livers. In addition, senescent activated stellate cells generated during liver damage were shown to be preferentially killed by NK cells both *in vitro* and *in vivo*. In doing so, immune clearance of senescent cells prevented excess fibrosis which occurred when cells were unable to enter the senescent state. Other than possibly acting as an anti-cancer mechanism, senescent cells until this point were frequently considered as only detrimental to the tissue in which they reside. However, it is now apparent that senescent cells could also play beneficial roles and that this is likely dependent upon the biological context. Following on from these findings, a role for cell senescence in other fibrotic models were also reported (Fitzner et al., 2012). In addition, physiological functions for senescent cells such as during wound healing, regeneration, embryonic/placental development and skeletal bone regulation during late puberty, slowly began to emerge (Chuprin et al., 2013; Demaria et al., 2014; Li et al., 2017; Muñoz-Espín et al., 2013a; Storer et al., 2013; Yun et al., 2015). However, the possibility that the aforementioned studies are instead reporting non-senescent cells displaying biomarkers in common with senescent cells cannot be ruled out.

Sagiv et al later investigated the mechanisms promoting NK mediated killing of senescent cells. Granule exocytosis, but not death-receptor-mediated apoptosis, was shown to be required for NK cell-mediated killing of senescent cells (Sagiv et al., 2013). Mice with defects in granule exocytosis accumulated senescent stellate cells within the liver following damage leading to increased fibrosis. In a follow-up study, Sagiv et al also investigated the mechanisms by which NK cells specifically recognise and target senescent cells. Ligands of an activating Natural Killer (NK) cell receptor (NKG2D), MICA and ULBP2 were found to be consistently upregulated following induction of replicative senescence, oncogene-induced senescence, etoposide-induced senescence and were found to be required for efficient NK-mediated cytotoxicity towards senescent cells (Sagiv et al., 2016). During liver fibrosis, senescent activated stellate cells were found to accumulate in mice lacking the NKG2D receptor. Other studies have reported the upregulation of NKG2D ligands following induction of cell senescence in tumour cells (Antonangeli et al., 2016; Iannello et al., 2013).

Whilst mechanistically, NK cells have been the primary focus to date in regard to immune surveillance of senescent cells, additional innate immune cells also appear important. Lujambio et al went on to publish findings focused on macrophage responses towards senescent cells. This study demonstrated that p53-expressing senescent stellate cells release factors which promote macrophage polarisation towards a tumour-inhibiting M1 state capable of targeting senescent cells in cultures (Lujambio et al., 2013). Findings by Hall et al suggest that alginate-encapsulated senescent cells may induce a M2-like

phenotype in peritoneal macrophages of mice as evident by elevated expression of arginase 1 (Arg1) (Hall et al., 2016). M2 macrophages are also capable of phagocytosis and so have the potential to also induce cell death in senescent cells. In addition, the induction of macrophages into M2a, M2b, and M2c subtypes by senescent cells may also occur and so further investigations are required. Other studies have also suggested that macrophages play a role in immune surveillance of senescent cells. An earlier study by Hall et al showed that alginate beads containing senescent cells promote macrophage recruitment when transplanted into mice (Hall et al., 2016). Findings from Yun et al, who has pioneered cell senescence research within the salamander model, suggests that macrophages are important for efficient clearance of senescent cells during salamander limb regeneration (Yun et al., 2015). Interestingly, macrophages may also promote induction of cell senescence in tumour cells as a possible anti-cancer mechanism (Reimann et al., 2010).

Senescent cells induce an adaptive immune response

In 2011, Kang et al demonstrated that pre-malignant senescent hepatocytes undergo immune-mediated clearance by CD4⁺ T cells (Kang et al., 2011). The presence of major histocompatibility complex class II (MHCII) molecules on senescent hepatocytes appeared to be important for direct killing of pre-malignant senescent hepatocytes. However, MHCII expression was not sufficient for inducing proliferation of naïve CD4⁺ T cells. In addition, killing of pre-malignant senescent hepatocytes by CD4⁺ T cells required the presence of monocytes/macrophages. Macrophage depletion in mice via gadolinium treatment greatly reduced immune surveillance of senescent cells. An impairment in immune clearance of pre-malignant senescent hepatocytes resulted in the development of hepatocellular carcinomas. This study again demonstrated the importance of immune surveillance of senescent cells in tumour suppression. Whilst senescent cells may promote T cell recruitment for their clearance, T cells may also induce cell senescence in cancer cells as a mechanism to limit cancer progression (Braumüller et al., 2013; Rakhra et al., 2010).

