Cellular senescence in osteoarthritis pathology

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Summary

Cellular senescence is a state of stable proliferation arrest of cells. The senescence pathway has many beneficial effects and is seen to be activated in damaged/stressed cells, as well as during embryonic development and wound healing. However, the persistence and accumulation of senescent cells in various tissues can also impair function and have been implicated in the pathogenesis of many age-related diseases. Osteoarthritis (OA), a severely debilitating chronic condition characterized by progressive tissue remodeling and loss of joint function, is the most prevalent disease of the synovial joints, and increasing age is the primary OA risk factor. The profile of inflammatory and catabolic mediators present during the pathogenesis of OA is strikingly similar to the secretory profile observed in ‘classical’ senescent cells. During OA, chondrocytes (the sole cell type present within articular cartilage) exhibit increased levels of various senescence markers, such as senescence-associated beta-galactosidase (SAβ Gal) activity, telomere attrition, and accumulation of p16INK4a. This suggests the hypothesis that senescence of cells within joint tissues may play a pathological role in the causation of OA. In this review, we discuss the mechanisms by which senescent cells may predispose synovial joints to the development and/or progression of OA, as well as touching upon various epigenetic alterations associated with both OA and senescence.

Key words: cellular senescence; epigenetics; osteoarthritis.

Introduction

Osteoarthritis (OA)

Osteoarthritis (OA) is the most prevalent disease of synovial joints (around 4.7% of global population for knee and hip OA alone), afflicting many millions worldwide with pain and disability (Cross et al., 2014), and thus represents an enormous healthcare and socioeconomic burden. Advancing age is a major risk factor, thus the burden of OA is set to increase dramatically as populations continue to age. Gender is also recognized as a contributing factor, with the female population generally being at a higher risk of developing OA. Females are seen to develop more severe knee and hand OA compared to their male counterparts, especially when ≥55 years old (Srikanth et al., 2005). It has been implied that hormones may play a role in the increased incidence of OA in females, particularly a postmenopausal decrease in estrogen levels. Other risk factors contributing to the burden of OA are summarized in Fig. 1.

A generalized structure for a synovial joint is illustrated in Fig. 2, characterized by the presence of connective tissues such as articular cartilage, subchondral bone, ligaments, and in some joints menisci (fibrocartilage structures that provide stability and load dispersal), encapsulated by the synovial membrane (Fig. 2A). A joint affected by OA exhibits progressive degeneration of the articular cartilage, formation of bony peripheral outgrowths (osteophytes), changes in subchondral bone and thickening of both the synovium and ligaments (Fig. 2B), and in many cases synovial inflammation (synovitis), which is thought to be an important driver of early pathology (Benito et al., 2005). Pathologic roles for multiple tissues in deteriorating joint function therefore define OA as a whole joint disease, driven by various biomechanical and inflammatory factors. There are currently no treatments available to effectively prevent or reverse progressive joint damage; therefore, new and innovative treatments are urgently required to improve treatment options. This will require continued improvements in our understanding of the molecular mechanisms underlying OA pathology.

