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Review article

Aging, articular cartilage chondrocyte senescence and osteoarthritis

James A. Martin & Joseph A. Buckwalter*
*Department of Orthopaedics, University of Iowa, Iowa City, IA 52242, USA; *Address for correspondence (01008 Pappajohn Pavilion, Department of Orthopaedics, University of Iowa College of Medicine, Iowa City, IA 52242, USA (e-mail: joseph-buckwalter@uiowa.edu; fax: +1-319-356-8999)*

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Abstract

The incidence of osteoarthritis (OA), the disease characterized by joint pain and loss of joint form and function due to articular cartilage degeneration, is directly correlated with age. The strong association between age and increasing incidence of osteoarthritis (OA) marks OA as an age related disease. Yet, like many other age related diseases, OA is not an inevitable consequence of aging; instead, aging increases the risk of OA. Articular cartilage aging changes that may lead to articular cartilage degeneration include fraying and softening of the articular surface, decreased size and aggregation of proteoglycan aggrecans and loss of matrix tensile strength and stiffness. These changes most likely are the result of an age related decrease in the ability of chondrocytes to maintain and repair the tissue manifested by decreased mitotic and synthetic activity, decreased responsiveness to anabolic growth factors and synthesis of smaller less uniform aggrecans and less functional link proteins. Our recent work suggests that progressive chondrocyte senescence marked by expression of the senescence associated enzyme beta-galactosidase, erosion of chondrocyte telomere length and mitochondrial degeneration due to oxidative damage causes the age related loss of chondrocyte function. New efforts to prevent the development and progression of OA might include strategies that slow the progression of chondrocyte senescence or replace senescent cells.

A wide variety of chronic human impairments and diseases first develop in middle age and then become more severe with time. As the population ages, the incidence, prevalence and impact of these impairments and diseases increase. Concern over the effects of aging on health has led physicians, scientists and the public to direct their attention toward improving understanding of age-related changes in tissues and organ systems and the role of these changes in causing disease and disability (Hayflick 1996; Clark 1999). As a result, dramatic progress has been made in defining age related alterations in the tissues that form brain, bone, heart, blood vessel and skeletal muscle and in developing approaches to decrease the adverse effects of these changes. Far less attention has been paid to the age related changes in articular cartilage that increase the risk of synovial joint degeneration, the pathological process that begins with loss of articular cartilage structure and function and that leads to the clinical syndrome of osteoarthritis (Buckwalter et al. 1993a, b, 1995, 1997a, b; Buckwalter et al. 2000). Yet, no disease is more closely correlated with advancing age than osteoarthritis (Buckwalter et al. 1995, 1997b; Buckwalter and Lappin 2000; Buckwalter et al. 2000, 2001a), few age related diseases impose a greater burden on the health care system (Praemer et al. 1999), and no disease causes loss of mobility for more people (Praemer et al. 1992, 1999).

Age and the incidence of osteoarthritis

After age 40, the incidence of osteoarthritis rises dramatically with every passing decade (Praemer et

al. 1999; Buckwalter et al. 2000, 2001a, b). The obvious mechanical functions of synovial joints and the strong correlations between a history of intense joint use and the prevalence of osteoarthritis (Buckwalter 1995; Buckwalter and Mankin 1997b) have led to the widespread concept that mechanical wear causes osteoarthritis; that is, with use joints wear out (Gorman 1996). This concept leads to the conclusions that development or progression of osteoarthritis cannot be prevented unless people restrict their activity to an unreasonable degree and that the best approaches to management of the disease are symptomatic treatments for patients with mild or moderate osteoarthritis and joint replacement for patients with severe disease. An alternative view is that normal joint use over a life time does not cause the joint degeneration responsible for osteoarthritis (Buckwalter and Mankin 1997b), instead aging increases the risk of osteoarthritis by compromising the ability of articular cartilage chondrocytes to maintain or restore the tissue (Buckwalter and Mankin 1997b; Buckwalter et al. 2000). The implications of this conclusion are that the aging changes responsible for the increased risk can be identified and that the potential exists to develop strategies that will decrease the risk or slow the progression of osteoarthritis. Determining which of these views is correct requires understanding of relationships between the articular cartilage degeneration that leads to osteoarthritis and articular cartilage aging.