Another study also suggests that oncogene-induced senescence (OIS) in primary human melanocytes can activate aspects of the adaptive immune response. van Tuyn et al have shown that during OIS, melanocytes express MHC class II antigen presentation molecules which can activate T cell proliferation *in vitro* (van Tuyn et al., 2017). *In vivo*, non-proliferating, oncogene expressing melanocytes localize to skin-draining lymph nodes where they induce T cell proliferation and an antigen presentation gene expression signature. Senescent melanocytes are thought to form within

benign melanocytic nevi which are known to persist for decades (Joselow et al., 2017), suggesting that immune surveillance mechanisms are not active. The authors of this study suggest that benign human nevi represent a subset of OIS melanocytes that have been selected for downregulation of MHC II via evasion of MHC II-mediated immune-editing, providing a possible explanation for their persistence.

Immune surveillance of senescent cells during reproductive processes

In recent years there has been emerging interest in the role of cell senescence during processes associated with pregnancy. In 2013, three papers were published demonstrating a potential role for senescent cells in embryonic and placental development (Chuprin et al., 2013; Muñoz-Espín et al., 2013b; Storer et al., 2013). Two back-to-back papers published in *Cell* suggested that senescent-like cells function to promote tissue remodelling during embryonic growth and patterning in mice, a process which appears to be associated with macrophage clearance (Muñoz-Espín et al., 2013a; Storer et al., 2013).

In a study focused on placental development, fusion-induced cell senescence was reported to function in the formation of the syncytiotrophoblast, which serves as the maternal/foetal interface at the placenta (Chuprin et al., 2013). Fusion-induced senescence promoted the upregulation of secretory components associated with immune surveillance. It was suggested that cytokines secreted from these senescent cells may function in placental growth during pregnancy, protecting the foetus from pathological organisms and facilitating immune cell interaction (Burton and Krizhanovsky, 2014).

More recently, Egashira et al reported that macrophages contribute towards the clearance of senescent cells in the mouse postpartum uterus (Egashira et al., 2017). Depletion of macrophages in the postpartum mice increased the number of senescent cells in the uterus. In p53-deleted mice, uterine senescent cells increased with the authors suggesting that uterine p53 deficiency may affect the distribution of a macrophage subpopulation, leading to impaired clearance of senescent cells. However, p53-deleted mouse models are often used in studies to prevent cell senescence, so an increase in this instance is perplexing and requires further study. Excessive uterine senescent cells were associated with a decrease in second pregnancy success rate in a preterm birth model. In another study, findings by Brighton et al suggest that acute decidual cell senescence governs endometrial rejuvenation and remodelling at embryo transplantation (Brighton et al., 2017). During this process, uterine natural killer cells were shown to selectively target and clear senescent decidual cells through granule exocytosis.

Although the senescent programme may play important physiological functions during pregnancy, under other contexts it may contribute to pathological processes during pregnancy (reviewed in Velarde & Menon 2016; Cox & Redman 2017).

Cellular senescence: immunosuppressive responses

Whilst a number of studies have demonstrated a positive physiological impact by which senescent cells promote immune clearance, other studies have suggested that a senescence-mediated immune response can also be immunosuppressive and tumour promoting. In 2014, a study conducted by Di Mitri et al demonstrated that senescence induction in PTEN null prostate tumours in mice promoted an infiltration of Gr-1+ myeloid cells which protected a fraction of proliferating tumour cells from cell senescence, thereby sustaining tumour growth (Di Mitri et al., 2014). During the same year the another study was published reporting that Pten-loss induced cell senescence in tumours promoted an immunosuppressive secretome that contributed to tumour growth and chemoresistance (Toso et al., 2014).

Ruhland et al also went on to provide evidence suggesting that senescent cells can establish an immunosuppressive microenvironment that drives tumorigenesis (Ruhland et al., 2016). In this study, senescent stromal cells were shown to drive localised increases in suppressive myeloid cells that contributed to tumour promotion. In particular, interleukin-6, a common component of the senescent secretome, was responsible for inducing the increase in suppressive myeloid cells and their ability to inhibit anti-tumour T cell responses.