Cellular senescence

In 1961, Leonard Hayflick and Paul Moorhead first described the phenomenon known as ‘cellular senescence’, a form of ‘senescence at the cellular level’ (Hayflick & Moorhead, 1961), stating that primary human fibroblasts have a restricted lifespan of around 50 cell divisions in culture. This was once believed to be purely an in vitro phenomenon caused by cell culture shock; however, many research groups observed senescent cells in premalignant tissues, and this was soon discovered to be an important process in vivo (Dimri et al., 1995; Serrano et al., 1997; Sherr & DePinho, 2000; Michaloglou et al., 2005; Narita & Lowe, 2005). Cellular senescence is now considered a signal transduction process that results in cells entering a stable state of growth arrest while remaining metabolically active. Senescent cells most commonly enter this stable state in G1 phase, or early S phase, of the cell cycle (Di Leonardo et al., 1994; Ogryzko et al., 1996; Serrano et al., 1997; Herbig et al., 2004). However, senescent cells have also been observed to undergo arrest in G2 phase (Mao et al., 2012). Senescence ultimately results in the loss of cellular replicative capacity due to the inability of these cells to express genes required for proliferation (Dimri et al., 1994, 1996). Senescence is not characterized by a specific set of markers, but rather by association with a collection of cellular phenotypes that often coexist in a stressed cellular environment, such as altered morphology, chromatin structure and gene expression patterns, and an activated DNA damage response (d’Adda di Fagagna et al., 2003; Di Micco et al., 2006, 2011; di Adda di Fagagna, 2008; Rodier et al., 2009). Senescent cells secrete a variety of inflammatory cytokines, growth factors and many more soluble and insoluble factors known as the senescence-associated secretory phenotype (SASP) (Campisi, 2005), or the senescence-messaging secretome (SMS) (Kulman & Peep, 2009). These factors are secreted into the cell microenvironment, with cytokines such as IL-6 and IL-8 enforcing the stable growth arrest of senescent cells (Acosta et al., 2008; Kulman et al., 2008). Various features of senescent cells, such as the SASP, can...
cause damage to surrounding tissue (Burton et al., 2007). SASP secreted by senescent cells can alter the tissue microenvironment, while the senescence of stem or progenitor cells can impair tissue regeneration (Koobatian et al., 2015). Cells undergo senescence in response to various detrimental stimuli, including but not limited to oncogene activation; radiation; oxidative stress; shortened telomeres; and unscheduled DNA replication. Senescence is known to evoke tumor suppression, and it is widely accepted that senescence functions as a protective mechanism against cancer due to its ability to induce the proliferation arrest of damaged cells (Michaloglou et al., 2005; Dhomen et al., 2009; Goel et al., 2009). Aside from cancer, senescence-associated growth arrest is also important in normal physiological processes such as wound healing (Krizhanovsky et al., 2008).

Over the past decade, many studies have linked cellular senescence to aging (Krishnamurthy et al., 2004; Baker et al., 2008, 2013, 2016) and age-related pathologies (Baker et al., 2011), thus leading to an overlap in research between the fields of disease processes and gerontology.

**Cellular senescence and disease**

In healthy individuals, the body utilizes various systems that help to prevent and/or repair cellular, molecular, and physiological damage to cells. However, these repair systems become progressively weaker during aging and the organism becomes more vulnerable to the development of a variety of diseases. Mammals such as mice, baboons, and humans have been reported to accumulate senescent cells as they age (Dimri et al., 1995; Krishnamurthy et al., 2004; Jeyapalan et al., 2007). Moreover, the accumulation of senescent cells in tissues contributes to both aging and the promotion of age-related diseases (Krishnamurthy et al., 2004; Baker et al., 2008, 2016). For example, researchers have observed the presence of senescent cells (endothelial-like cells, vascular smooth muscle cells, and macrophage-like cells) in mice induced to develop atherosclerosis (Childs et al., 2016). Senescent vascular endothelial cells are present in human atherosclerotic lesions and contribute to atherogenesis (Minamino et al., 2002). In the context of OA, senescent cells were observed near the osteoarthritic lesions, but not in intact cartilage from the same patients and normal donors (Price et al., 2002; Erusalimsky & Kurz, 2005). Consistent with this, transplanted senescent cells induce an OA-like state in mice (Xu et al., 2016).

We discuss below the potential mechanisms by which accumulation of senescent cells may predispose articular joints to the development and/or progression of OA. We will also discuss the role of epigenetic changes in senescent cells in the context of OA pathology and highlight potential epigenetic treatment options.

**Senescence in osteoarthritis pathology**

Although there are multiple joint tissues and cell types involved in OA pathology, chondrocytes have been the focus of the vast majority of studies to date that address a role for senescence. Chondrocytes are the only cell type present in articular cartilage, a highly specialized avascular and aneural tissue whose structural and mechanical properties are largely defined by the two predominant extracellular matrix (ECM) components, type II collagen, and aggrecan. Chondrocytes are responsible for producing and maintaining this ECM and receive nutrients and external chemical signals from the synovial fluid via secretions of fibroblast-like synoviocytes of the intimal synovial layer.

