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Graphical Abstract

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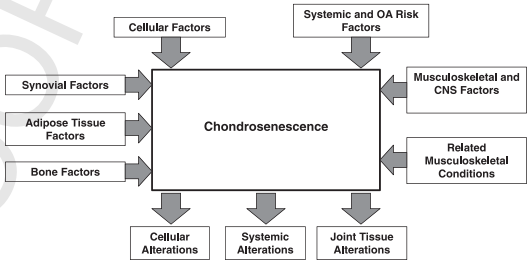
Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis

Ali Mobasheri*, Csaba Matta, Róza Zákány, Giuseppe Musumeci



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Highlights

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Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis

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- Aging and inflammation contribute to the development and progression of arthritic and musculoskeletal diseases.
- "Inflammaging" refers to low-grade inflammation that occurs during physiological aging.
- "Chondrosenescence" refers to the age-dependent deterioration of chondrocyte function and is intimately linked with inflammaging.
- A better understanding of chondrosenescence may lead to the development of new therapeutic and preventive strategies for osteoarthritis.

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Review

Chondrosenescence: Definition, hallmarks and potential role in the pathogenesis of osteoarthritis

Q1 Ali Mobasheri[a,b,c,*], Csaba Matta[a,d], Róza Zákány[d], Giuseppe Musumeci[e]

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ABSTRACT

Aging and inflammation are major contributing factors to the development and progression of arthritic and musculoskeletal diseases. "Inflammaging" refers to low-grade inflammation that occurs during physiological aging. In this paper we review the published literature on cartilage aging and propose the term "chondrosenescence" to define the age-dependent deterioration of chondrocyte function and how it undermines cartilage function in osteoarthritis. We propose the concept that a small number of senescent chondrocytes may be able to take advantage of the inflammatory tissue microenvironment and the inflammaging and immunosenescence that is concurrently occurring in the arthritic joint, further contributing to the age-related degradation of articular cartilage, subchondral bone, synovium and other tissues. In this new framework "chondrosenescence" is intimately linked with inflammaging and the disturbed interplay between autophagy and inflammasomes, thus contributing to the age-related increase in the prevalence of osteoarthritis and a decrease in the efficacy of articular cartilage repair. A better understanding of the basic mechanisms underlying chondrosenescence and its modification by drugs, weight loss, improved nutrition and physical exercise could lead to the development of new therapeutic and preventive strategies for osteoarthritis and a range of other age-related inflammatory joint diseases. Aging is inevitable but age-related diseases may be modifiable.

Contents

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1. Introduction

Aging is a natural and inevitable process by which living organisms approach the twilight of their existence. Human aging is a complex physiological process, which is accompanied by the gradual decline and adaptation of different body systems to the unavoidable passage of time. It is characterized by a progressive loss of structure, function, co-ordination and physiological integrity, leading to impaired homeostasis in all systems [1]. Aging is a risk factor for a variety of chronic health problems including cancer, diabetes, cardiovascular and neurodegenerative disorders. Advancing age is also a risk factor for arthritic and musculoskeletal diseases. There are common factors or "hallmarks" associated with each of these diseases. For example, there are six hallmarks associated with cancer (see [2,3]). Aging itself is characterized by nine hallmarks [1]. These include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication. Current research is attempting to examine the relative contributions of the hallmarks of aging and the links between them in order to identify pathways and targets that can be exploited to promote healthy aging and develop new, more effective and more targeted drugs and treatments with minimal side effects for diseases known to be associated with aging (Fig. 1).

One of the hallmarks of aging is cellular senescence. Normal cells possess a finite lifespan. Cells are continually exposed to a variety of harmful exogenous and endogenous factors that may induce stress and cause reversible or irreversible damage leading to complete recovery or cell death, respectively [4]. Unlike cancer cells, normal cells do not divide indefinitely due to a process known as cellular or replicative senescence [5]. Cellular senescence was formally described by Hayflick more than 50 years ago as a process that limited the growth and proliferation of normal human cells in culture [6]. Therefore, in cultured cells *in vitro*, replicative senescence limits the proliferation of normal cells, causing them to irreversibly arrest growth and adopt striking changes in cell form and function [7]. However, *in vivo* in aging adult tissues, senescent cells simply accumulate within tissues. Replicative senescence may contribute to ageing but it has been proposed that this process evolved to protect higher eukaryotes, particularly mammals, from developing cancer [8]. However, paradoxically, in older organisms, senescent cells may have the undesired effect of contributing to age-related pathologies and may actually contribute to carcinogenesis [9]. Cellular senescence is an important mechanism for preventing the proliferation of potential cancer cells and it is increasingly recognized as a critical feature of mammalian cells to suppress tumorigenesis, acting alongside cell death programs [5,10].