In the same year, Eggert et al reported that senescence-associated immune responses can both promote tumour suppression and tumour progression depending on the context (Eggert et al., 2016). The CCL2-CCR2 axis was shown to mediate myeloid cell accumulation in mice livers following induction of senescent cells, which acted as tumour suppressive in the early stages of liver tumorigenesis. However, when senescent cells are present in later phases of tumour development, the combination of senescent cells and tumour cells instead promotes tumour progression. The senescent secretome in this instance promoted the growth of liver cancer by inhibiting NK cell function through the accumulation of immunosuppressive immature myeloid cells.

Whilst much more research is required to determine the mechanisms promoting an immunosuppressive phenotype in senescent cells, it is likely dependent upon the genetic background of the cells, the mode of senescence induction and whether senescent cells have persisted and

undergone changes that may be attributed to a chronic response rather than a beneficial acute response. Experimentally underexplored is the concept of acute and chronic cell senescence. Acute senescence, referring to the early activation of the senescence program for a limited time-frame is likely beneficial, functioning in physiological processes. However, if senescent cells are not removed, they may develop a chronic phenotype associated with persistent inflammation which promotes age-related inflammatory diseases. In the cancer microenvironment, chronic inflammation can promote an immunosuppressive environment (Wang and DuBois, 2015), a process that likely extends to a senescence microenvironment.

Cell senescence of immune cells

Cell senescence of immune cells should not be confused with immunosenescence, the ageing of the immune system, which although may include induction of cell senescence, also includes an array of age-related changes unrelated to cell senescence. It is also important to note that the term “senescent” is often used interchangeable with “aged” by immunologists discussing immune cell ageing (see figure 1 for our definition of the terminology). The absence of separation regarding genuine mechanisms of immune cell ageing with those of the cell senescent program can lead to confusion. An awareness of immune cell senescence is important because such changes likely impact their response towards senescent cells of non-immune origin.

T cells: The majority of research to date focused on immune cell senescence has been undertaken on T cells. Like cell senescence in non-immune cells, T cell senescence is associated with irreversible growth arrest and a pro-inflammatory secretome that can contribute towards ageing and age-related diseases (Chou and Effros, 2013). However, the possibility that the senescence program may also be utilised by T cells during physiological immune responses cannot be ruled out.

Regulatory T cells (Tregs) play a role in regulating or suppressing other cells of the immune system. Ye et al has demonstrated that one way in which Tregs appear to promote immune suppression is through the induction of cell senescence in responder naïve and effector T cells (Ye et al., 2012). In addition, Montes et al reported that soluble factors from tumours can induce human T cells to develop a senescent-like phenotype that is also associated with the suppression of responder T cell proliferation (Montes et al., 2008). Such an immunosuppressive response likely functions in both physiological and pathological processes depending on the biological context. For example, Tregs have been shown to be beneficial through facilitating cutaneous wound healing (Nosbaum et al., 2016),

but can also be detrimental by promoting tumour development (Beyer and Schultze, 2006). Cancer can be considered as a persistent wound healing response (Schäfer and Werner, 2008), which may explain the role of immune cells in both these contexts. Ramello et al went on to demonstrate that senescent T cells also promote the secretion of pro-inflammatory cytokines and angiogenic factors by human monocytes/macrophages (Ramello et al., 2014).

Natural Killer cells: In addition to T cells, benefits of NK cell senescence have been reported. Rajagopalan and Long have provided evidence to suggest that induction of NK cell senescence by CD158d functions to reprogram NK cells in order to promote vascular remodelling during pregnancy (Rajagopalan and Long, 2012). CD158d, a receptor for soluble HLA-G (produced by foetal trophoblast cells during early pregnancy) was shown to trigger a DDR in NK cells leading to the appearance of biomarkers associated with cell senescence, including p21 expression and a senescent secretome. This senescent secretome promoted vascular remodelling and angiogenesis, indicating that NK cell senescence in this instance is beneficial for favouring reproduction. However, tissue remodelling and angiogenesis mediated by a senescent secretome in other contexts can be detrimental (Oubaha et al., 2016).