Senescent cells exhibit a SASP that enables them to communicate with other cells, as well as the microenvironment, stimulating neighboring cells to senesce (Acosta et al., 2013). One characteristic feature of SASP is enhanced production of vascular endothelial growth factor (VEGF), a signal protein that promotes blood vessel formation via the processes of vasculogenesis and angiogenesis. VEGF and its cognate receptors are expressed in OA cartilage and may contribute to dysregulated osteogenesis and the formation of osteophytes (Pflander et al., 2001; Hashimoto et al., 2002; Enomoto et al., 2003). Chondrocyte SASP is known to include production of matrix-degrading proteases including the matrix metalloproteinases MMP-1, and -13 (Philipot et al., 2014). MMP-13 is thought to be central to the irreversible degradation...
of the cartilage type II collagen lattice in OA, partly on the basis of exogenous expression or deficiency in murine studies (Neuhold et al., 2001; Little et al., 2009).

Obesity is a major risk factor in OA, and oxidative stress resulting from excess adiposity (Keaney et al., 2003; Furukawa et al., 2004) could therefore contribute to disease partly through reactive oxygen species (ROS)-induced pathways. Excess adiposity is also associated with an increased accumulation of senescent cells and associated SASP factors (Schafer et al., 2016), as proposed (Tchkonia et al., 2010). Further, exercise prevents diet-induced cellular senescence as well as the SASP within visceral adipose tissue (Schafer et al., 2016). This suggests a possible mechanism whereby exercise-mediated health benefits may be mediated by the prevention of senescence.

**Senescence in OA chondrocytes & cartilage**

It is thought that cellular senescence may play a significant role in the pathology of OA, with OA chondrocytes exhibiting a variety of senescent-associated phenotypes (discussed below and Fig. 3). Despite recent traction for views of OA as a whole joint disease rather than merely dysfunctional cartilage, chondrocytes remain regarded as key players in OA pathology and are understood to exhibit during disease a perturbation of the normal balance between synthesis and degradation of extracellular matrix (ECM) components. This involves upregulating the production of matrix-degrading metalloproteinases such as MMP-13, exogenous activity of which was sufficient to recapitulate key OA features in mice (Neuhold et al., 2001). Senescence of chondrocytes would be expected to lead similarly to shifting of the balance between ECM synthesis and degradation, through metalloproteinase components of the SASP response. Moreover, enhanced degradation of cartilage ECM by chondrocytes during OA may be partly due to demethylation of CpG sites in the promoter regions of genes encoding key cartilage-degrading proteases, and therefore contributing to disease progression by increasing their production (Roach et al., 2005).

Aging and OA are not always interdependent, and many cases of OA in younger adults stem from joint injury (Gelber et al., 2000). However, as cellular senescence can result from a chronically stressed environment, it remains an interesting possibility that posttraumatic OA may be characterized or even partly triggered by an accumulation of senescent cells within damaged tissue. This view was supported, albeit in vitro, by the observation that mechanical stress accelerated chondrocyte senescence through increased oxidative stress (Martin et al., 2004a). Chondrocyte senescence is observed to be triggered by oxidative stress (Martin et al., 2004b) and is expected to contribute to the abnormal inflammatory environment present in OA. Chondrocytes exhibit very low metabolic activity and are well adapted to the hypoxic conditions of the joint, although exacerbated hypoxia may drive synovial inflammation in rheumatoid arthritis (RA) (an age-related autoimmune inflammatory joint disease characterized by joint destruction, chronic inflammation, and dysfunction of innate and adaptive immune responses) contributing to pathology (Ng et al., 2010). As synovitis is an acknowledged feature of OA, it is plausible that enhanced hypoxia could play a similar pathologic role in this scenario (Girotmanolaki et al., 2003). Similarly, chronic oxidative stress experienced by other joint tissues during disease may also lead to cellular senescence.

