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An overview of underlying causes and animal models for the study of age-related degenerative disorders of the spine and synovial joints

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Abstract

As human lifespan increases so does the incidence of age-associated degenerative joint diseases, resulting in significant negative socioeconomic consequences. Osteoarthritis (OA) and intervertebral disc degeneration (IDD) are the most common underlying causes of joint-related chronic disability and debilitating pain in the elderly. Current treatment methods are generally not effective and involve either symptomatic relief with non-steroidal anti-inflammatory drugs and physical therapy or surgery when conservative treatments fail. The limitation in treatment options is due to our incomplete knowledge of the molecular mechanism of degeneration of articular cartilage and disc tissue. Basic understanding of the age-related changes in joint tissue is thus needed to combat the adverse effects of aging on joint health. Aging is caused at least in part by time-dependent accumulation of damaged organelles and macromolecules, leading to cell death and senescence and the eventual loss of multipotent stem cells and tissue regenerative capacity. Studies over the past decades have uncovered a number of important molecular and cellular changes in joint tissues with age. However, the precise causes of damage, cellular targets of damage, and cellular responses to damage remain poorly understood. The objectives of this review are (1) to provide an overview of the current knowledge about the sources of endogenous and exogenous damaging agents and how they contribute to age-dependent degenerative joint disease, and (2) highlight animal models of accelerated aging that could potentially be useful for identifying causes of and therapies for degenerative joint diseases.

Keywords

Synovial joints; intervertebral disc; DNA repair; aging; oxidative damage

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Introduction

Joints are anatomical structures that are essential for mobility and flexibility. Diseases of the joint therefore lead to reduced mobility and chronic pain [1]. Degenerative changes of the joint result in osteoarthritis, spinal stenosis and disabling chronic pain, and the prevalance of these diseases is increasing as humans live longer [2]. Although the etiology of joint degeneration is complex and multifactorial, aging is clearly the number one risk factor for the initiation and progression of degenerative joint diseases [3, 4]. Together OA and IDD are the most common cause of chronic disability in the elderly [5]. However, currently treatment is largely limited to symptomatic relief with non-steroidal anti-inflammatories and surgical joint replacement because the molecular mechanisms of degeneration of articular cartilage and intervertebral discs are still not completely understood. Given that the fraction of the U.S. population >65 years of age is anticipated to double in the next 25 years [6], it is imperative to identify the causes of joint degeneration. This would enable more targeted and less invasive therapies aimed at keeping the elderly mobile and functional.

With aging there is an inevitable loss of tissue homeostasis leading to impaired ability of that tissue to respond to stress [7]. With time, damage occurs to cells, organelles and macromolecules, leading to cell death and senescence. The types of damage that are implicated in driving aging include accumulation of damaged proteins, mitochondrial damage and dysfunction, telomere shortening, DNA damage, attrition of quality control mechanisms (autophagy, DNA repair, etc.), and the loss of multipotent stem cells and tissue regenerative capacity [8–10]. The most abundant source of endogenous damage is reactive oxygen species derived from mitochondria or inflammatory cells [11, 12]. Remarkably, very little is known about the contribution of these factors to degenerative joint disease. The purpose of this review is to provide a current overview of what is known about aged joints and highlight animal models of accelerated aging that are anticipated to be of value for identifying causes and therapies for degenerative joint diseases.

Aging Characteristics of Intervertebral Discs

Situated between the bony vertebrae, intervertebral discs (IVDs) are spinal joints that function primarily to provide support and flexibility to the otherwise rigid vertebral column. Young healthy IVDs consist of the fibrous annulus fibrosus (AF) surrounding the central gelatinous nucleus pulposus (NP). AF tissue, composed of highly organized lamellae or sheets of collagen fibrils, functions mainly to bear tensile forces generated during bending or twisting. NP tissue, on the other hand, serves to counteract compressive loads and is made up of loosely organized networks of collagen type II and elastin fibers that enclose a gel-like substance of proteoglycan and water [13]. IVDs consist of mostly extracellular matrix (ECM) sparsely populated with fibrochondrocytes in AF tissue and chondrocyte-like and notochordal cells in the NP. Mostly avascular, IVDs exchange nutrient and waste products with its surrounding environment primarily by diffusion via a network of vascular channels perforating the osseous vertebral end plate [14].

