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

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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.

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.

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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

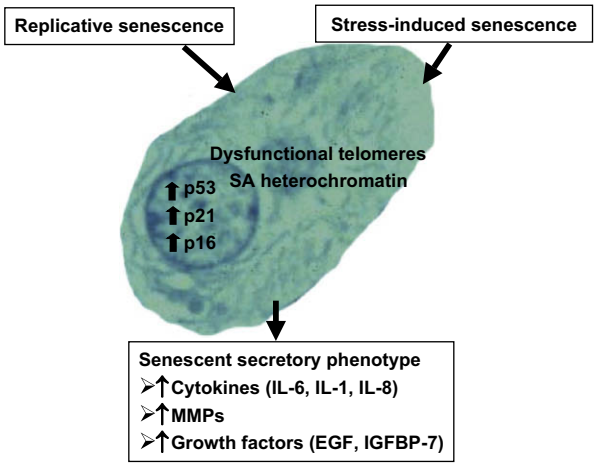

Fig. 1. Cell senescence. There are two major types of cell senescence-replicative (intrinsic) and stress-induced (extrinsic). Senescence is associated with telomere dysfunction, formation of SA heterochromatin, and increased expression of p53, p21, and p16. The senescent secretory phenotype is characterized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