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Review
Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix

R. F. Loeser*

Section of Molecular Medicine, Department of Internal Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Summary

Objective: Age-related changes in multiple components of the musculoskeletal system may contribute to the well established link between aging and osteoarthritis (OA). This review focused on potential mechanisms by which age-related changes in the articular cartilage could contribute to the development of OA.

Methods: The peer-reviewed literature published prior to February 2009 in the PubMed database was searched using pre-defined search criteria. Articles, selected for their relevance to aging and articular chondrocytes or cartilage, were summarized.

Results: Articular chondrocytes exhibit an age-related decline in proliferative and synthetic capacity while maintaining the ability to produce pro-inflammatory mediators and matrix degrading enzymes. These findings are characteristic of the senescent secretory phenotype and are most likely a consequence of extrinsic stress-induced senescence driven by oxidative stress rather than intrinsic replicative senescence. Extracellular matrix changes with aging also contribute to the propensity to develop OA and include the accumulation of proteins modified by non-enzymatic glycation.

Conclusion: The effects of aging on chondrocytes and their matrix result in a tissue that is less able to maintain homeostasis when stressed, resulting in breakdown and loss of the articular cartilage, a hallmark of OA. A better understanding of the basic mechanisms underlying senescence and how the process may be modified could provide novel ways to slow the development of OA.
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Key words: Aging, Cell senescence, Chondrocyte, Cartilage, Oxidative stress.

Introduction

The prevalence of osteoarthritis (OA) rises directly with age and it is the most common cause of chronic disability in older adults[1,2]. However, it is important to note that OA is not an inevitable consequence of aging; it is not a simple "wearing out" of the joints; and aging-related changes in the joint can be distinguished from those due to disease. Not all older adults develop OA and not all joints are equally affected. Although the relationship between aging and the development of OA is incompletely understood, it is becoming apparent that aging changes in the musculoskeletal system contribute to the development of OA by working in conjunction with other factors such as obesity, joint injury, and genetics. From studies of surgically induced OA in young animals[3], it is also apparent that OA-like changes in the joint can develop without a significant contribution of aging. Thus aging and OA are inter-related but not inter-dependent.

OA is best characterized as joint failure due to progressive changes in several components of the musculoskeletal system that include, but are not limited to, the articular cartilage. Other joint structures, including the bone, muscle, synovium, and soft tissues (ligaments, tendons, and in the knee the menisci) are altered in OA but have not been as extensively studied as the articular cartilage, especially in regards to aging. This review focuses on how aging affects the articular cartilage but many of the concepts discussed will likely apply to other joint tissues as well.

*Address correspondence and reprint requests to: Richard F. Loeser, Section of Molecular Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA. Tel: 1-336-713-4259; Fax: 336-716-1214; E-mail: rloeser@wfubmc.edu

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Methods

The PubMed database until February 1, 2009 was searched using the search terms: aging, cell senescence, chondrocytes, cartilage, or OA. Articles, published in English, were selected for review based on their relevance to the topic of aging changes in chondrocytes or cartilage that might contribute to the development of OA.

Results

CELL SENESCENCE

The term senescence is derived from the Latin word senescere which means to grow old or to wane. Classical descriptions of cell senescence most often refer to the loss of the ability of mitotic cells to further divide in culture after a period of 30–40 population doublings, often referred to as the "Hayflick limit"[4]. It could be argued that this form of "replicative senescence", resulting from an arrest in cell-cycle progression, is an *in vitro* artifact of cell culture. However, *in vivo* relevance of replicative senescence, for at

least some tissues such as skin, is supported by findings that fibroblasts isolated from older humans or animals reach replicative senescence sooner than cells isolated from younger individuals[5]. In addition, at least some of the changes exhibited by cells that have undergone replicative senescence can be found in cells in older adults, such as the findings of shortened telomeres and the formation of senescence-associated (SA) heterochromatin[5].

Cell senescence may have evolved as a mechanism to prevent cells with damaged DNA from being replicated and thus to prevent tumor formation. Replicative senescence is associated with changes in DNA structure and function including a shortening in the telomeres accompanied by telomere dysfunction[6,7]. Telomeres are found at the ends of chromosomes and are incompletely replicated during mitosis such that with each cell division a portion of the end of the telomere is lost resulting in telomere shortening. The discovery of telomere shortening with each cell division and the finding that a loss in telomere function could cause cell-cycle arrest provided a mechanism for a biological clock that over time would result in replicative senescence.