Astrocytes: Biomarkers of cell senescence have also been observed in response to hydrogen peroxide treatment in human and rodent astrocytes (Bitto et al., 2010). Stress-induced astrocytes in culture underwent growth arrest, morphological alterations, expressed SA-beta-Gal activity and increased p21, p53 and p16 expression. A similar finding was observed for astrocytes following extended cell culture. In a follow-up study, Crowe et al monitored the transcriptional changes associated with stress-induced senescence in human astrocytes, reporting that brain-expressed genes, including those involved in neuronal development and differentiation, were down-regulated in senescent astrocytes (Crowe et al., 2016). Astrocyte senescence also appears to occur in vivo. Bhat et al assessed biomarkers of cell senescence in astrocytes of brain tissue from aged individuals and patients with Alzheimer's disease (AD) (Bhat et al., 2012). A significant increase in p16^{INK4A}-positive astrocytes was observed in subjects aged 35-50 years and 78-90 years when compared with foetal tissue samples. In culture, it was also shown that beta-amyloid could trigger cell senescence, suggesting that senescent astrocytes may increase the risk of sporadic AD. In another study related to HIV therapy, it was reported that highly active antiretroviral therapy (HAART) increases biomarkers associated with cell senescence (cell cycle arrest, SA-Beta-Gal activity, p21 expression) in primary cultures of human astrocytes (Cohen et al., 2017). It was suggested that HAART-induced senescent astrocytes may play a role in neurocognitive impairment observed in HIV-infected patients.

Macrophages: Evidence for cell senescence in macrophages to date is less convincing. In response to hyperoxia (95% O₂), RAW264.7 macrophages have been shown to undergo cell cycle arrest associated with increases in p21^{CIP1} and p53 accumulation, characteristic features of cell senescence (Nyunoya et al., 2003). In another study, acrylamide was shown to induce a senescence-like response in RAW264.7 and mouse peritoneal macrophages, including cell cycle arrest, altered morphology and SA-Beta-Gal activity (Kim et al., 2015). Progressive telomere shortening and induction of cell cycle arrest has also been observed in rat microglia cells, a type of macrophage found in the brain (Flanary and Streit, 2004). However, whilst it can be assumed that cell cycle arrest in this instance is related to cell senescence, further characterisation is required. Caldeira et al have shown that after a very short period (16 Days) in culture, mouse microglia cells displayed altered morphology and increased SA-beta-Gal activity typical of senescent cells (Caldeira et al., 2014). Interestingly, NFκB activity which is normally elevated in non-immune senescent cells was decreased in culturally aged microglia cells in this instance. However, analysis of cell cycle does not appear to have been undertaken and since SA-beta-Gal activity has been observed in culturally aged but growth competent cells (Walters et al., 2016), it cannot be assumed that such cells are senescent. Finally, SA-beta-Gal activity and p16^{INK4A} expression in macrophages has also been reported to be elevated in ageing mice, with the authors suggesting that such cells are a subclass of macrophages rather than being senescent (Hall et al., 2016).

Recognition of senescent cells by macrophages: possible mechanisms

Whilst there is currently little understanding concerning the mechanisms governing macrophage mediated recognition of senescent cells, the processes are probably not specific to senescent cells. Rather, the more characterised molecular mechanisms associated with macrophage recognition in other cell models such as during cancer immunosurveillance and apoptotic cell clearance may also be pertinent for senescence surveillance.

Apoptotic cells have been shown to preferentially express specific cell surface antigens which can be recognised by naturally occurring antibodies (IgMs) that enable phagocytosis by macrophages. As such, it can be speculated that senescent cells may also express specific cell surface antigens which would not only provide insights into the mechanisms mediating immune clearance, but would also provide a means to specifically identify senescent cells in tissues. In a study focused on identifying antibodies which specifically target senescent cells, researchers identified the IgM clone 9H4 which recognises an oxidised form of membrane-bound vimentin (malondialdehyde (MDA) -modified

vimentin) expressed on the surface of senescent human fibroblasts (Frescas et al., 2017). Whilst not the focus of the study, the authors speculate that MDA-modified vimentin may serve as an “eat me” signal leading to phagocytosis by macrophages (Figure 2).