Articular cartilage degeneration and osteoarthritis

One of the first events in articular cartilage degeneration is disruption or alteration of the molecular structure and composition of the matrix (Buckwalter and Mankin 1997b). Some of the early matrix changes include loss of proteoglycans and an increase in water concentration. The tissue damage stimulates a chondrocytic synthetic and proliferative response that may maintain or even restore the articular cartilage. This chondrocytic response may continue for years, however, in instances of progressive joint degeneration the chondrocytic anabolic response eventually declines and the imbalance between chondrocyte synthetic activity and degradative activity leads to progressive thinning of articular cartilage. Even in the early stages of joint degeneration the stiffness of the articular cartilage declines and its permeability increases (Buckwalter et al. 2000). These alterations may further accelerate the loss of articular cartilage.

Articular cartilage degeneration does not progress relentlessly in all cases. The response of the synovial joint to articular cartilage degeneration can, in some instances, restore a form of cartilaginous surface (Buckwalter et al. 2000, 2001a, b). It is not clear how frequently this occurs, but studies of small groups of patients confirm that even in individuals with complete loss of articular cartilage the potential exists for spontaneous restoration of a cartilaginous surface that functions effectively for years (Buckwalter et al. 2000, 2001a, b). Thus far the characteristics of patients in whom this response occurs have not been defined, but this phenomenon deserves further study.

Articular cartilage aging

Articular cartilage undergoes significant structural, matrix composition, and mechanical changes with age, but these changes differ in many respects from those seen in osteoarthritic joints (Roth et al. 1980; Buckwalter et al. 1993a, b; Bullough et al. 1993; Buckwalter et al. 1994; Mow et al. 1995; Buckwalter and Lane 1996; Buckwalter et al. 2000; Verzijl et al. 2000). Although the aging changes in the cartilage matrix are almost certainly closely related to progressive change in cell function, that is an age related decline in the ability of the cells to maintain the tissue, this relationship has not been clearly defined.

The most obvious structural change in articular cartilage with age is increasing articular surface fibrillation (Koepp et al. 1999; Buckwalter et al. 2000). This condition is more common in some joints than others, but appears to be a universal age related process. The majority of patients with articular surface fibrillation do not develop joint pain or dysfunction. Thus, age-related articular cartilage fibrillation does not necessarily lead to the progressive articular cartilage degeneration responsible for osteoarthritis.

The most striking articular cartilage matrix changes in with age are alterations in aggregating proteoglycans or aggrecans, molecules that consist of central protein cores with multiple covalently bound chondroitin and keratan sulfate chains and that give articular cartilage its stiffness to compression, resilience and durability (Buckwalter et al. 1982; Buckwalter and Rosenberg 1983; Buckwalter et al. 1985; Thonar et al. 1986; Buckwalter et al. 1994). The size of proteoglycan aggregates (molecules formed by the non-covalent association of multiple aggrecans with a hyaluronan filament) decreases significantly with age,

because the aggrecan molecules become shorter, as do their chondroitin sulfate chains, and because the mean number of aggrecans in each aggregate decreases (Figure 1). These age-related changes might be due to degradation of the proteoglycans in the matrix, alterations in proteoglycan synthesis, or both. Evidence from studies of bovine chondrocytes *in virto* suggests that there is an age-related change in aggrecan synthesis that leads to the production of shorter more variable aggrecan protein cores and chondroitin sulfate chains (Buckwalter et al. 1985; Thonar et al. 1986a, b; Buckwalter et al. 1994). Likewise, *in virto* experiments show that aggregates assembled in cultures of older chondrocytes are smaller and more irregular than those assembled in cultures of younger chondrocytes (Buckwalter et al. 1994). Some of these changes in aggregates may be caused by age related alterations in link proteins (the small proteins that stabilize the association between aggrecans and hyaluronan) (Buckwalter et al. 1986; Buckwalter and Rosenberg 1988; Buckwalter et al. 1994; Tang et al. 1996). Other age related changes in the molecular composition and structure of the articular cartilage matrix include increased collagen cross-linking and decreased water concentration (Buckwalter et al. 1993a, b; DeGroot et al. 1999; Verzijl et al. 2000).

Presumably as a result of changes in the molecular composition and organization of the matrix, the mechanical properties of articular cartilage deteriorate with age (Kempson 1980, 1982, 1991; Roth et al. 1980; Mow et al. 1995). The best documented changes include declines in tensile stiffness and strength that may make the tissue more vulnerable to injury and development of progressive degeneration.