The disc appears to undergo age-related degenerative changes earlier in life than other tissue[15]. Aging-relate changes include increased number and size of fissures, the presence of granular debris, and neovascularization from the outer aspect of the anulus inwards [15]. Age also closely correlates with the progressive loss of disc water and matrix proteoglycan [13]. In particular the NP becomes more fibrous as the PG content diminishes, leading to cracks and fissures. Ossification and thinning of the cartilaginous end plate, microfractures in the adjacent subchondral bone, and bone sclerosis, are also found with increasing age [16].

Analysis of cadaveric human vertebrae revealed a drastic reduction in the number of vascular channels in the vertebral end plate between individuals six and thirty months of age [15]. Because disc physiological vacularity virtually disappears by the age of 16[15], this likely contributes to a reduction in nutrient supply to the disc, accumulation of cellular waste products, and increasingly acidic environment (pH 6.3–6.6) that severely compromises cell function or causes cell senescence and death [17]. With aging, there is also a loss of disc matrix proteoglycan proteins and accumulation of degraded matrix molecules. Loss of disc matrix proteoglycans inevitably leads to loss of hydration, resulting in altered biomechanics and pathologic outcomes such as spine stiffness, spinal stenosis, and disabling chronic back pain [13, 18]. Identifying the mechanisms that drive the loss of endplate vasculature and IVD matrix with age will provide new opportunities for preventing, delaying or ameliorating IDD.

Aging Characteristics of Articular Cartilage

Synovial joints consist of two or more bones covered with hyaline cartilage and the synovial membrane that contains the cells that secrete synovial fluid. There is usually a plate of strong subchondral bone immediately underneath to the cartilage lining. A fibrous joint capsule surrounds the ends of the bones in the joint and contains the synovial fluid-filled cavity. This organ is comprised of specialized tissues that enable a frictionless environment essential for movement and transfer of load from one bone to another. Compared to normal healthy disc tissues which are avascular except for the outermost annulus fibrosus, articular cartilage in synovial joints is much more vascular.

Osteoarthritis (OA) is the most common form of arthritis that is characterized by loss of joint form and function due to progressive articular cartilage degeneration. Articular cartilage is mostly composed of water and ECM proteins, with a sparsely distributed population of highly specialized chondrocytes embedded within a matrix that make up less than 2% of the volume of the tissue. The major matrix proteins in cartilage are the proteoglycans and collagen. Aggrecan is the key proteoglycan responsible for the resiliency of the tissue, while type II collagen provides tensile strength [19]. Structural changes of the joint caused by OA are visible on standard x-rays include narrowing of the joint space due to cartilage loss, formation of osteophytes at the joint margins, and bony sclerosis or increased density or thickness of the bone just underneath the articular cartilage.

Other aging-related changes in articular cartilage include fibrillation of the articular surface, decrease in the size and aggregation of aggrecans and increased collagen cross-linking [20]. These lead to the loss of tensile strength and joint stiffness. Aggrecan complexes shrink with age and are structurally altered due to proteolytic modification of the core protein as well as changes in the length and abundance of glycosaminoglycan side-chains [21]. These changes are most likely the consequence of altered chondrocyte function. Aged chondrocytes characteristically have decreased ECM protein synthesis and reduced responsiveness to anabolic growth factors; they also produce less link proteins, smaller and less uniform aggrecan [20, 21]. Recent work suggests that there is progressive senescence of articular cartilage chondrocytes, as evident by increased expression of the cell senescence markers P16/INK4A and senescence-associated beta-galactosidase (SA-beta-gal) and decreased telomere length with age [22, 23].

Aging and oxidative stress as an etiologic factor of joint diseases

Aging is caused at least in part by the time-dependent accumulation of cellular and molecular damage leading to a progressive decline in functional reserve [8, 24]. Traditionally it was assumed that continuous mechanical wear and tear is the primary basis of joint damage. However, there is growing evidence for oxidative damage, a known driver of cell