However, cell senescence appears to be much more complex than simple cell-cycle arrest occurring after a finite number of cell divisions. Progressive telomere shorting due to repeated cycles of cell division does not explain senescence in post-mitotic cells such as neurons, or quiescent cells such as chondrocytes. More recently, attention has been drawn to other forms of cell senescence sometimes referred to as ''extrinsic'' or ''stress-induced'' senescence as opposed to the intrinsic senescence resulting from replication. Stress-induced senescence can occur from diverse stimuli including ultraviolet radiation, oxidative damage, activated oncogenes, and chronic inflammation[7,8]. Oxidative damage to DNA can directly contribute to stress-induced senescence and, because the ends of chromosomes are particularly sensitive to oxidative damage, can result in telomere shortening similar to that seen with replicative senescence[6,7].

Stress-induced senescence due to oxidative stress fits quite well with one of the long-standing theories of aging first proposed by Harman in the 1950s that invoked free radicals, or reactive oxygen species (ROS), as mediators of aging[9]. Oxidative stress has been found to induce cell senescence *in vitro* and there is *in vivo* evidence for age-related oxidative stress in many tissues[5]. As additional evidence for a role of ROS in aging, increased expression of the anti-oxidant enzyme catalase in mitochondria of transgenic mice can extend life-span and reduce age-related changes in tissues such as the heart[10,11]. However, extension of life-span could not be reproduced in transgenic mice overexpressing catalase in peroxisomes[12], suggesting that the source of ROS may be important in aging.

The concept that ROS contribute to cell senescence by causing direct damage to proteins, lipids, and DNA is evolving to include the role of ROS in regulating cell signaling pathways that promote senescence[13]. ROS are generated by intracellular enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and 5-lipoxygenase in response to activation of specific cell signaling pathways. These ROS serve as secondary messengers that regulate signal transduction by activating redox-sensitive kinases and inhibiting redox-sensitive phosphatases[13,14]. Insufficient levels of ROS can be detrimental to certain signaling pathways, such as the epidermal growth factor (EGF) pathway that regulates cell proliferation, while excessive levels of ROS may inhibit pathways, such as the insulin signaling pathway, through activation of the stress-induced kinase JNK[13,15]. A direct role for ROS in mediating senescence has been demonstrated through a positive feedback loop where mitogenic signaling that includes activation of protein kinase Cdelta (PKCδ) by ROS cooperates with the $p16^{INK4A}$ pathway to promote senescence[16].

Senescent cells exhibit altered activity and expression of regulatory proteins that control growth and proliferation (Fig. 1). These include p53 and the cyclin-dependent kinase inhibitors $p21^{CIP1}$, and $p16^{INK4A}$ [5,8]. Activation of p53 occurs from DNA damage or from telomere shortening and serves to inhibit cell-cycle progression. Activated p53 increases the expression of p21 which contributes to senescence. As p21 declines in senescent cells, p16 is increased which appears to serve a more long-term role in the inhibition of cell-cycle progression through inhibition of retinoblastoma protein[5]. The permanent state of cell-cycle arrest is also related to epigenetic changes that include the formation of foci of heterochromatin referred to as senescence-associated heterochromatin foci or SAHFs that include histone variants such as the macro-H2A[17]. SAHFs and macro-H2A are used as markers for senescent cells as are findings of increased p16 expression[17,18].

Senescent cells have also been found to have increased levels of the lysosomal enzyme β-galactosidase that is detectable at pH 6 rather than the normal pH 4.5[19]. Detection of β-galactosidase activity at pH 6 has been referred to as senescence-associated (SA) β-galactosidase (SA-βgal). Detection of activity at pH 6 is thought to be due to an increase in lysosomal mass and is not specific to cell senescence since it has been noted in immortalized cells, tumor cell lines, and even in normal cells under certain cell culture conditions[5,19,20]. We have noted positive staining for SA-β-galactosidase in the immortalized chondrocyte cell line C28I2 indicating it is not a specific marker for chondrocyte senescence (unpublished observation).