Mammalian organisms contain two types of cells: post-mitotic cells, which never divide, and mitotic cells, which have the capacity to divide. Examples of post-mitotic cells include nerve, muscle, and fat cells, most of which persist for life. Mitotic cells include epithelial and stromal cells of organs such as the skin. Post-mitotic and mitotic cells differ in their proliferative capacity, and thus they may age by different mechanisms [11]. Skin is an organ that clearly shows the signs of aging. Senescent keratinocytes and fibroblasts accumulate with age in human skin. Senescent skin cells possess a unique phenotype and exert long-range, pleiotropic effects [11]. They express a distinctive set of degradative enzymes, growth factors and pro-inflammatory cytokines [11]. Therefore, a few senescent cells in tissues such as skin might be able to compromise its function and integrity. The same principle may apply to several other tissues where a small number of senescent cells may interfere with the physiological functions of that tissue.

In this paper we review the published literature that support the concept of "chondrocyte senescence" may have similar effects in aging articular cartilage. We propose the term "chondrosenescence", define it as the age-dependent deterioration of chondrocytes and highlight its hallmarks and how they affect the phenotype of these cells and their specialized functions. We also propose the concept that a small number of senescent chondrocytes may be able to take advantage of the inflammatory tissue microenvironment and the inflammaging and immunosenescence that is concurrently occurring in the arthritis patient, further contributing to the age-related degradation of articular cartilage and other joint tissues. In this new framework chondrosenescence is intimately linked with inflammaging and the disturbed interplay between autophagy and inflammasomes [12]. This refined definition contextualizes the pro-inflammatory phenotype of chondrosenescent cells during the aging process and in age-related joint diseases, implicating mitochondrial dysfunction [13,14], oxidative stress and activation of inflammasomes [15]. The release of soluble and insoluble factors secreted by senescent chondrocytes further contributes to the inflammatory microenvironment that is believed to drive the catabolic degradation of extracellular matrix (ECM) macromolecules in articular cartilage. Since these molecules may be viewed as biochemical markers (biomarkers) of chondrosenescence, we also provide a brief overview of markers that may be used to identify and characterize chondrocytes *in vitro* and *in vivo*.

2. Aging and inflammation—"Inflammaging"

"Inflammaging" is defined as "low-grade chronic systemic inflammation established during physiological aging" [16]. The aging phenotype, is characterized by immunosenescence, and is explained by an imbalance between inflammatory and anti-inflammatory pathways, which results in a "low grade chronic pro-inflammatory status" [17]. Inflammaging is thought to be driving force behind many forms of age-related pathologies, such as neurodegeneration, atherosclerosis, metabolic syndrome, diabetes mellitus and sarcopenia [16]. There is also increasing evidence to suggest that inflammaging is also associated with inflammatory diseases of the musculoskeletal system (*i.e.* osteoporosis, osteoarthritis and rheumatoid arthritis) [18–20]. In this context, humans and animals must maintain homeostasis as they age despite incessant attack from both intrinsic and extrinsic stimuli [21]. Increased longevity results in a reduced capacity to mount inflammatory responses to infections and coordinate efficient anti-inflammatory responses to antigens and other noxious agents in