senescence, in aged disc and articular cartilage, as a principal driving force of joint degeneration [25, 26]. Immunomorphological analysis revealed a higher level of carboxymethyl-lysine (CML; a biomarker of oxidized protein) in degenerated intervertebral discs from aged patients compared to young normal discs [27]. Similarly, NF-κB, a transcription factor activated in response to cellular stress, including oxidative stress, is increased in old discs [27]. The source of reactive oxygen species (ROS) driving oxidative damage includes free radicals generated from radiation, by-products of oxidative phosphorylation, as well as from cellular response to pro-inflammatory cytokines [28, 29]. ROS are produced by chondrocytes in response to stimulation by numerous cytokines and growth factors, including IL-1, TNF-α, TGF-β, as well as by integrin stimulation with fibronectin fragments [30, 31]. IL-1-dependent production of ROS is implicated in causing DNA damage in chondrocytes [32]. Although cells in articular cartilage and disc tissue reside in an environment with a low oxygen tension, they are aerobic and utilize oxidative phosphorylation. In addition, aged disc and articular cartilage tissue accumulate fissures, resulting in neovascularization and exposure of the otherwise hypoxic resident cells to higher oxygen tension and oxidative stress [33]. Compared to young rats, old rats have increased levels of intracellular ROS in their cartilage [26]. Age-related oxidative stress was also reported to make human and rat chondrocytes more susceptible to cell death [34]. Recently, dysfunction of chondrocyte mitochondria, the primary site of ROS production from cellular aerobic metabolism, was implicated in the establishment and progression of OA [35].

Increased levels of ROS can contribute to aging changes in cells and tissues by damaging proteins, lipids, and DNA. One marker of protein oxidation is nitrotyrosine. Nitrotyrosine is formed by the reaction of protein tyrosine residues with peroxynitrite (ONOO–) [36]. Peroxynitrite (ONOO2) is a potent damaging nitrating and oxidizing agent produced *in vivo* that is likely to account for much of the cytotoxicity commonly attributed to nitric oxide as it is formed by a rapid reaction of nitric oxide (NO) with oxygen radical superoxide (O2•−). Nitrotyrosine levels are elevated in aged human and monkey cartilage compared to young and in cartilage from OA patients [37]. Increased nitrotyrosine levels in chondrocytes correlate with a reduced anabolic response to IGF-I stimulation[37]. This suggests that oxidative damage may contribute to impaired response of cartilage to growth factor stimulation. Insulin-like growth factor (IGF-1) is critical for the normal development and growth of cartilage in childhood and the maintenance of cartilage in adults. In healthy cartilage, IGF-I induces expression of collagen type II and proteoglycan core protein [38]. However, chondrocytes in arthritic cartilage of humans and animals have decreased anabolic responses to IGF-1 [37, 39]. Consistent with these observations is the fact that excess NO reduces chondrocyte responsiveness to IGF-I and that treating chondrocytes with the oxidant H_2O_2 inhibits proteoglycan synthesis in vitro [40].

Perhaps the most convincing evidence of age-associated oxidative damage in disc and articular cartilage is the accumulation of advanced glycation end products (AGEs), produced by nonenzymatic glucosylation and oxidation of proteins and lipids [41, 42]. The long half-life of collagen (>100 years for collagen type II) [43] makes it particularly susceptible to progressive accumulation of AGEs. The best characterized AGEs in cartilage and disc are pentosidine and carboxymethyllysine. The former is found in collagen and directly increases with donor age in human cartilage. Pentosidine, which cross-links collagen molecules, might play an important role in increased collagen stiffness and the weakening of cartilage biomechanics with old age [41]. Pentosidine formation in cartilage is also associated with altered or reduced chondrocyte anabolic activity such as synthesis of matrix collagen and proteoglycans [44]. Hence AGE accumulation might affect chondrocyte function in addition to cartilage biomechanics. Correlation between increased OA severity and cartilage AGE

levels might provide the first *in vivo* evidence for a molecular mechanism by which aging may predispose an individual to OA [45].