In addition to causing cell-cycle arrest due to an increase in expression of genes that inhibit proliferation, the changes that occur in senescent cells can also result in the increased production of cytokines, growth factors, and matrix

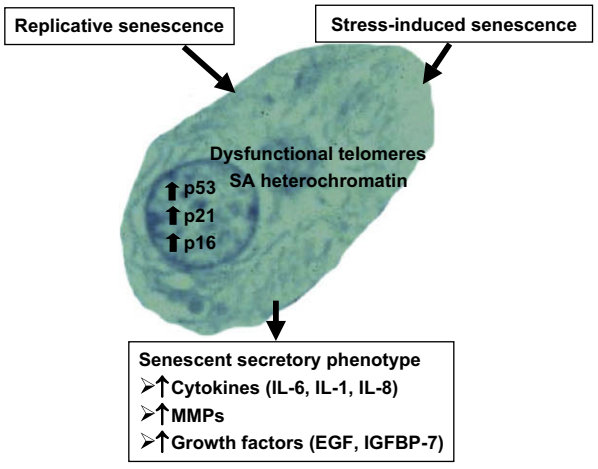

Fig. 1. Cell senescence. There are two major types of cell senescence-replicative (intrinsic) and stress-induced (extrinsic). Senescence is associated with telomere dysfunction, formation of SA heterochromatin, and increased expression of p53, p21, and p16. The senescent secretory phenotype is characterized by increased production of cytokines, MMPs and growth factors such as EGF or growth factor binding proteins such as IGFBP-7.

metalloproteinases (MMPs; Fig. 1). Sometimes referred to as the senescent secretory phenotype[8,18], this form of cell senescence may be particularly relevant to the development of OA. The senescent secretory phenotype is characterized by the increased production of cytokines including interleukin-6 (IL-6) and interleukin-1 (IL-1), MMPs, and growth factors such as EGF. Recent studies have also provided evidence for a role of the IL-8 receptor chemokine XC receptor2 (CXCR2)[21] and insulin-like growth factor binding protein-7 (IGFBP-7)[22] in senescence, suggesting autocrine loops of secreted proteins contribute to cell senescence. The accumulation of cells expressing the senescent secretory phenotype can also contribute to tissue aging through damage to the extracellular matrix, such as seen with the degradation of dermal collagen due to an age-related increase in collagenase[8,18].

CHONDROCYTE SENESCENCE

Chondrocytes from older adults exhibit many of the changes that are typical of cell senescence (Table I). Chondrocytes will divide in cell culture and after multiple passages will exhibit telomere shortening characteristic of replicative senescence[23]. Evidence of telomere shortening in chondrocytes has also been reported in cells isolated from older adults[24]. But because adult articular chondrocytes rarely, if ever, divide in normal tissue *in vivo*[25,26], it would seem unlikely that they would experience telomere shortening due to classical replicative senescence *in vivo*.

It is much more likely that chondrocyte senescence is the extrinsic type induced by chronic stress. There is evidence that telomere shortening noted in chondrocytes could be due to DNA damage from ROS[27,28]. Interestingly, shorter telomere length in peripheral leucocytes was associated with radiographic hand OA in a cross-sectional study[29]. This association might also suggest a link between systemic oxidative stress, telomere erosion, and OA.

As discussed further below, an increase in chondrocyte ROS levels can be aging-related[30,31]. However, ROS generated from excessive mechanical loading and/or stimulation by cytokines could also contribute to DNA damage and subsequent telomere shortening[32,33]. The lack of cell division in normal adult articular cartilage and the lack of a ready supply of local progenitor cells in cartilage suggest that the chondrocytes present in the cartilage of an older adult are likely to be the very same cells that were present decades earlier. In contrast to many other tissues in the body that experience a regular turnover of cells, the long lifetime of chondrocytes would make these cells particularly susceptible to the accumulation of changes from both aging and extrinsic stress.

Evidence of cell senescence in tissues from older adults can be obtained by examining for the presence of senescence markers. These markers currently include histological staining for SA-βgal, SA heterochromatin, increased p53, p21, and p16 and reduced Wnt2[18]. Staining for SA-βgal has been shown to be present in articular chondrocytes from older adults[24] and in OA chondrocytes[34]. Chondrocyte SA-βgal staining, as well as telomere shortening, has also been noted after treatment *in vitro* with IL-1β or H_2O_2 consistent with stress-induced senescence[35]. Staining for SA-βgal needs to be interpreted with caution since this marker of cell senescence is not specific and can be influenced by factors such as cell culture[20]. As noted above, we have seen positive staining in immortalized cells that would not be considered senescent.