Recent work has shown that in addition to the structural, matrix composition and mechanical changes in articular cartilage with age, articular cartilage cell function also changes. The mitotic and synthetic activity of human articular cartilage chondrocytes decline with age (Bolton et al. 1999; Martin and Buckwalter 2001), and rat articular cartilage chondrocytes show an age related decline in anabolic response to insulin-like growth factor I (IGF-I) (Martin et al. 1997; Martin and Buckwalter 2000). IGF-I appears to be an important anabolic factor in articular cartilage and thus presumably has a critical role in stimulating the chondrocyte synthetic activity that preserves articular cartilage (Martin and Buckwalter 2000). The age related decline in the anabolic response of articular cartilage chondrocytes to IGF-I may be associated with increased expression of insulin

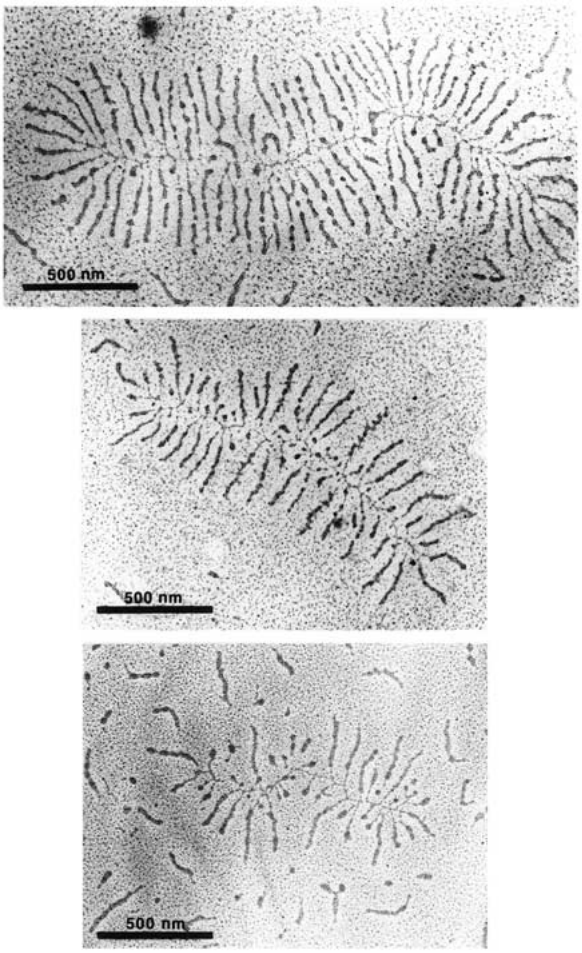

Figure 1. Age related changes in proteoglycans. Electron micrographs of bovine cartilage proteoglycan aggregates. Top: Fetal proteoglycan aggregate. Middle: Proteoglycan aggregate from skeletally immature animal. Bottom: Proteoglycan from skeletally mature animal. Aggregates consist of a central hyaluronan filament with multiple attached aggrecans. The aggrecans as demonstrated in these electron micrographs consist of protein cores with collapsed glycosaminoglycan chains covering most of the protein core. Notice that with increasing age the hyaluronan filaments and aggrecans shorten and the length of the aggrecans varies more. Measurement of molecules from animals and humans of different ages confirms these observations (Buckwalter et al. 1994).

growth factor binding proteins (Martin et al. 1997). An age related increase in the production of these binding proteins could decrease the ability of the chondrocytes to maintain or repair the articular cartilage matrix.

If articular cartilage aging and the articular cartilage degeneration responsible for osteoarthritis are distinct processes, how can the striking increase in the incidence of articular cartilage degeneration with age be explained? The answer appears to be

that the structural, molecular, cellular and mechanical aging changes in articular cartilage increase the vulnerability of the tissue to degeneration. Furthermore, the evidence that articular cartilage chondrocytes synthesize smaller more irregular aggrecans and are less responsive to anabolic cytokines with increasing age suggests that older articular cartilage is less able to repair and restore itself (Martin et al. 1997; Martin and Buckwalter 2000). Studies of the risk of osteoarthritis following joint injuries support this concept. They show that the risk of developing post-traumatic osteoarthritis following an intra-articular fracture of knee increases as much as three to four fold after 50 years of age (Volpin et al. 1990; Honkonen 1995). Thus, articular cartilage aging does not cause osteoarthritis, but the age-related metabolic and phenotypic decline of the chondrocytes increases the risk of articular cartilage degeneration and limits the ability of the cells to repair the tissue once degenerative changes occur. However, identifying a possible relationship between aging changes in chondrocyte function and the risk of developing osteoarthritis does not explain why the aging changes occur. Lack of such an explanation makes it difficult to develop new strategies for preventing the development of osteoarthritis or slowing its progression.