Animal models of OA and IDD

Animal models of human diseases are vital for research aimed at identifying disease mechanism and therapeutic targets for disease prevention and treatment. Although spontaneous models of IDD and OA such as the Hartley guinea pig exist[46, 47], the classic models of OA and IDD have generally involved surgical manipulation to create injury-induced OA- and IDD-like degenerative changes in rats, rabbits, dogs, and sheep [3, 48]. For example the rabbit model of annular puncture-induced IDD is well established [49]. Transgenic and knockout mice with defective expression of matrix metalloproteinases (MMPs), ECM proteins, transcription factors, and angiogenic factors have been recently created to investigate the mechanisms that control cartilage development and joint pathology [3, 50, 51]. The current mouse models of OA and IDD are summarized in Tables 1 and 2, respectively (See Supplementary Material). Mice harboring deletion of the anti-catabolic gene *Timp3* or postnatal overexpression of constitutively active MMP13 exhibit OA-like pathology [52, 53]. Developmental defects or mutations in a number of cartilage matrix genes (aggrecan, biglycan, fibromodulin, matrilin-3, *Col2a1*, *Col19a1, Col11a1*) result in degenerative cartilage changes in adult mice [54, 55]. For example, premature onset of OA was reported in the Disproportionate micromelia (Dmm) mouse, which has a deletion of C-propeptide encoding a region of *Col2a1*, and resulting in defective cartilage matrix [56]. In addition, several well-defined genetic defects have been reported to show premature onset of OA. Deficiency in the alpha1 integrin subunit is associated with early deregulation of cartilage homeostasis and accelerated development of OA [57]. Accelerated development of aging-associated OA is observed in mice deficient in osteopontin, a noncollagenous ECM protein that interacts with integrin receptors to promote cell adhesion, chemotaxis and signal transduction [58]. Thus, disruption of genes involved in the synthesis or remodeling of the cartilage matrix generally leads to degenerative joint diseases. This illustrates the importance of ECM homeostasis in maintaining joint integrity and function, but does not reveal the mechanism behind loss of matrix homeostasis in OA.

Accelerated aging animal models to study bone and joint aging

In orthopaedics, aging-related diseases include IDD, OA, osteoporosis, and poor healing of bone fractures. Therefore, studying these diseases in aged organisms is extremely important to accurately identify mechanisms of pathogenesis and the impact of old age on treatment modalities. Unfortunately, this is implausible due to the time and cost needed for aging studies even in mice (3 years). Hence models of accelerated aging offer another unique tool for studying aging-related joint and bone disease. Recently, numerous mouse models of accelerated aging have been developed. Many of these mimic human progerias, or diseases of accelerated aging, and include spontaneous and premature onset of aging-related bone and joint disease (Table 3 in Supplementary Material). Hence, the mice are predicted to be useful for rapidly testing hypotheses about causes and treatments of aging-related orthopaedic complications and to have translational relevance.

Role of DNA damage in aging and Ercc1 model of accelerated aging for orthopaedic research

Damaged DNA, unlike proteins or other macromolecules which are degraded and replaced, must be repaired in order to maintain normal cellular function. Each cell in an organism is subjected to tens of thousands of DNA lesions every day due to the inherent chemical instability of DNA, metabolic byproducts that can covalently modify DNA, and environmental exposure to tobacco smoke, UV light, radiation and so forth [8]. As a consequence, all organisms have evolved elaborate mechanisms to repair DNA damage. But

these are likely imperfect over the lifetime of complex organisms, leading to time-dependent accumulation of DNA damage. DNA damage is hypothesized to be a major driving force behind aging based on the fact that inherited defects in DNA repair invariably lead to accelerated aging of one or more organ systems [9].

Werner syndrome is a classic accelerated aging syndrome in which patients succumb to cancer or cardiovascular disease by the 4th decade of life. Werner syndrome is caused by inherited defects in WRN protein required for telomere maintenance and replicating damage genomes. Mouse models of Werner syndrome have osteoporosis and pathological fractures by 8 months of age [59]. Mouse models of XFE progeroid syndrome, caused by reduced expression of XPF-ERCC1 DNA repair nuclease, spontaneously develop osteoporosis and IDD within the first 6 months of life [60]. Mice expressing mutant p53, a key regulator of the cellular response to genotoxic stress, exhibit premature aging and significant osteopenia, causing suppression of both bone formation and resorption [61]. A knockout mouse for the ataxia telangiectasia mutated (*Atm*) gene is also recognized as a model of premature aging, and shows severe osteopenia and decreased bone formation [62]. These models strongly support the conclusion that DNA damage, if not repaired, promotes bone and joint degeneration.

Hutchinson-Gilford progeria syndrome (HGPS) is a systemic disease of dramatically accelerated aging, caused by mutation in lamin A, a nuclear structural protein. Both HGPS patients and mouse models of the disease have significantly reduced bone density, kyphosis and pathologic fractures [63]. The classic model of accelerated senescence-prone (SAMP) mice is an inbred strain of animals that exhibit shorter lifespan and an earlier onset of age-associated pathological phenotypes similar to several geriatric disorders observed in humans, including degenerative joint disease [64]. Klotho mice, which are produced by inactivation of the KL gene encoding a type-I membrane protein that is related to β-glucuronidases, exhibit a variety of age-related degenerative diseases including arteriosclerosis, skin atrophy, osteoporosis, osteopenia and emphysema [65]. Klotho mice represent the first established mouse aging model in which the defective gene encodes an extracellular protein rather than a nuclear molecule [65]. Trichothiodystrophy is caused by inherited mutations in genes that encode proteins required for nucleotide excision repair, and is characterized by accelerated aging of the nervous and skeletal systems. Mice that model trichothiodystrophy spontaneously develop kyphosis and osteoporosis within one year of age [66]. Interestingly, the severity of age-related changes in the cartilage of the premature aging trichothiodystrophy (TTD) mice were reported to be no greater than those observed in wild-type littermates, in striking contrast with bone and many other tissues [67]. The authors suggested that difference in vasculature and thereby oxygen tension in cartilage and bone might explain such segmental aging characteristics.