Importantly, Dai *et al*[35]. have provided evidence that stress-induced senescence *in vitro* is also accompanied by an increase in chondrocyte p53 and p21 expression as additional markers of the senescent phenotype. The senescence marker $p16^{INK4A}$ has also been examined and found to be present at greater levels in OA chondrocytes relative to age-matched normal tissue which in turn had higher levels than fetal tissue[36]. In the latter study, siRNA knockdown of $p16^{INK4A}$ was found to promote chondrocyte proliferation and matrix gene expression consistent with a link between senescence and the reduction in the ability of chondrocytes to proliferate and repair the matrix.

There is mounting evidence that chondrocytes can exhibit features of the senescent secretory phenotype which has important implications for the role of chondrocyte senescence in the development and progression of OA. When compared to cells isolated from young tissue donors, human articular chondrocytes from older adults were found to secrete more MMP-13 into the media after stimulation with either IL-1β or fibronectin fragments[37]. Isolated human chondrocytes were also found to produce more IL-1[37] and more IL-7[38] with increasing donor age and, like IL-1 and fibronectin fragments, IL-7 can also induce MMP-13 production[38]. MMP-13 serves as a major mediator of type II collagen cleavage[39,40]. Studies have shown increased immunostaining for MMP-3 and MMP-13 in cartilage with aging[41] as well as an age-related accumulation of collagen neoepitopes representing denatured or cleaved collagen[42,43]. Development of the senescent secretory phenotype also might explain the cross-sectional observation of an increase in type II collagen degradation products in the urine of healthy adults after about age 50 years[44].

Another feature of chondrocyte senescence is a decline in the proliferative and anabolic response of chondrocytes to growth factor stimulation. An age-related loss in the normal mitogenic response to several different growth factors has been noted including transforming growth factor-beta (TGF-β), basic fibroblast growth factor (bFGF), platelet-derived growth factor (PDGF), insulin-like growth factor-I (IGF-I), as well as those contained in fetal bovine serum[45]. IGF-I is well known to stimulate cartilage matrix synthesis and there is substantial evidence for an age-related decline in the ability of IGF-I to stimulate proteoglycan and collagen production[46–48]. TGF-β is another important cartilage anabolic factor and studies in equine chondrocytes have noted an age-related decline in response[49]. While the anabolic response to TGF-β was maintained in cartilage from aging mice, the ability of TGF-β to counteract the anti-anabolic affects of IL-1 was lost[50]. The ability of bone morphogenic protein-6 (BMP-6) to stimulate proteoglycan synthesis has also been shown to decline with age in human chondrocytes[51]. Finally, a comparison of young and old bovine chondrocytes revealed that the cells from older animals produce less functional matrix when cultured with 10% serum[52].

Table I
Features of chondrocyte senescence

Senescence feature	References
Telomere shortening	23,24,27,28
↑ SA-βgal, p53, p21, p16	24,35,36
↑ Cytokine and MMP production	37,38,41–43
↑ Oxidative stress/damage	30,31,35,75
↓ Growth factor response	45–52
↓ Growth factor production	53,54
↑ Cell death	26,31,53,59–62,64

Besides a loss in the ability of chondrocytes to respond to growth factor stimulation, there is also evidence for an age-related reduction in the levels of certain growth factors in cartilage. In the mouse, levels of TGF-β2 and TGF-β3 but not TGF-β1 decline with age as does the level of the TGF-β receptors I and II[53]. It has been shown that the expression and amount of osteogenic protein-1 (OP-1) (also called BMP-7) present in human cartilage declines significantly with age[54]. The reduction in OP-1 levels may be due to a recently discovered age-related increase in methylation of the chondrocyte OP-1 promoter[55]. DNA methylation and histone acetylation are two common epigenetic mechanisms that serve to regulate the level of gene transcription. The extent of DNA methylation in gene promoters that are rich in CpG sequences (such as the OP-1 promoter) can be altered with aging or in disease states and result in altered gene expression[56]. In general, an increase in promoter methylation will result in reduced gene expression.