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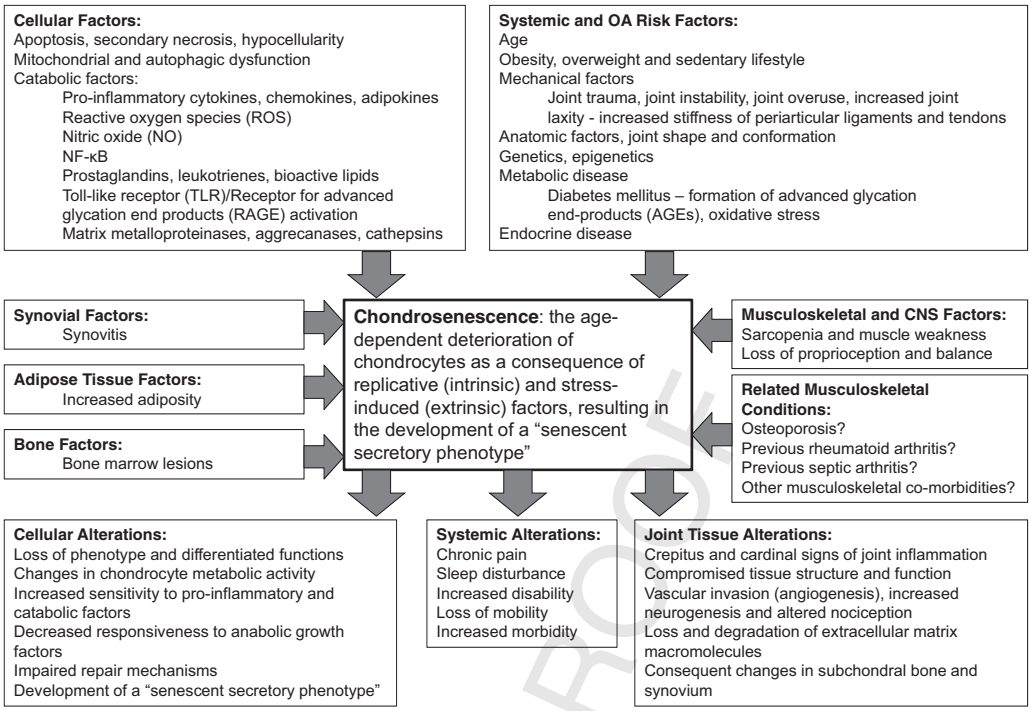

Fig. 1. *The hallmarks of chondrosenescence.* Chondrosenescence is defined as the age-dependent deterioration of chondrocytes as a consequence of replicative (intrinsic) and stress-induced (extrinsic) factors, resulting in the development of a "senescent secretory phenotype". The intrinsic and extrinsic factors involved are listed.

our food and environment. Molecular evidence points to a disturbed interplay between autophagy and inflammasomes [12]. Declined autophagic capacity in aging cells impairs the process of cellular housekeeping. This leads to protein aggregation, accumulation of misfolded proteins and the formation of dysfunctional mitochondria, which increases the generation of reactive oxygen species (ROS) thus promoting oxidative stress. Recent studies indicate that oxidative stress can induce the assembly of multiprotein inflammatory complexes called the inflammasomes [15]. Nod-like receptor protein 3 (NLRP3) is the major immune sensor for cellular stress signals. NLRP3 inflammasome-dependent inflammatory responses are triggered by a variety of signals of host danger, including infection, tissue damage and metabolic dysregulation [13,14]. Inflammatory signals activate inflammasome-dependent responses and caspases, predominantly caspase-1, which cleaves the inactive precursors of interleukins, thus stimulating their elevated secretion and activity [12]. Consequently, these cytokines provoke inflammatory responses and accelerate the aging process by inhibiting autophagy, which is believed to be a protective mechanism in cartilage. Autophagy may be a protective or homeostatic mechanism in normal cartilage [22]. However, in OA it is associated with a reduction and loss of Unc-51-like kinase 1 (ULK1), an inducer of autophagy, Beclin1, a regulator of autophagy, and microtubule-associated protein 1 light chain 3 (LC3), which executes autophagy and a increased chondrocyte apoptosis (see subsequent sections) [23].