When compared to isolated chondrocytes from normal cartilage, OA chondrocytes positively express a variety of senescence-associated markers, for example, telomere attrition (Martin & Buckwalter, 2001); activated DNA damage response (DDR); ROS secretion; SAβGal activity (Price et al., 2002); increased p16ink4a expression (Zhou et al., 2004); accumulation of MMPs induced by pro-inflammatory cytokines (Billinghurst et al., 1997; Shlipov et al., 1997; Fig. 3). This has led to speculation that the integrity and function of the cartilage becomes impaired due in part to the age-related accumulation of senescent chondrocytes. In 2001, Martin and Buckwalter described telomere erosion in OA chondrocytes (Martin & Buckwalter, 2001). However, in normal articular cartilage, the rate of chondrocyte mitosis is very low (Aigner et al., 2001). This limited proliferative capacity in normal cartilage would suggest that other stress factors, such as oxidative stress (Yudoh et al., 2005) and abnormal mechanical loading (Harbo et al., 2012, 2013), may contribute to the shortening of telomeres of OA chondrocytes, contributing to pathology via premature senescence. Premature senescence of chondrocytes could then be a consequence of both intrinsic (limited replicative capacity in situ) and extrinsic (stress-induced) factors provoking the age-dependent deterioration of chondrocytes. On the other hand, as proliferation of chondrocytes is actually increased in OA cartilage (Aigner et al., 2001), this might help explain observed telomere erosion in disease. In knee OA, Gao et al. (2016) demonstrated a correlation between SAβGal expression and disease severity of patients. They investigated the levels of SAβGal expression in normal cartilage compared with OA cartilage of differing severity (mild, moderate, and severe). In normal articular cartilage, no staining was observed. However, in lesions taken from mild, moderate, and severely damaged knee OA cartilage, they observed SAβGal staining in a subset of chondrocytes close to the lesion. It is important to highlight that the elevation of SAβGal in cultured cells isolated from diseased joints is not a reliable indicator of pathological involvement of senescence in vivo. Hence, more functional experiments are required to investigate role ofsenescent cells in the in vivo development of OA.

Chondrocyte turnover is thought to be a rare event in cartilage; however, these cells proliferate when removed from the tissue and placed in culture. In human OA lesions, senescent cells are often found near clusters of cells, indicating increased mitotic activity prior to senescence (Price et al., 2002). Further, senescent cells are known to accumulate in tissues as we age and the mere presence of them in a disease context could be a consequence of the normal aging process. For example, Martin and Buckwalter (2001) showed that an association between OA and aging is due in part to replicative senescence of chondrocytes in vivo. However, evidence for direct involvement of senescent cells in cartilage damage comes from the senescent cell transplantation mouse model (Xu et al., 2016). In a recent study, Xu et al. injected either senescent or nonsenescent cells into the knee joint area of mice. Authors showed that transplanting senescent cells into the knee region caused pathological features suggestive of OA (Xu et al., 2016). More specifically, knee joints injected with senescent cells exhibited severe articular cartilage damage at the lateral and medial tibial plateaus, as well as the femoral condyles. This would suggest targeting senescent cells might be an attractive therapeutic modality for treatment of OA. However, it is not yet fully understood how the mechanisms of chondrocyte senescence contribute to cartilage degradation, and further mechanistic studies are urgently needed.

**Senescence in the bone microenvironment**

Recently senescent cells have been identified within the bone microenvironment (Farr et al., 2016). Comparing the presence of senescence and SASP markers in young (6 month) and old (24 month) mice, osteoblasts and osteocytes retrieved from trabecular and cortical skeletal tissue in older animals showed an increase in expression of p16ink4a, a cell cycle inhibitor seen to increase with age (Krishnamurthy et al., 2004;
Telomere attrition
(Price et al., 2002; Farr et al., 2016)

↑ p16ink4a expression
(Zhou, Lou and Zhang, 2004)

↑ SAβgal activity
(Price et al., 2002; Gao et al., 2016)

Activated DNA damage response
(Price et al., 2002)

Fig. 3 A comparison of the different characteristics observed in cell types found within joints of healthy subjects and patients with OA.

Waaijer et al., 2012; Burd et al., 2013; Farr et al., 2016), concomitant with an increase in senescent osteocytes present within the bone cortex. Telomere dysfunction-induced foci were also more prevalent in osteocytes from old mice. These findings suggest age-related bone loss could be, in part, caused by cellular osteocyte senescence, given the vital role of these cells in bone remodeling.