The potential for epigenetic regulation of chondrocyte gene expression is just beginning to be explored. Although changes in DNA methylation were not found to directly regulate aggrecan expression in normal aged or OA cartilage[57], inhibition of OP-1 promoter methylation *in vitro* resulted in an increase in both IGF-I and aggrecan expression, most likely from autocrine stimulation from an increase in OP-1 expression[55]. In contrast to the age-related increase in chondrocyte OP-1 promoter methylation, Roach *et al*[58]. found that in OA chondrocytes there was a decrease in the methylation of promoter sites in several matrix degrading enzymes including MMP-3, MMP-9, MMP-13, and a disintegrin and metalloproteinase with thrombospondin motif-4 (ADAMTS-4) that could explain their increased expression in OA. Most recently, levels of the high-mobility group box (HMGB) protein 2, which is expressed in the superficial zone of cartilage, have been shown to decline with age in human and mouse articular cartilage[59]. HMGB2 is a nonhistone chromatin protein that can serve as a transcriptional regulator. Taniguchi *et al*[59]. demonstrated that deletion of HMGB2 in transgenic mice resulted in the early onset of OA-like changes in the superficial zone of cartilage that were associated with an increase in susceptibility of chondrocytes to cell death. Further studies on epigenetic regulation of chondrocyte gene expression may provide novel insights into the changes in chondrocyte gene expression noted in aging and OA.

Chondrocyte senescence can contribute to a decline in chondrocyte numbers due to increased cell death, although the extent of cell death with aging or in OA has varied among studies[60–62]. In human hip cartilage, a 30% fall in cell density between the ages of 30 and 70 years has been reported[63]. In femoral head cartilage from rats, a 46% decline in cell numbers was noted in old compared to young adult rats[31]and an age-related increase in apoptotic chondrocytes in rat cartilage has been reported as well[64]. In mice a similar age-related reduction in cell numbers in the medial tibial cartilage was seen[53]. However, a study of human knees found less than 5% cell loss with aging[26].

There are certainly reasons to expect an age-related increase in death of chondrocytes including the decline in growth factor activity, the loss of survival promoting matrix proteins, and the increase in oxidative damage. As noted above, the response of chondrocytes to IGF-I declines with age and IGF-I is an important autocrine survival factors in cartilage[65]. Although matrix alterations occur with aging, it is not known if these affect the ability of either type II collagen[66] or fibronectin signaling through the α5β1 integrin[67] to promote chondrocyte survival. Oxidative damage from ROS could also contribute to chondrocyte death. Levels of ROS increase in cartilage with aging and chondrocytes from older adults are more susceptible to ROS-mediated cell death[30]. There is also evidence, at least in mouse cartilage, for an age-related decline in the anti-apoptotic protein Bcl-2 and the Bcl-2 associated-athanogene-1 (Bag-1)[68] that could increase the susceptibility of chondrocytes to cell death. Finally, the age-related decline in HMGB2, discussed above, may also make chondrocytes more susceptible to cell death[59]. Because of a lack of replacement cells in cartilage, any loss of cells due to cell death could have important negative consequences.

THE ROLE OF OXIDATIVE STRESS IN CHONDROCYTE SENESCENCE

Oxidative stress may play a major role in the link between aging and the development of OA (Fig. 2). Oxidative stress results when the amount of ROS exceeds the anti-oxidant capacity of the cell. This can be due to either increased production of ROS or decreased levels of anti-oxidants and in aging both are often responsible[13]. Glutathione is a major intracellular anti-oxidant that also participates in regulating redox signaling events. An increase in levels of oxidized glutathione can be a sign of oxidative stress[5]. Evidence for an age-related increase in oxidative stress in human chondrocytes was obtained by finding an increase in the ratio of oxidized to reduced glutathione in isolated cells[30]. Increased levels of intracellular ROS were also detected in cartilage from old rats when compared to young adults[31]. Importantly, age-related oxidative stress was found to make human chondrocytes[30] and rat chondrocytes[31] more susceptible to cell death mediated by oxidants. As additional evidence for oxidative stress playing a role in chondrocyte senescence, chondrocyte senescence *in vitro* was associated with oxidative stress[69] and exogenous addition of ROS to cultured chondrocytes was found to induce markers of the senescent phenotype[35].