3. The inflammatory microenvironment of chondrocytes

Chondrocytes exist in an avascular microenvironment, with low nutrient and oxygen levels [24,25]. Although chondrocytes rely on glycolysis [26], some of the metabolic functions of these cells are oxygen dependent [27,28]. Oxygen is mainly supplied by diffusion from the synovial fluid [24,29]. Consequently, the lack of oxygen means that chondrocytes display a metabolism adapted to anaerobic conditions [27,28,30]. There is little published information about the regulation of antioxidant enzymes within cartilage. Equally little is known about the transport of antioxidants from the circulation to chondrocytes. However, transport of nutrients, oxygen and antioxidants to chondrocytes is thought to occur by diffusion from subchondral bone [31] and the synovial microcirculation [32]. The role of subchondral bone in the pathogenesis of cartilage damage has been underestimated [31]. There is increasing evidence that vascular pathology plays a role in the initiation and/or progression of OA [33]. In pathological conditions, oxygen tension in synovial fluid is subject to fluctuation as blood flow may be reduced by venous occlusion and stasis, vascular shunt and fibrosis in synovium and/or by the development of microemboli in the subchondral vessels [33]. In response to oxygen variations induced through ischemia/reperfusion injury, mechanical stress, immunomodulatory and inflammatory mediators, chondrocytes produce abnormal levels of reactive oxygen species (ROS) that are generally produced by immune cells [27,28,34]. The main ROS produced by chondrocytes are NO and superoxide anion that generate derivative radicals, including peroxynitrite and hydrogen peroxide (H_2O_2) [35,36]. NO and its redox derivatives appear to have a number of different functions in both normal and pathophysiological joint conditions [37]. Low NO concentrations have protective effects on other cell types and the literature that deals with this area is beyond the scope of this review. Chondrocytes stimulated with pro-inflammatory cytokines produce large amounts of NO, which has been implicated in OA and has the capacity to inhibit extracellular matrix production by interfering with important autocrine and paracrine factors [38]. The published literature suggests important roles for NO in inflammation and pain associated with OA but this area is highly controversial and more work needs to be done to clarify the role of NO in joint health and disease [39]. NO is synthesized by nitric oxide synthase (NOS) enzymes. Chondrocytes express both endothelial (eNOS) and inducible (iNOS) forms of the enzyme. NO production is stimulated by cytokines (*i.e.* IL-1β, TNF-α), interferons (*i.e.* interferon γ (IFN-γ) and lipopolysaccharides (LPS). In fact the increased expression of iNOS and cyclo-oxygenase-2 (COX-2) in OA is largely due to the increased expression of pro-inflammatory cytokines, particularly IL-1β, which act in an autocrine/paracrine

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fashion to perpetuate a catabolic state that leads to progressive destruction of articular cartilage [40]. In contrast NO production is inhibited by growth factors such as transforming growth factor β (TGF-β).

In healthy cartilage chondrocytes possess robust defence mechanisms against attack by NO, free radicals and reactive oxygen species (ROS). However, responses to ROS generation will be dependent on redox status at the cellular level and influenced by systemic levels of inflammatory mediators, if present. When the oxidant level does not exceed the reducing capacities of cells, ROS are strongly involved in the control of cellular functions including signal transduction. In contrast, in some pathological situations, when the cellular antioxidant capacity is insufficient to detoxify ROS, oxidative stress may occur that degrade not only cellular membranes and nucleic acids but also extracellular components including proteoglycans and collagens. This is likely to happen in certain OA phenotypes. Furthermore, ROS can modify proteins by oxidation, nitrosylation, nitration or chlorination of specific amino acids, leading to impaired biological activity, changes in protein structure and accumulation of damaged proteins in the tissue.

A further point that needs to be made in connection with oxidative stress is the fact that redox sensitive transcription factors (*e.g.* NF-κB) are upregulated, which might result in an uncontrolled inflammatory response. Oxidative stress may also cause cell death and release of cellular content into extracellular environment, activating clearance mechanisms in the microenvironment. Altogether, degradation products and cellular content containing oxidized molecules may contribute to the exacerbation of synovial inflammation and form a vicious circle, constituted by newly formed ROS and further degradation products.

The enzyme complex NADPH, which catalyzes the reduction of molecular oxygen to superoxide anion radicals, produces superoxide anion radicals. Biochemical studies have shown that chondrocytes express the large subunit of the flavocytochrome of NADPH oxidase [41]. Even immortalized human chondrocyte-like cells lines express various components of the NADPH oxidase complex [42]. Articular chondrocytes also appear to express cell-specific components of NADPH oxidase complex such as p22phox, p40phox, p47phox, p67phox and gp91phox [41].

4. Hallmarks of chondrosenescence

There are common factors or "hallmarks" associated with every chronic disease. Six different hallmarks have been associated with cancer [2,3] and aging itself has at least nine hallmarks [1]. However, there are no published papers that have specific hallmarks listed as being associated with chondrosenescent cells in OA. However, before we list these hallmarks, it is appropriate to address this fundamental question: *what is the impact of cartilage aging?*