Chondrocyte senescence

The causes of aging changes in cells remain controversial, but it is increasingly clear that these changes culminate in cell replicative senescence, a state of irreversible cell cycle arrest. Although loss of the ability of cells to divide is an accepted measure of senescence, cell function may begin to deteriorate before cells reach cell cycle arrest. Abnormal protein synthesis, altered growth factor responses, and longer population doubling times, appear well in advance of outright growth arrest in continuously dividing somatic cell populations (Campisi 1999). This suggests that cell populations begin to drift toward senescence relatively early in their replicative lifespans and that this loss of cell function might contribute to aging changes in tissue structure and function.

The possibility that chondrocyte begin to lose their ability to maintain articular cartilage well before they reach replicative senesence is consistent with our recent finding that senescent chondrocytes accumulate with age in articular cartilage (Martin and Buckwalter 2001), and with the correlation between increasing age and incidence of osteoarthritis. If chondrocyte senescence has an important role in the development and progression of osteoarthitis, understanding of the mechanisms of chondrocyte senescence will be important in devising new approaches to the prevention and treatment of this disease. A number of hypotheses have been proposed to explain how cells become senescent with age, however, most current studies focus on two fundamentally different mechanisms: replicative exhaustion marked by telomere erosion and oxidative damage.

Telmomere erosion

The telomere erosion hypothesis suggests that cell aging is regulated by an intrinsic genetic ‘clock’ associated with erosion of telomeres (Blackburn 1991; Campisi 1999). Telomeres, DNA sequences at the ends of chromosomes, are necessary for chromosomal replication but they also may help maintain normal chromosome function by preventing enzymatic degradation and clumping of chromosomes. The growth of most somatic cell populations is sharply limited by telomere erosion, which proceeds in increments with each round of cell division. A long history of vigorous mitotic activity in a cell population results in telomere exhaustion, a form of DNA damage that induces senescence (Allsopp et al. 1992, 1995; Allsopp and Harley 1995).

Chromosomes from young, normal somatic cells have relatively long telomeres of $>$ 9 kilobase pairs (kbp) but these are eroded at the rate of 100–200 base pairs (bp) with each cell division cycle. Erosion beyond the minimum length necessary for DNA replication (5–7.6 kbp) results in cell cycle arrest, a condition referred to as replicative senescence. Most cell types reach cell cycle arrest after a characteristic number of population doublings. This fundamental barrier to unbridled growth, the Hayflick limit (Hayflick and Morehead 1961; Hayflick 1965, 1996), is common to somatic cells that lack telomerase, an enzyme responsible for replacing telomere sequences. The Hayflick limit for human fibroblasts has been estimated at $\sim$60 population doublings while the estimated limit for human chondrocytes is $\sim$35 doublings. In contrast, many cancer cell lines, in which the ‘telomerase’ enzyme is active, are virtually immortal. In telomerase-negative cells, telomere length can be viewed as a cumulative history of preceding cell division as well as a predictor of future capacity to divide.

The role of chondrocyte turnover in cartilage aging and disease has not been systematically studied due in part to the difficulty of assessing the *in vivo* replicative history of chondrocytes. Terminal restriction fragment length analysis of telomeres offers a simple means to overcome this problem as cell-turnover should be detectable as an age-related decline in average telomere length. If telomere erosion causes senescence, telomere length should correlate with phenotypic measures of senescence. Based on these rationales we hypothesized that telomere length in human articular cartilage chondrocytes declines as a function of age as phenotypic measures of senescence (loss of DNA synthetic activity and increasing expression of senescence-associated beta-galactosidase activity) increase.

To test this hypothesis, we measured senescence markers in human articular cartilage chondrocytes from 27 donors ranging in age from one to 87 years (Martin and Buckwalter 2001). The markers included senescence-associated beta-galactosidase (SA β-gal) activity (Dimri et al. 1995), mitotic activity measured by ^{3}H-thymidine incorporation, and telomere length (Counter et al. 1992). Beta-galactosidase expression increased with age (r = 0.84, P = 0.0001) and mitotic activity declined (r = –0.77, P = 0.001) (Figure 2A) indicating that senescent chondrocytes accumulate with cartilage age. Mean telomere length (MTL) also declined with age (r = –0.71, P = 0.0004) (Figure 2B), suggesting that age-related telomere erosion leads to chondrocyte senescence.