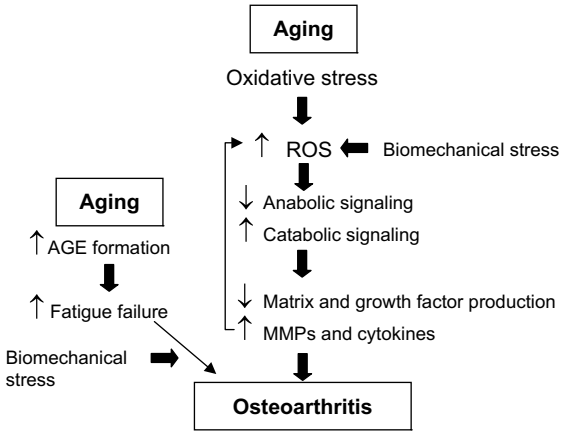

Fig. 2. Theoretical model for the relationships of aging, oxidative stress, and the development of OA. Aging-related oxidative stress as well as abnormal biomechanical stress results in increased levels of ROS in chondrocytes. The increase in ROS modulates anabolic and catabolic signaling pathways resulting in reduced matrix synthesis, inhibition of growth factor expression, and increased production of MMPs and cytokines that lead to matrix loss and OA. Aging also results in increased formation of AGEs which causes increased fatigue failure of the cartilage that when stressed also contributes to the development of OA.

There is also evidence for reduced levels of anti-oxidant enzymes in cartilage with aging and in OA that would contribute to chondrocyte oxidative stress. In chondrocytes from aged rats, catalase, but not superoxide dismutase or glutathione peroxidase, was found at lower levels than in young adults[31]. Proteomic studies of human articular chondrocytes found a decrease in mitochondrial superoxide dismutase with aging[70] as well as a decrease in OA cells when compared to cells from normal tissue[71]. Although not studied in aging, cartilage from adults with OA also had less extracellular superoxide dismutase than normal cartilage[72] and gene array studies performed with RNA isolated from OA cells revealed a decreased expression of superoxide dismutase and glutathione peroxidase[73].

Increased levels of ROS can contribute to aging changes in cells and tissues by causing oxidative damage to proteins, lipids, and DNA. One marker of protein oxidation is the presence of nitrotyrosine which can be detected using anti-nitrotyrosine antibodies. Nitrotyrosine is created by the reaction of protein tyrosine residues with peroxynitrite ($ONOO^-$) formed when the ROS superoxide ($O_2^{\cdot-}$) and nitric oxide ($NO^{\cdot}$) react[74]. Increased immunostaining for nitrotyrosine has been noted with aging in normal human and monkey cartilage[75]. Nitrotyrosine has also been detected in OA tissue[28,72,75]. In monkey cartilage, the presence of positive immunostaining for nitrotyrosine correlated with a reduced anabolic response to IGF-I in chondrocytes isolated from nearby tissue, suggesting that oxidative damage may be one mechanism for the reduced growth factor response[75]. In addition, excess levels of NO, a reactive nitrogen species, have also been found to reduce the chondrocyte response to IGF-I[76]. Likewise, earlier studies noted that treatment with H_2O_2 inhibits chondrocyte proteoglycan synthesis[77].

The source of ROS contributing to oxidative stress and oxidative damage can include both free radicals generated as by-products of aerobic metabolism as well as ROS generated in response to specific stimuli such as growth factors and cytokines. Although chondrocytes live in an environment with a low oxygen tension, they do consume oxygen and therefore exhibit aerobic metabolism[78]. ROS have been shown to be produced by chondrocytes in response to stimulation by cytokines and growth factors, including IL-1, TNF-α, FGF and TGF-β[79–82] as well as by integrin stimulation with fibronectin fragments[83]. IL-1 stimulation of ROS has been associated with chondrocyte DNA damage[33]. Production of ROS by chondrocytes may also have an important physiologic role *in vivo*. A recent study showed that in the growth plate ROS regulate proliferation and the initiation of hypertrophy[84]. The latter finding suggests a potential connection between ROS production in articular cartilage and chondrocyte hypertrophy observed in OA.

The underlying mechanisms by which oxidative stress contributes to chondrocyte senescence have not been well defined. Studies in other cell types have provided evidence that oxidative stress contributes to senescence through modulation of the activity of specific cell signaling pathways[5,85]. As noted above, this can be due to modulation of the activity of a number of redox-sensitive kinases and phosphatases. The activity of MAP kinase pathways, which include ERK, JNK, and p38, may be particularly important. Caveolin-1 is an integral membrane protein that serves as a scaffold and can regulate cell signaling pathways involved in senescence[5]. Caveolin-1 has been found to play a role in chondrocyte senescence induced by IL-1 and H_2O_2 through activation of the p38 MAP kinase[35]. We have recent evidence that ROS can contribute to chondrocyte IGF-I resistance and reduced proteoglycan synthesis by causing an imbalance in the activity of the phosphoinositide-3 (PI-3) kinase-Akt pathway, which is necessary for chondrocyte proteoglycan synthesis, and the mitogen-activated protein kinase/extracellular regulated kinase (MEK-ERK) MAP kinase pathway, which inhibits proteoglycan synthesis (Yin *et al.*, unpublished data). Because IGF-I can also stimulate chondrocyte anti-oxidant capacity[82], resistance to IGF-I could further contribute to a redox imbalance.