Clearly, age is one of the most important risk factors for OA, followed by obesity, joint trauma, joint instability, genetic factors, underlying metabolic or endocrine disease. However, as we age, our tissues undergo age-related changes. These include changes in metabolic activity, mitotic activity, decreased sensitivity of chondrocytes to growth factors (especially changes in chondrocyte transforming growth factor β (TGF-β) signalling [43]), decreased responsiveness to anabolic mechanical stimuli and impaired "repair mechanisms [44]. Age-related changes in articular cartilage can contribute to the development and progression of OA. However, the degeneration of normal articular cartilage "is not simply the result of aging and mechanical wear" [45]. Nevertheless, aging modifies the structure and function of articular cartilage and other joint tissues such as subchondral bone, muscle, soft tissues, synovial membrane, and synovial fluid. In aging articular cartilage chondrocytes exhibit an age-related decline in proliferative and synthetic capacity while maintaining the ability to produce pro-inflammatory mediators and matrix degrading enzymes [46]. 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.

5. The role of apoptosis in joint inflammation

Apoptosis, also known as programmed cell death (PCD) is the physiologically regulated process of dell death that occurs in multicellular organisms during embryonic development. Various definitions have been proposed for apoptosis [47,48] since the term was initially introduced by Kerr, Wyllie and Currie [49]. Research carried out over the last twenty-five years has demonstrated a clear role for apoptosis in a variety of chronic diseases including joint inflammation and arthritis. The demonstration of macrophage phagocytosis of aging neutrophils in joint inflammation was one of the first studies that linked apoptosis to arthritis [50]. Interestingly, the authors of this original study proposed that apoptosis in the synovial joint may represent a mechanism for the removal of neutrophils during inflammation, a process that may serve to limit the degree of joint injury during inflammation [50]. A few years later apoptosis was observed in the synovium in rheumatoid arthritis (RA) [51]. Firestein and colleagues studied RA synovial tissue (ST) to determine if and where apoptosis occurs *in situ*. They used immunohistochemical techniques to show that DNA strand breaks occur mainly in macrophages, although some fibroblast-like cells in the RA synovium were also labelled. They also proposed that pro-inflammatory cytokines regulate this process, and the cytokine profile in RA (high interleukin 1β (IL-1β), high tumour necrosis factor α (TNF-α) and low interferon γ (IFN-γ) along with local oxidant injury might conspire to favour induction of apoptosis in the synovium [51]. This was one of the first reports of "inflammaging" in the synovial joint, although this term was not specifically mentioned at this stage. Further evidence for apoptosis in RA was provided by ultrastructural studies that demonstrated Fas and Bcl-2 expression in synovial fibroblasts from patients with RA [52]. Fas is an important cell surface receptor belonging to the TNF receptor superfamily, also known as CD95, that induces apoptosis on binding Fas ligand and Bcl-2 is an integral outer mitochondrial membrane protein that blocks apoptosis and its increased abundance is a reflection of apoptotic activity in tissues. Therefore, observations of Fas antigen, Fas ligand and the tumour suppressor protein p53 over-expression in RA synovial tissue [53,54] and accumulation of soluble Fas ligand in serum and synovial fluid of patients with RA [55] added further molecular evidence for the involvement of apoptosis in joint inflammation and the accumulation of soluble Fas in the joint cavity of RA patients was proposed as a mechanism that may inhibit apoptosis and exacerbate the inflammatory process [55]. The expression of Bcl-2 is thought to result in extended life of matrix degrading synovial fibroblasts at the site of synovial invasion into cartilage and bone in RA joints [52]. Indeed, identification of apoptotic changes in osteocytes in pathological human bone indicated a functional role for apoptosis in remodelling in joint disease [56]. Increased synovial apoptosis and focally regulated endothelial proliferation in the synovium pointed to microvascular dysfunction as a mechanism for facilitating persistent synovitis in RA [57].

Transgenic mice lacking collagen II were found to exhibit increased apoptosis and led to the suggestion that apoptosis may also play a role in degenerative joint diseases such as OA in which there is extensive cartilage loss [58]. Hashimoto et al., studied