Accelerated aging animal models to study intervertebral disc aging

Much progress has been made in cartilage and disc research using animal models. However, there are currently few rapid models of age-related IDD to investigate how aging brings about degenerative changes in discs. The sand rat and chondrodystrophoid dog represent spontaneous models of IDD, but have undefined genetic causes [68]. Thus the availability of small economical animal models of age-associated IDD would accelerate the development of effective therapeutics to improve the health of discs in aged individuals. Niedernhofer and coworkers created a mouse strain modeled after human progeria by genetically deleting the gene *Ercc1*[69]. Loss or reduced expression of XPF-ERCC1 DNA repair endonuclease leads to accelerated aging of the epidermal, nervous, hepatobiliary, endocrine, immunologic, renal, hematopoietic and musculoskeletal systems in humans and mice [60, 69]. Mice expressing ~10% of the normal complement of this this nuclease have a maximum lifespan of ~7 months [70] compared to their wild-type counterparts, which have a maximum lifespan

of >3 years. These features make the ERCC1-deficient mice strain attractive for studying OA, IDD and osteoporosis within the context of an aged organism, rather than in the context of a juvenile organism as is often the case with genetic disease models.

Indeed ERCC1-deficient mice were recently demonstrated to have age-related IDD, including loss of disc height and degenerative structural changes in their vertebral bodies similar to those reported for old rodents [60]. Compared to their wild-type littermates, ERCC1-deficient mice exhibit premature loss of disc PG, reduced matrix PG synthesis, and enhanced apoptosis and cell senescence [60]. These mice also exhibit early onset of osteoporosis. ERCC1-deficient mice therefore offer a rapid and accurate model of spontaneous age-related orthopaedic complications and are anticipated to be useful for probing the molecular basis of joint and bone aging, especially the phenomenon of DNA damage-induced cell senescence, as well as testing therapeutics.

Concluding remarks

Chronic pain and disability due to IDD and OA are the most common age-associated conditions of humans, especially in the developed countries where the aging population is rapidly expanding. Aging was traditionally assumed to contribute to the development of joint degeneration through sequential events of mechanical wear and tear affecting the matrix. Emerging evidence, however, suggest a mechanism in which oxidative damage-induced cellular senescence is the primary driver of age-related joint degeneration. According to this new model, joint tissue accumulates molecular damage over time from continually exposure to the stresses of living activities (mechanical loads, inflammation, smoking, endogenous oxidative stress, etc.), which eventually leads to tissue dysfunction. Senescent cells are compromised in their capacity for matrix repair and synthesis, and thus tissue homeostasis is lost and tipped toward further matrix catabolism by any additional assaults. To date, it is still unclear what drives chondrocyte senescence.

This new model of joint aging predicts that the major determinant of joint degeneration is the level of cellular senescence. With this new knowledge, Martin and Buckwalter [20] astutely pointed out that “whatever treatment we envisage for osteoarthritis, we must take into account that we are dealing with aged/(pre)senescent cells with accumulated DNA damage and hence altered gene expression program that no longer have the ability of their juvenile counterparts to counteract the many mechanical, inflammatory, oxidative, and other assaults to the tissue”. Nevertheless, many outstanding questions remain. It is not clear how various stressors, i.e., mechanical damage, oxidative DNA damage, inflammatory stress, smoking, etc. drive cellular senescence in joint and bone tissues. Nor is the mechanism by which senescent cells drive disc and bone degeneration known. Using accelerated aging models will hasten research. Likewise, using models in which the primary insult is well-defined (e.g., unrepaired DNA damage) will help focus mechanistic hypotheses. Investigating these questions, will yield numerous novel targets for therapeutics aimed at delaying the onset or attenuating disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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