Autophagy

Autophagy is a cellular process thought to be a mechanism for cell survival when cells become stressed, for example under hypoxia or nutrient deprivation, in which cells degrade dysfunctional proteins and macromolecules and recycle them to produce the necessary raw materials for protein synthesis (Narita et al., 2011). There is an increasing interest in the role of autophagy in cartilage biology; this process may provide a key link between aging, cell survival, and OA. For instance, autophagy appears to be constitutively active in articular cartilage but decreases with age; an increase in apoptotic chondrocyte death was associated with a decline in autophagy and increased cartilage damage (Carames et al., 2010, 2015). Furthermore, chondrocyte-specific deficiency of the autophagy factor ATG5 was recently shown to promote age-related OA features in mice, concomitant with increased chondrocyte apoptosis (Bouderlique et al., 2016).

Advancing age is associated with dysregulated autophagy in cells such as cardiac myocytes (Terman et al., 2003); this dysregulation appears to result in oxidative stress and subsequent cellular senescence (Wu et al., 2009; Toshima et al., 2014). It is possible that these processes observed in other cell types also play a role in the cellular senescence
observed in OA. Age-related loss of skeletal muscle is a major cause of
movement impairment during later stages of human life (García-Prat
et al., 2016). It is thought that stem cells in the muscle lose their
regenerative function as we age and contribute to structural and
functional decline of the muscle tissue, reviewed in detail (Grounds,
2014). In 2016, García-Prat et al. (2016) described in detail the
relationship between cell survival and autophagy in muscle stem/
progenitor cells, with physiologically aged satellite cells undergoing
senescence due to a decrease in autophagy. Young satellite cells entered
senescence due to increased mitochondrial dysfunction, oxidative stress,
and failure of proteostasis. By restoring the autophagy pathway in aged
cells, these workers were able to show the reversal of senescence and
restoration of regenerative functions (García-Prat et al., 2016). Extrapolating
to the context of OA, this suggests that targeting improved
autophagy in joint tissues could provide a potential therapy that may
lead to a decrease in inflammation, along with enhanced regeneration of
joint tissues. This is a tempting idea given not only the poor regenerative
capacity of articular cartilage in OA, but also with regard to senescence
suppression strategies aimed at enhancing the capacity for potential use
of autologous chondrocyte populations for tissue regeneration/engi-
neering applications (Ashraf et al., 2016).

It is clear from the studies described above that an increased
understanding of the senescent program in relation to OA would provide
beneficial insight into the molecular mechanisms occurring within OA
joints, which could serve to broaden the spectrum of therapeutic
opportunities for the treatment of this debilitating disease. Furthermore,
a renewed effort to understand cellular senescence in joint tissues other
than cartilage might contribute to a more holistic view of the role of this
process in OA pathology.

Epigenetic changes in OA and cellular senescence

Both OA and cellular senescence are characterized by various epigenetic
changes thought to contribute to altered cellular phenotypes and disease
progression. Typically, epigenetic mechanisms can be clustered into
three categories: DNA methylation involving the methylation of CpG
islands; histone modifications such as acetylation, methylation, ubiqui-
tination, and phosphorylation; and regulatory micro RNAs (small
noncoding sequences involved in gene expression). We have already
discussed various striking similarities of senescent cells with cells found in
noncoding sequences involved in gene expression). We have already
mentioned the importance of histone modifications in OA (McClurkin et al.,
2013). Histone modifications are dynamic and can be modified by enzymes
such as histone deacetylases (HDACs), histone acetyltransferases (HATs),
and histone methyltransferases (HMTs). These modifications can affect
transcriptional activity by altering chromatin structure and influencing
the accessibility of DNA to transcription factors.

In OA, DNA methylation has also been implicated in the regulation of
gene expression. DNA methylation involves the addition of a methyl group
(—CH3) to cytosine residues in DNA, typically at CpG dinucleotides. This
methylation can lead to the silencing of gene expression by forming a
methylation-CpG island (MCpG) complex that recruits DNA methyltransferases
(DNMTs) to further methylate the DNA. DNA methylation is a dynamic
process that can be reversibly modified by enzymes such as Ten-1 (Tet),
which catalyzes the demethylation of 5-methylcytosine (5mC) to 5-hydroxymethylcytosine
(5hmC), allowing for the modulation of gene expression. In OA, DNA
methylation has been shown to be increased in cartilage and synovial tissues
and is associated with the expression of inflammatory genes.