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Fas/Fas ligand expression and induction of apoptosis in chondrocytes from normal and OA cartilage [59]. They found that subpopulations of chondrocytes express Fas and are susceptible to Fas-induced apoptosis and Fas-mediated chondrocyte loss may contribute to cartilage degradation in OA [59] and RA [60]. Blanco et al., used FACS analysis and the TUNEL technique to show that OA chondrocytes indeed die by apoptosis and proposed increased apoptosis as a possible pathogenic pathway for OA [61]. Linking chondrocyte apoptosis and cartilage degradation in OA suggested that apoptosis and extracellular matrix depletion in articular cartilage are anatomically linked and may be mechanistically related [62]. Taken together, these studies revealed that apoptotic chondrocyte death plays an important role in the pathogenesis of OA and could be targeted for the development of new therapeutic strategies. Further mechanistic insight came from clinical and laboratory animal studies from the Lotz group in La Jolla that demonstrated a role for nitric oxide (NO) or antibody to Fas undergo cell death by apoptosis [63] (reviewed in [64]). Another elegant study by the Lotz group revealed that apoptotic bodies isolated from NO-treated chondrocytes or cartilage contain alkaline phosphatase and NTP pyrophosphohydrolase activities and can precipitate calcium [65]. This was the first study that implicated chondrocyte-derived apoptotic bodies in the pathologic cartilage calcification seen in aging and OA.

According to the recent literature there is a significant decrease in chondrocyte abundance in articular cartilage with aging and a moderate to strong positive correlation exists between the degree of cartilage damage and chondrocyte death by apoptosis [66]. Although there is a strength relation between chondrocyte apoptosis and cartilage degeneration in human osteoarthritis (OA), in 40–60-year-old donors' cartilages there are unusually high numbers of apoptotic chondrocytes also in macroscopically normal cartilage [66].

6. Morphological features of chondrocyte apoptosis

Cells that undergo apoptosis exhibit a characteristic pattern of morphologic changes, including cell shrinkage, condensation, fragmentation of the nucleus and bubbling of the plasma membrane, known as "blebbing," and chromatin condensation and nucleosomal fragmentation [47]. These morphological features have been described in chondrocytes from murine models of OA [67] and in human OA samples [68]. Various methods have been published for evaluating them [69]. These features have also been described in hypertrophic chondrocytes in the growth plate [70] but the relevant literature is beyond the scope of this review. Some investigators have even proposed the term 'chondroptosis' to reflect the fact that chondrocytes may undergo apoptosis in a non-classical manner [71]. However, the term 'chondroptosis' has not been widely used or adopted. Freshly isolated chondrocytes from human OA cartilage exhibit morphological evidence of apoptosis, with clear cytoplasmic, cell-surface blebs, altered nuclear shape, apoptotic bodies and a parallel loss of nuclear volume [72].

7. Targeting apoptotic pathways in OA

Chondrocyte apoptosis is a challenging target for the development of therapeutic interventions for OA because of the potentially harmful systemic effects that pharmacological and biological regulators of apoptosis may have, especially the potential for development of tumours. However, the joint is more isolated from systemic regulation than many other organs. Therefore, the death receptor, mitochondrial and endoplasmic reticulum pathways and the major cellular pathways that regulate apoptosis could be targets of innovative new treatments. Of all the elements involved in the apoptosis of chondrocytes, caspase inhibition has been studied with the greatest detail (reviewed in [73]). However, caspases are not the only targets. There are other molecular targets with the capacity to modulate mitochondrial function and these have already been reviewed [73].