The relevance of loss of telomere length to an *in vivo* age related decline in chondrocyte function rests on proof that *in vivo* chondrocyte turnover rates are sufficient to cause telomere erosion. Short term DNA labeling studies indicate that chondrocyte mitoses are relatively rare in normal cartilage. Although this apparent rate of turnover is too slow to result in significant telomere erosion over the short term, decades of turnover might well be sufficient. Furthermore, mitotic activity increases several-fold following cartilage injury (Buckwalter and Mankin 1997; Buckwalter et al. 2001a), therefore repetitive joint injury could accelerate telomere erosion. Increased mitotic activity during cartilage degeneration may also speed up the accumulation of senescent, growth-arrested chondrocytes in end-stage osteoarthritis. These observations suggest that, in many cases, *in vivo* chondrocyte turnover is sufficient to result in biologically significant telomere erosion.

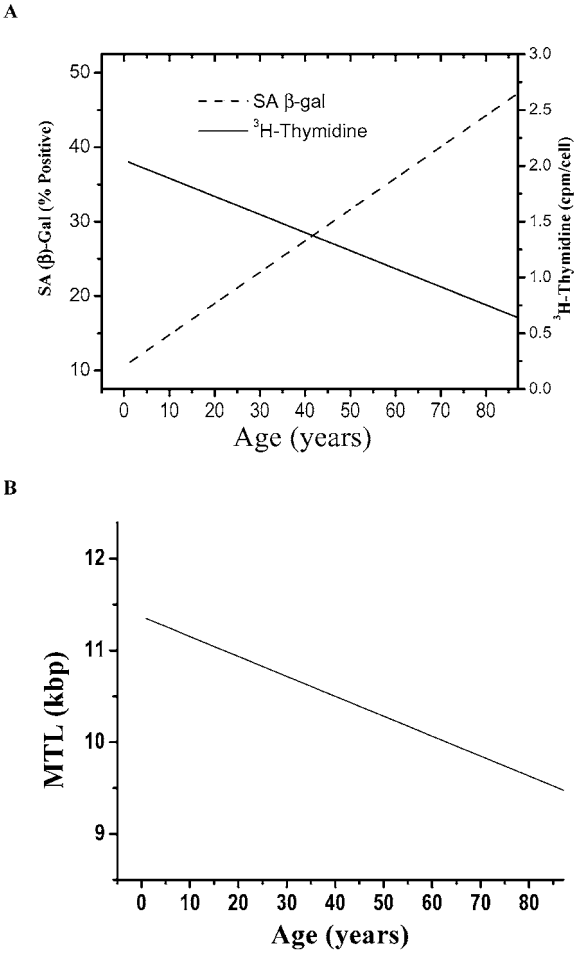

Figure 2. Aging, senescecence, and telomere erosion in articular chondrocytes. Results of linear regression analysis of chondrocytes from donors of various ages (1–87 years). (A) senescence-associated β-galactosidase activity (% positive staining cells) and ^{3}H-thymidine uptake over (cpm/cell/12 hours). (B) Mean telomere length in chondrocytes from the same set of donors shown in A.

Oxidative damage

Although the evidence for an association between telomere erosion and chondrocyte senescence is strong, cumulative cell damage from oxidative stress provides an alternative explanation for chondrocyte senescence (Dumont et al. 2000; Toussaint et al. 2000). Stress-induced senescence is manifested in age-related degeneration of mitochondria (Golden and Melov 2001). Mitochondria provide metabolic energy in the form of ATP and NADH via respiration, supporting the anabolic and catabolic activities that cells require for tissue maintenance. Respiration uses

oxygen as a terminal electron acceptor and mitochondria also serve the essential function of protecting cells from the toxic effects of oxygen and its free-radical derivitives. Thus, mitochondrial damage both limits energy production and exposes cells to oxidative damage. Additional downstream effects of mitochondrial damage include increased reliance on glycolysis which restricts RNA and DNA synthesis. Numerous studies have shown age-related declines in the respiratory activity of tissues such as bone, skeletal muscle, liver, and brain (Arnheim and Cortopassi 1992). The decline is due to progressive age-dependent decrease in the number and activity of mitochondria (Beckman and Ames 1997, 1999; Kopsidas et al. 1998). This phenomenon is related to alterations in the mitochondrial genome. Deletions, duplications and point mutations that disrupt expression of electron transport complex proteins accumulate with age (Arnheim and Cortopassi 1992; Lee et al. 1997; Kang et al. 1998). These events lead to increasing free radical production as a consequence of faulty electron transport. Oxidatively damaged mitochondria can initiate apoptosis through caspase activation but may also lead to a state of irreversible growth arrest that is superficially similar to replicative senescence (Toussaint et al. 2000).