Oxidative stress may also contribute to chondrocyte senescence by promoting endoplasmic reticulum (ER) stress. ER stress has been shown to down-regulate expression of cartilage matrix proteins including collagen type II and aggrecan and to increase chondrocyte apoptosis[86,87]. Whether ER stress increases with age in cartilage has not been determined. Further studies on redox regulation of cell signaling in chondrocytes, as well as on ER stress, should help to better define the mechanism of oxidative stress-induced chondrocyte senescence and may provide new targets for slowing the aging process in cartilage.

AGING IN THE CARTILAGE MATRIX

Age-related changes in the cartilage matrix have been reported that could be important in contributing to the development of OA (Table II). There is evidence from magnetic resonance imaging (MRI) studies that the articular cartilage in the knee thins with aging, particularly at the femoral side of the joint[88] and at the patella[89]. Cartilage thinning is consistent with a gradual loss of cartilage matrix with aging as well as a decrease in cartilage hydration and cellularity. A recent study of human femoral cartilage demonstrated an age-related decrease in cellularity and glycosaminoglycan content that could contribute to weakening of the tissue[90]. Age-related changes in the size, structure, and sulfation pattern of aggrecan have also been reported[91–94]. Aggrecan's abundant negatively charged sulfates, which are very hydrophilic, are responsible for maintaining the high content (about 70–80%) of water in cartilage. Aging changes in aggrecan likely contribute to a loss in cartilage resiliency and hydration[95]. There is also evidence for an age-related accumulation of aggrecan fragments containing the hyaluronic acid binding region[96]. The aggrecan fragments that remain bound to hyaluronic acid can occupy the space where a newly synthesized complete aggrecan molecule would normally bind and thus result in smaller proteoglycan aggregates being present with increasing age[91].

The aging cartilage matrix appears to be particularly susceptible to the accumulation of advanced glycation end-products (AGEs). AGEs are formed by reducing sugars such as glucose, fructose or ribose, reacting with lysine or arginine residues in a process of non-enzymatic glycation[97]. The low turnover rate of type II collagen, calculated to be over 100 years[98], allows for the accumulation of AGEs that have been noted in human knee tissue[97,99]. AGE

Table II
Aging changes in the cartilage matrix

Aging change	References
↑ AGE formation	97–101,103
↓ Hydration	95
↓ Aggrecan size	91–94
↑ Collagen cleavage	42–44
↑ Fatigue failure	90,95,100
↓ Growth factor levels	53,54
↑ Matrix calcification	108–111

formation in collagen can result in increased cross-linking. The most common AGE-related cross-link is pentosidine which has been found to be present in cartilage in increasing amounts with age[98,100,101]. Formation of excessive collagen cross-links affects the biomechanical properties of cartilage resulting in increased stiffness making the cartilage more brittle[102] and increasing the susceptibility of the tissue to fatigue failure[100].

In addition to altering the biomechanical properties of cartilage, increased levels of AGEs in cartilage may also affect chondrocyte function. An association has been noted between AGE formation and a decline in chondrocyte anabolic activity[103]. The mechanism by which AGEs affect chondrocyte function may include a direct interaction with cell receptors such as RAGE (the Receptor for Advanced Glycation End-products). RAGE is expressed by chondrocytes and RAGE levels in cartilage increase with both aging and the development of OA[104]. Stimulation of chondrocyte RAGE by AGE-albumin, produced *in vitro*[105], or by S100 proteins, which can also bind RAGE and are present *in vivo*[104,106,107], results in increased production of MMPs as well as a modulation of the chondrocyte phenotype to hypertrophy. Because increases in MMP production and chondrocyte hypertrophy are hallmarks of OA, signaling through RAGE could play an important role in connecting age-related changes in the matrix to the development of OA. Chondrocyte RAGE signaling requires ROS[107], providing another link between oxidative stress, aging, and OA.