8. Age-related alterations in chondrocyte calcium signalling pathways

Calcium signalling is extraordinarily diverse and versatile, affecting almost all cellular functions including metabolism, proliferation, differentiation, and apoptosis [74]. While calcium homeostasis of differentiating and mature chondrocytes has been partially characterized [75], little is known about age-related changes of these pathways in senescent chondrocytes. It has long been established that alterations in Ca^{2+} homeostasis, including mitochondrial Ca^{2+} overload, are linked to aging [76]. For example, in senescent detrusor as well as cerebral arterial smooth muscle cells, calcium signals with decreased amplitudes but with increased durations were observed, reflecting on disturbances in both Ca^{2+} influx (*e.g.* inhibition of voltage-operated calcium influx, increased calcium mobilization by ATP) and elimination pathways [77,78]. This suggests that alterations in Ca^{2+} signalling can also be expected in ageing chondrocytes. A recent study that evaluated and compared gene expression profiles using microarrays in knee joint tissues from younger and older adult mice after experimentally induced OA, interesting alterations were found [79]. Among the genes with altered expression in older mice compared to younger animals, genes involved in Ca^{2+} signalling were significantly represented. The genes that were downregulated in older mice included regulatory molecules: the histidine-rich calcium binding protein (Hrc), which is a regulator of ER calcium sequestration, and the versatile intracellular regulatory protein S100B; the ER calcium release channel RyR1; the alpha2/delta1 (*CACNA2D1*) and gamma6 (*CACNG6*) regulatory subunits of voltage-gated calcium channels; the sodium/calcium exchanger involved in calcium elimination pathways (NCX3); and a calcium-regulated ion transporter, the large conductance calcium-activated potassium channel ($K_{Ca}1.1$; *KCNMA1*). At the same time, several other subsets of genes involved in calcium homeostasis were found to be upregulated in older mice *vs.* younger animals, including ionotropic purinergic receptors $P2X_1$, $P2X_4$, $P2X_7$, and the transient receptor potential cation channel, subfamily C, members 1 and 6 (*TRPC1, TRPC6*). Disturbed calcium homeostasis in senescent chondrocytes is likely and increased expression of purinergic receptors in aged chondrocytes, similar to what has been observed in ageing myocytes, is of particular importance, considering the key role of these receptors in the calcium homeostasis of chondrocytes [80,81]. It is therefore clear that we are far from identifying biomarkers among the members of the calcium toolkit that would be reasonable indicators for chondrosenescence. However, the fact that there *are* alterations in the mRNA expression of molecules involved in calcium signalling implicates that research into this field may shed more light on the process of chondrosenescence.

9. Alterations in the chondrocyte channelome in aging chondrocytes

Ion channels that enable ion transport across the plasma membrane are vital components of cellular homeostasis. It is now evident that chondrocytes are characterized by an ever-expanding complement of ion channels referred to as the chondrocyte channelome [82]. The resting membrane potential (RMP), which is known to control the mRNA expression of cartilage matrix components [83] in chondrocytes, is maintained by plasma membrane

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ion transporters. Since altered activity of Na^+ channels and ATP-sensitive K^+ (K_{ATP}) channel has been reported in other tissues in various aging models [84,85], it is also realistic to expect age-related changes in the chondrocyte channelome. Indeed, changes have already been observed in K_{ATP} channel expression in OA chondrocytes and the function of K_{ATP} channels appears to be impaired in OA chondrocytes [86] (see the following section for more details).

In the previously mentioned microarray study that compared gene expression profiles in knee joint tissues from younger and older mice [79], alterations in the expression of genes encoding ion channels and other transporters were detected. Most importantly, the α2 isoform of the Na^+, K^+-ATPase (*ATP1A2*) was reported to be downregulated in aged cartilage. Apart from VOCC subunits and $K_{Ca}1.1$, other transporter subunits including Na_Vb_4 voltage-gated sodium channel, or Na^+/Ca^{2+} exchanger 3 (NCX3) were also downregulated. In contrast, several transporters were upregulated in aged cartilage *vs.* younger controls, for example the ligand-gated purinergic receptors $P2X_1$, $P2X_4$, $P2X_7$, as well as TRPC1 and TRPC6. Furthermore, the cation transporter ATPase type 13A2 (*ATP13A2*) was also found to be affected [79]. It is important to note that these data only show that there are alterations of these molecules at the mRNA expression level; however, there are no data available regarding the protein level expression or function of these molecules.

Therefore, it is only possible to consider these molecules as potential biomarkers of chondrosenescence when protein expression and functional data become available.

9.1. The role of mitochondrial dysfunction, anaerobic metabolism and oxidative stress in chondrosenescence

Cellular senescence and mitochondrial dysfunction have both been listed among the nine tentative hallmarks of aging in different organisms [1]. In tissues where cells regularly replicate, gradual telomere shortening ultimately leads to definite arrest of cell cycle [87].