Studies in our laboratory with adult human articular cartilage chondrocytes suggest that mitochondrial degeneration is associated with *in virto* chondrocyte senescence. Flow cytometric analysis using mitochondria-specific stains showed a decline in numbers of mitochondria (10-nonyl acridine orange stain) and mitochondrial membrane potential ($DiOC_6$ stain) with long-term *in virto* growth (Korchak et al. 1982; Maftah et al. 1990). Using long-distance PCR (Kovalenko et al. 1997) we also found an increase in DNA rearrangements that culminated in loss of the full-length mitochondrial genome as cells approached growth arrest (Figure 3). Although it remains to be seen if mitochondrial degeneration increases with aging *in vivo*, our data suggest that functional lifespan of chondrocytes may be limited by mitochondrial aging. Additional support for this hypothesis comes from studies which show that chondrocytes produce potentially damaging levels of oxygen-free radicals in response to inflammatory cytokines (Oh et al. 1998). Moreover, we have shown that free radical production in cartilage explants is stimulated by physiologic mechanical stress. These findings suggest that environmental stress plays a role in cartilage aging by contributing to free-radical production that could accelerate mitochondrial degeneration.

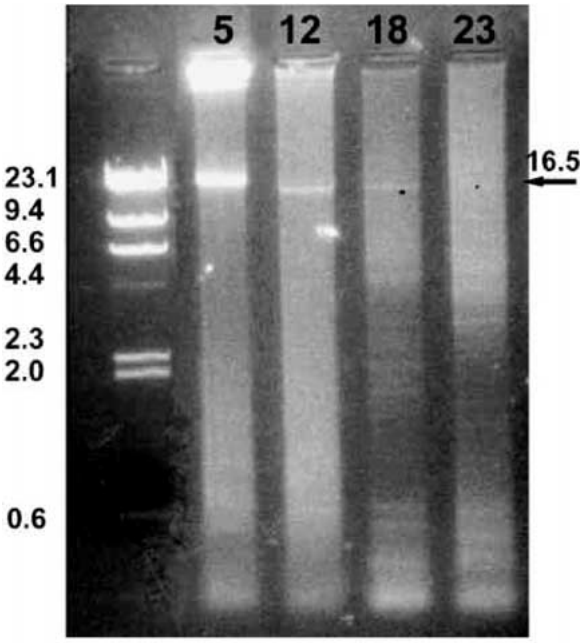

Figure 3. Mitochondrial genome degeneration in serially passaged chondrocytes. Ethidium bromide-stained agarose gel showing the products of long-distance polymerase chain reaction amplification of the mitochondrial genome at 5, 12, 18, and 23 population doublings. The position of the full-length mitochondrial genome (16.5 kbp) is indicated. DNA molecular weight standards (lamda HindIII) are shown on the left.

The role of mitochondria in cartilage degeneration remains unclear, however, the hypothesis that mitochondrial degeneration contributes to this process is well supported by genetic studies which link premature aging syndromes with heritable mutations in the mitochondrial genome (Wallace 2001).

Conclusions

Age dependent changes in articular cartilage increase the risk of the synovial joint degeneration that causes the clinical syndrome of osteoarthritis. In addition, these changes may adversely affect the outcomes of attempts to repair or regenerate articular cartilage. Our data strongly suggest that chondrocyte senescence contributes to the risk of cartilage degeneration by decreasing the ability of the cells to maintain and repair the tissue, and that the most likely mechanisms of chondrocyte senescence are telomere erosion and oxidative damage. Some of the variability in the risk of developing osteoarthritis among individuals may be due to differences in onset of chondrocyte senescence,

possibly related to genetically determined differences or to differences in exposure to environmental factors, such as oxidative stress and joint mechanical injuries that increase the rate of chondrocyte turnover. Is it possible to slow, or temporarily reverse at least some of the age-related changes in articular cartilage that increase the probability of joint degeneration? Once degenerative changes have developed, can they be stabilized or reversed? Will it be possible to develop methods that predictably produce functional durable cartilagenous articular surfaces for middle age and older people with joint injuries and joint degeneration? Further study will answer these questions. However, the observations developed thus far strongly suggest that better understanding of the aging changes in articular cartilage and how these changes influence the ability of the tissue to maintain and regenerate itself will lead to improved methods of preserving and restoring articular surfaces for middle-aged and older individuals. In particular, new efforts to prevent the development or progression of OA might include strategies that delay the onset of chondrocyte senescence or replace senescent cells.

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