Despite the fact that type II collagen has a very long half-life in cartilage, there is evidence for an age-related increase in collagen turnover. Type II collagen degradation was noted to increase with age in macroscopically normal ankle cartilage[43]. In that study, the ratio of cleaved/denatured type II collagen was examined and revealed a highly significant positive correlation with age ($r = 0.78$, $P < 0.0001$) suggesting a disassociation between cleavage and denaturation with aging. An increase in cleavage without a similar level of denaturation could be due to the age-related increase in collagen cross-linking from AGEs.

An age-related increase in calcification of the articular cartilage, and the menisci in the knee, has been demonstrated radiographically[108,109]. This may be related to an increase in the activity of transglutaminase, an enzyme involved in the biomineralization process[110] and to an increase in inorganic pyrophosphate production in response to transforming growth factor-β stimulation[111]. Despite a strong association among age, chondrocalcinosis, and the presence of OA[108,109], the precise role of cartilage matrix calcification in the development of OA is not clear, in part due to the number of older people with asymptomatic chondrocalcinosis[112].

AGING AND THE LOSS OF HOMEOSTASIS

If aging does not directly cause OA, how does it contribute to the development and/or progression of the disease? It is now fairly well accepted that in OA, at least in the articular cartilage, an imbalance exists in anabolic and catabolic pathways that favors matrix degradation[113–115]. We have proposed[116], that aging changes including excessive levels of ROS could play an important role in tipping the balance of anabolic and catabolic signaling (Fig. 2). An alteration in the level of anabolic and catabolic activity represents a loss in homeostasis. Many of the chronic degenerative conditions associated with aging appear to result from an age-related loss in the ability of cells and tissues in the body to maintain homeostasis, particularly when put under stress[117].

The obvious stress for joint tissues is the mechanical stress that results from joint loading and motion. It is clear that excessive or abnormal mechanical stresses play a central role in the development of OA[118]. Under conditions where an anatomically normal joint is stressed, healthy joint tissues appear to be very capable of adapting to stress. As an example of successful adaptation, the chronic repetitive loads endured by long distance runners do not appear to result in OA later in life[119,120]. The development of OA occurs in joints that are unable to maintain homeostasis. Excessive loads, particularly when placed on a malaligned joint, overwhelm the homeostatic mechanisms leading to OA[118,121,122].

OA is rare in young adults and even serious joint injuries usually don't manifest as OA until years later, suggesting that young joint tissues can compensate, to some degree, to abnormal mechanical stress. But with aging, the ability to compensate and maintain homeostasis declines. Older adults who experience a joint injury develop OA much more rapidly than younger adults with a similar injury[123]. Likewise, older adults who develop inflammatory arthritis, such as rheumatoid arthritis, exhibit more rapid joint destruction relative to younger adults[124]. The age-related changes in the chondrocyte and in the cartilage matrix described above likely result in a tissue that cannot adequately maintain homeostasis when stressed resulting in matrix destruction and loss.

Conclusions

As we learn more about the basic biology of aging and how aging affects joint tissues such as the articular cartilage, the links between aging and the development of OA are becoming more apparent. It is unlikely that OA is a direct consequence of aging joints but rather aging affects the ability of the articular cartilage, and likely other joint tissues as well, to maintain homeostasis when stressed. The aging chondrocyte's ability to produce and repair the extracellular matrix is compromised due to a decline in growth factor activity. This appears to be related to both a decline in the local availability of growth factors, including BMP-7 and TGF-β, as well as a decline in the chondrocyte's response to stimulation with growth factors such as IGF-I. The latter findings suggest that growth factor therapy, being developed as a way to stimulate cartilage matrix production and repair, may not work well in older adults.

Chondrocyte senescence is associated with an increased production of inflammatory mediators and matrix degrading enzymes characteristic of the senescent secretory phenotype. The aging cartilage matrix likely contributes to these changes in chondrocyte function and also contributes to a loss in homeostasis due to altered biomechanical properties. Age-related oxidative stress and damage may play a central role in cartilage aging through modulation of cell signaling pathways that regulate anabolic and catabolic activity. Although the use of general anti-oxidants as therapies for aging-related diseases has not met with much success to date, it is possible that modulating the activity of a specific set of redox-regulated pathways may be more efficacious. Further studies aimed at elucidating the mechanisms that contribute to chondrocyte and cartilage aging should uncover new ways to slow the aging process in joint tissues and postpone the development of OA.

Conflict of interest

None declared.

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