In articular cartilage, where chondrocytes, the only resident cell type of the tissue are quiescent and rarely, if ever, divide under physiological conditions replicative senescence would seem unlikely. Nonetheless, telomere shortening was detected in chondrocytes isolated from articular cartilage of older adults [88]. This "non-replicative" telomere erosion can be the result of various external and internal stressors such as continuous mechanical load or hypoxia. Articular chondrocytes are embedded into an avascular extracellular matrix and supplied by oxygen and nutrients from synovial fluid by slow diffusion. Although direct measurement of O_2 tension in articular cartilage is difficult, it does not exceed the O_2 level of synovial fluid, which has been estimated to be around 5–6% [89]. Metabolism of articular chondrocytes is well adapted to this hypoxia and elevation of oxygen content of their environment does not result in increased oxygen consumption [90]. The vital role of this unique oxygen homeostasis in development and maintenance of cartilage has been demonstrated by the observation that hypoxia-inducible factor-1(HIF-1) was indispensable for survival of chondrocytes [91]; whether the expression level of HIF-1 changes with ageing of chondrocytes has not been elucidated yet. Hypoxia-induced genes include glucose transporters (GLUT1, GLUT3), which are important for anaerobic metabolism [25,92,93]. Articular chondrocytes express multiple isoforms of these facilitative GLUTs and some have been shown to be regulated by growth factors and cytokines [25,93,94]. Although hypoxia, growth factors and cytokines are involved in regulating the overall glucose transport capacity of human chondrocytes, recent studies have shown that ATP-sensitive potassium [K(ATP)] channels are present in chondrocytes [95] and are involved in the regulation of GLUT1 and GLUT3 abundance in these cells. [86]. Therefore, K(ATP) channels are components of a broad glucose sensing apparatus that modulates glucose transporters and allows chondrocytes to adjust to varying extracellular glucose concentrations. However, the function of K(ATP) channels seems to be impaired in chondrocytes from OA cartilage [86]. In addition, there is evidence for altered GLUT1 expression in OA chondrocytes and this may reflect a possible contribution of altered glucose metabolism in the pathogenesis of this disease [25,96]. One likely contributor to this scenario is altered mitochondrial metabolism in OA. Another contributing factor is the low density of chondrocytes within cartilage. Chondrocytes occupy 1–2% of the volume of the tissue in mature adult human articular cartilage, which is approximately one tenth compared to that in other tissues [97].

10. Conclusions

The incidence of OA, the age-related inflammatory joint disease characterized by pain and loss of synovial tissue structure and function due to articular cartilage degeneration, is steadily rising across the world as the aging population expands. Therefore health and social care systems across the globe need to prepare for a "tsunami" of OA cases in the next two decades. Although there is a very strong association between age and increasing incidence of OA, the disease itself is not an inevitable consequence of aging; instead, aging increases the risk of OA. This is a very important point and is often overlooked in the scientific literature. A characteristic of OA is deviant behaviour of chondrocytes in diseased articular cartilage [43]. OA chondrocytes resemble terminally differentiated chondrocytes in the growth plate and actively produce pro-inflammatory cytokines and matrix degrading enzymes [43,46]. These catabolic factors result in further cartilage degeneration. Progressive chondrocyte senescence is also characterized by expression of senescence-associated markers, erosion of chondrocyte telomere length and mitochondrial dysfunction due to oxidative damage causing the age related loss of chondrocyte function [98]. In appropriate joint loading and mechanical stresses associated with abnormal loads on the joint considerably increased the production of oxidants and soluble factors that sustain the chondrosenescent phenotype [99]. Chondrosenescence and OA are intimately linked and the premature senescence accounts for age-related decline in chondrocyte function and indicate that mechanically induced oxidative damage plays a role in this process [99]. Poor cartilage repair in older patients is likely to be limited by the inability of older chondrocytes to form new mechanically competent cartilage. Chondrosenescence *in vivo* contributes to the age-related increase in the prevalence of OA and decrease in the efficacy of cartilage repair [44]. Chondrosenescence directly affects the extracellular matrix, resulting in a tissue that is functionally impaired and less able to maintain homeostasis when stressed, resulting in breakdown and loss of the articular cartilage, a classic hallmark of OA [46]. Age-related senescence and loss of muscle and bone mass are also likely to be important as are sarcopenia and increased bone turnover may also contribute to the development of OA [100]. A better understanding of the basic mechanisms underlying senescence and how the process may be modified could provide novel approaches to slow the development of OA and lead to the development of new therapeutic strategies that may delay the onset of chondrocyte senescence or replace senescent cells [101].

Contributors

Ali Mobasheri: Conceived the concept of chondrosenescence, drafted and submitted the commissioned paper. Csaba Matta:

Drafted two sections, read, edited and approved the submission; made a significant intellectual contribution to the manuscript. Róza Zákány: Drafted one sections, read, edited and approved the submission; made a significant intellectual contribution to the manuscript. Giuseppe Musumeci: Drafted one section, read, edited, and approved the submission; made a significant intellectual contribution to the concept of the manuscript.

Competing interests

The authors declare no competing interests.

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Conflict of interest statement

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