Senescent cells commonly display higher levels of ROS, likely owing to changes in cell metabolism and/or the presence of dysfunctional mitochondria, which can lead to the oxidation of phospholipids (Ademowo et al., 2017). During apoptosis, oxidatively modified lipids on the surface of cell membranes can function as pattern recognition ligands promoting macrophage recognition and phagocytosis via the scavenger receptor CD36 (Hazen, 2008), RAGE (Friggeri et al., 2011) and other class I and class II scavenger receptors (Canton et al., 2013). Macrophage CD36 binds to and internalises oxidised phospholipids and lipoproteins (Silverstein and Febbraio, 2009). Whether CD36 plays a role in the senescence surveillance by macrophages has yet to be determined (Figure 2). The naturally occurring antibody E06 is also known to specifically recognise oxidised phospholipids, functioning as an “eat me” signal for macrophages. Whilst this occurs during apoptosis, it raises the possibility that it may also function as a mechanism promoting senescence immunosurveillance by macrophages. However, Frescas et al (2017) claim that E06 failed to recognise senescent cells, but this may depend on how long cells have been senescent and so further research is required.

Surface expression of CD47 acts as a “don’t eat me” signal, sending inhibitory signals through SIRP α , a receptor expressed on the surface of macrophage, ensuring that healthy cells are not inappropriately phagocytosed. Therefore, the downregulation of CD47 would be required for macrophages to target damaged “self” cells (Liu et al., 2017). One study has demonstrated that induction of tumour cell senescence via c-Myc inactivation leads to the downregulation of CD47 which consequently promoted tumour regression (Casey et al., 2016). Whether CD47 downregulation in this instance is a specific response to c-Myc inactivation or activation of the senescence program is unclear. However, it would make biological sense to downregulate CD47 during cell senescence to enable removal of damaged “self” cells, but further research is required (Figure 2). In addition to CD47 in this study, the downregulation of programmed death-ligand 1 (PD-L1), a protein thought to play a role in immune suppression (Kleinovink et al, 2017), was also observed upon c-Myc inactivation-induced senescence.

Modified cell surface glycan’s are also potential senescence antigens that can be recognised by the C-type lectin receptor (CLR) family (Figure 2). One of the CLRs exclusively expressed by macrophages is the macrophage galactose-type lectin (MGL) (van Kooyk et al., 2015). N- and O-glycan surface protein modifications have been associated with senescent fibroblasts with an observed senescence-associated decrease in sialylation and increase in galactose exposure (Itakura et al., 2016). The

expression of the α -2,6-sialyltransferase I (ST6Gal I), which transfers sialic acid to galactose residues of N-glycans has also been shown to decrease during *in vitro* cell ageing (Tadokoro et al., 2006).

Beyond Senolytics: immunotherapy targeting senescent cells

Immunotherapeutic strategies already in development for combating cancer may one day be repurposed for targeting senescent cells for the alleviation of age-related diseases (see Figure 3). Discussed in detail elsewhere (Burton and Krizhanovsky, 2014; Childs et al., 2015; Muñoz-Espín and Serrano, 2014; van Deursen, 2014), such age-related diseases include diabetes, Alzheimer's, pulmonary fibrosis, cardiovascular disease and osteoporosis. In addition, identifying further molecular changes associated with senescent cells, especially cell-type specific alterations, would be advantageous for developing therapeutic approaches for targeting senescent cells. However, since senescent cells can also be beneficial, the elimination of acute senescent cells could be problematic. Therefore, the identification of therapeutic targets specific to chronic senescent which are absent in acute senescent cells would be highly desirable.

A recent study identified the expression of CD26/dipeptidyl peptidase 4 (DPP4) on the membrane surface of senescent fibroblasts which have undergone replicative senescence (Kim et al, 2017). This study assessed the potential use of DPP4 as a membrane target for promoting antibody-dependent cell-mediated cytotoxicity (ADCC) against senescent cells. ADCC is a natural mechanism of cell-mediated immune defence involving the activation NK cells by antibodies which has been adapted to promote NK cell-mediated ADCC in cancer immunotherapy (Wang et al., 2015). Kim et al demonstrated that targeting DPP4 via an ADCC assay *in vitro* preferentially sensitised senescent, but not proliferating cells to cytotoxicity by NK cells. These findings highlight a potential immunotherapeutic strategy for targeting senescent cells.

As stated previously, one of the mechanisms by which NK cells specifically recognise and kill senescent cells is via the surface expression of NKG2D ligands (Sagiv et al., 2016). Since many tumour cells also express NKG2D ligands, such ligands have been suggested to be a useful target for immunotherapeutic approaches in cancer (Spear et al., 2013), and so could be adapted for senescent cell clearance. For example, the use of engineered immune cells such as chimeric antigen receptor (CAR) T cells to target specific molecules on cancer cells has great potential as an anti-cancer therapy (Yu et al., 2017). As such, it may be possible to target senescent cells by engineering T cells to express a NKG2D CAR which recognise NKG2D ligands on the surface of senescent cells.