Future perspectives

This review explores the potential of the senescent phenotype to predispose
joint tissues to the development and/or progression of OA. Senescent cells have the ability to synergize with inflammation and
inflammaging, already present within the OA joint microenvironment, to
drive further cellular senescent conversion and compound existing age-
related tissue damage, thus exacerbating and accelerating joint destruc-
tion. During OA, chondrocytes are seen to become ‘activated’ and
express a variety of senescence-associated markers. The exact mecha-
nism by which senescence of chondrocytes and especially other joint

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cells such as synovial fibroblasts, osteoblasts, and osteocytes contribute to OA is not fully understood, although the accumulation of senescent chondrocytes in the joints is widely thought to impair cartilage integrity. Activated chondrocytes secrete a potent cocktail of cartilage-degrading MMPs into the cartilage matrix, driving the development and progression of OA (Price et al., 2002). A study demonstrating the presence of senescent osteoblasts and osteocytes within the bone microenvironment of aged mice suggests an interesting potential to target these cell types to delay, or even prevent, age-related bone loss (Farr et al., 2016).

Given this potential to play a key role in OA pathology, innovative treatments could be developed by gaining an understanding of the underlying mechanisms by which cellular senescence contributes to OA. Recent studies have exposed numerous common characteristics between OA cartilage and preneoplastic tissues, one of the most prevalent being cellular senescence. These recent findings have inspired researchers to explore the potential of using anticancer treatments to slow or prevent the development and/or progression of OA.

**Potential innovative therapeutic approaches**

Senotherapeutic agents are used to target specific properties of cellular senescence; more specifically, senolytics are used to target anti-apoptotic mechanisms and induce cell death within senescent cells (Zhu et al., 2015a,b; Chang et al., 2016). Senolytic drugs may therefore also be potentially used to provide an innovative therapeutic approach to treatment of various conditions. Dasatinib is currently used in the treatment of cancer. It is widely accepted that cancer cells and senescent cells share common anti-apoptotic characteristics, and the combination treatment of dasatinib and quercetin has already been observed to reduce the burden of senescent cells, as well as enhance cardiovascular function, in aged mice (Zhu et al., 2015b). These workers reported, in Ercc1<sup>−/−</sup> mice exhibiting an accelerated aging condition, that periodic administration of dasatinib and quercetin was seen to delay bone loss and neurological dysfunction and to enhance health span (Zhu et al., 2015b). We would emphasize that not all senolytic compounds are anticancer and not all anticancer compounds are senolytic. Even among senolytics there seems to be cell type specific responses, for example, dasatinib is more effective in killing senescent human preadipocytes than human umbilical vein endothelial cells (HUVECs), whereas quercetin is effective in killing senescent HUVECs rather than senescent adipocytes. More mechanistic work is needed to clarify the exact relationship of effective in killing senescent HUVECs rather than senescent adipocytes.

**Conclusion**

The research of recent years has established that OA is not simply a passive ‘wear and tear’ disorder, but rather a complex age-related disease involving various different effectors, ranging from inflammatory mediators to epigenetic alterations. This complexity has undoubtedly contributed to the current lack of effective treatment options for OA, with pain management and eventual joint replacement surgery a common endpoint for a large proportion of patients. In this review, we have highlighted the potential mechanisms by which senescent cells can predispose joints of the body to the development and/or progression of OA, and explored the potential of using senolytic drugs to target senescent cells present in OA. In addition, targeting the epigenetic alterations observed in OA provides a promising approach to treatment as, unlike genetic changes, epigenetic changes can be reversed. In conclusion, gaining a better understanding of molecular mechanisms by which the senescence pathway and epigenetic changes underpin OA pathogenesis could open up novel and innovative therapeutic approaches.

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**Conflict of interest**

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