An adaptation of cancer vaccines could also be considered for boosting immune clearance of senescent cells. Although a universal biomarker of cell senescence has not been identified, the exposure of senescent cell membranes to immune cells may evoke an immune response to antigens not yet identified. In one approach, senescence vaccines would involve the isolation of senescence specific antigens (SSAs) which are then exposed to dendritic cells, professional antigen presenting cells. In response to SSA uptake, dendritic cells process and express these antigens on their cell surface which can then be recognised by T cells. T cell interaction with these antigens promotes T cell activation, differentiation and ultimately killing of target cells.

As discussed previously, the senescence program can also be activated in immune cells, which likely contributes towards impaired immune function, contributing to senescent cell accumulation. In addition to impaired immune cell function, the persistence of senescent immune cells would likely take up valuable immunological space required for expansion of functionally competent immune cells. T cells for example undergo rapid expansion in response to antigenic stimuli followed by cell-mediated apoptosis (Chou and Effros, 2013), a process that may be prevented if senescent T cells are present. As such, another strategy for promoting the elimination of non-immune senescent cells may be the removal of senescent immune cells with the anticipation that such an approach would enhance immune function.

One possibility could be to filter the blood of the unwanted senescent immune cells, maybe through the use of antibodies linked to magnetic nanoparticles/beads (Chen et al., 2017; Rebo et al., 2010). Another strategy may be the use of senolytic drugs to specifically kill senescent immune cells or senescent hematopoietic stem cells (HSCs) which give rise to immune cells. Senolytic targeting of senescent HSCs in mice leading to rejuvenation of aged tissue stem cells has already provided promising results (Chang et al., 2015). Rather than eliminating senescent immune cells, it may one day be possible to rejuvenate senescent immune cells, reversing their detrimental phenotype to improve immune function. For example, inhibition of p38 signalling in CD8+ T cells which exhibit features of cell senescence, increased proliferation, telomerase activity, mitochondria biogenesis and fitness (Henson et al., 2014). Small molecules based on resveratrol have also been reported to rescue cells from cell senescence (Latorre et al., 2017).

The ability of immune cells to migrate and kill target cells can become compromised with age, suggesting that therapeutically boosting these responses may be beneficial for promoting senescent cell clearance. One approach that can be adapted within immune cells is the engineering of cells that recognise specific components of the senescent secretome. As proof of principle, Qudrat et al generated cells with a chimeric IL-6 receptor (IL6Rchi) that generates a Ca²⁺ signal in response to IL-6,

a component of the senescent secretome (Qudrat et al., 2017). When IL6Rchi was expressed with an engineered Ca²⁺-activated RhoA (CaRQ), it enabled directed migration to IL-6 in cells which normally have no such function. Furthermore, the expression of vesicular stomatitis virus glycoprotein G (VSVG) and herpes simplex virus type 1 thymidine kinase (TK) in these IL-6 seeking cells allowed cell-cell fusion to occur with target cells and consequently cell death upon administration of ganciclovir, an anti-viral drug.

Concluding remarks

Senescent cells can be both beneficial and detrimental. Therefore, any therapeutic targeting of senescent cells needs to take into consideration that such approaches may also be harmful. This may include senescent cell removal during pregnancy or injury. However, some findings suggest that acute senescence mechanisms may be preserved during lifelong removal of senescent cells in naturally aged mice, but further research is required (Baker et al., 2016). Another aspect to consider is the biological consequences to mass removal of senescent cells. Such mass removal may disrupt tissue integrity and/or activate a strong pro-inflammatory response depending on the mode of cell death. Other therapeutic strategies in conjunction with senescent cell removal such as implantation of stem cells to aid repair may have to be considered (Burton, 2009). Whilst, there is currently more concern with therapeutically eliminating senescent cells once they have appeared, future strategies focused on preventing their long-term persistence would be greatly desirable.

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Figure Legends and Tables

Figure 1: “cell ageing” compared with “cell senescence”

Figure 2: Possible mechanisms of senescent cell recognition by macrophages.

Figure 3: Possible immunotherapies targeting senescent cells.

Table 1: Phenotypic changes often associated with cell senescence in vitro.