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Age-Related Alterations in Signaling Pathways in Articular Chondrocytes: Implications for the Pathogenesis and Progression of Osteoarthritis – A Mini-Review

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Abstract
Musculoskeletal conditions are a major burden on individuals, healthcare systems, and social care systems throughout the world, with indirect costs having a predominant economic impact. Aging is a major contributing factor to the development and progression of arthritic and musculoskeletal diseases. Indeed, aging and inflammation (often referred to as 'inflammaging') are critical risk factors for the development of osteoarthritis (OA), which is one of the most common forms of joint disease. The term 'chondrosenescence' has recently been introduced to define the age-dependent deterioration of chondrocyte function and how it undermines cartilage function in OA. An important component of chondrosenescence is the age-related deregulation of subcellular signaling pathways in chondrocytes. This mini-review discusses the role of age-related alterations in chondrocyte signaling pathways. We focus our attention on two major areas: age-dependent alterations in transforming growth factor-β signaling and changes in protein kinase and phosphoprotein phosphatase activities in aging chondrocytes. A better understanding of the basic signaling mechanisms underlying aging in chondrocytes is likely to facilitate the development of new therapeutic and preventive strategies for OA and a range of other age-related osteoarticular disorders.

Introduction

Aging is a risk factor for a variety of chronic health problems, including cancer, diabetes, cardiovascular and neurodegenerative disorders. Advancing age is also a major risk factor for the development of arthritic and musculoskeletal diseases. Osteoarthritis (OA) is an age-relat-

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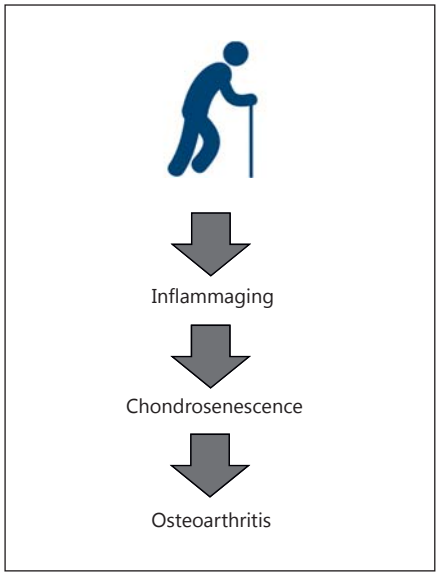

Fig. 1. Aging, inflammaging, and chondrosenescence in OA.

ed disease of synovial joints. It is characterized by the progressive deterioration and loss of articular cartilage with concomitant structural and functional changes in the entire synovial joint, including the synovium, periarticular ligaments, and subchondral bone. OA is actually one of the most common, costly, and disabling forms of joint disease, being far more common than rheumatoid arthritis, psoriatic arthritis, and other forms of joint disease. Age, obesity, and metabolic disease are major risk factors for the development of OA. Traditionally, OA was considered a noninflammatory 'wear and tear' disease. However, over the last few decades, this outdated view has been challenged with solid scientific and clinical evidence. OA is now generally accepted to be an inflammatory and biomechanical whole-organ disease that is influenced by a number of factors including 'inflammaging'. One of the hallmarks of inflammaging is cellular senescence. Evidence from the published literature supports the concept that 'chondrocyte senescence' or 'chondrosenescence' affects the functional phenotype of the cells, is intimately linked with 'inflammaging', and is involved in the pathogenesis and progression of OA [1] (fig. 1). This paper focuses on aging of chondrocytes in OA and the role of age-related alterations in chondrocyte signaling pathways. We turn our attention to the molecular mechanisms and subcellular pathways implicated in chondrocyte aging. We discuss two major areas of research: age-dependent alterations in transforming growth factor-β (TGF-β) signaling and changes in protein kinase and phosphoprotein phosphatase activities in aging chondrocytes and review the evidence supporting changes in these processes.

Age-Dependent Alterations in TGF-β Signaling in OA

Active TGF-β signaling is vital for the physiological maintenance of articular cartilage. Binding of TGF-β to its constitutively active type II receptor recruits the type I receptor, and the latter is thereafter phosphorylated and activated, a process that is necessary for TGF-β-mediated responses. The crucial role of active TGF-β signaling is highlighted by the promotion of chondrocyte hypertrophic differentiation and early OA development in mice expressing a truncated, kinase-defective TGF-β type II receptor in skeletal tissue [2]. Moreover, the TGF-β signaling protein Smad3 is absolutely essential for blocking hypertrophy in articular chondrocytes, both in mice and men [3, 4]. The presence of normal TGF-β signaling is crucial for the maintenance of articular cartilage; however, exposure of joint tissues in animal models to high active TGF-β levels leads to osteophyte formation, synovial fibrosis and most likely contributes to joint pain [5–8].

Crystals are common in OA joints and are thought to contribute to cartilage degradation and joint inflammation [9]. Moreover, increased mineralization of the cartilage matrix can occur in OA [10]. TGF-β is a known stimulant of inorganic pyrophosphate, a molecule essential for crystal formation. Work on porcine explants has shown that TGF-β strongly stimulates inorganic pyrophosphate elaboration in aged cartilage compared to young tissue [11]. A similar finding was reported comparing different human age groups (15–55 and 56–91 years) [12]. These results indicate that age-dependent changes in TGF-β responsiveness might contribute to crystal formation in aging joints.

Binding of TGF-β to its type II receptor results in the recruitment and phosphorylation of a type I receptor. In chondrocytes, TGF-β not only signals via the canonical type I receptor ALK5 (TGFBR1) but also via the ALK1 (ACVRL1) receptor [13, 14]. Signaling via ALK5 leads to activation of the Smad2/3 route, while ALK1 phosphorylation activates the Smad1/5/8 pathway. Using in vitro models, it has been shown that augmented expression of ALK1 increases matrix metalloproteinase-13 (MMP13) expression, while its inhibition reduces MMP13 expression. Moreover, reduced ALK5 expression elevates MMP13 expression levels. In human OA cartilage, ALK1 expression levels correlate positively with MMP13 expression [13]. These data indicate

that the balance between ALK5 and ALK1 is crucial to maintain cartilage homeostasis and that loss of ALK5 signaling will result in loss of cartilage integrity.

In classic murine studies carried out in the 1990s, the important observation was made that TGF-β effects on old cartilage are different from those on young cartilage [15]. TGF-β is able to counteract the effects of proinflammatory cytokine interleukin-1β (IL-1β) only in cartilage of young mice [16]. Aging is associated with a decreased expression of both ALK5 and phosphorylated Smad2/3 in articular cartilage which was in line with the observation that TGF-β does not block IL-1 effects in aged cartilage [17]. Since TGF-β can signal via either ALK5 or ALK1, investigation of the age-related changes in expression of ALK5 and ALK1 revealed that the ALK1/ALK5 ratio is significantly increased in old C57Bl/6 mice compared to young, favoring TGF-β signaling via the Smad1/5/8 route and the differentiation of chondrocytes into cells with an autolytic phenotype [13]. This observed change in the ALK1/ALK5 ratio has also been confirmed by others, for instance in the C57/BL1/6 (ICRFa) mouse strain and Dunkin-Hartley guinea pigs [18, 19]. The latter study [19] also demonstrated a progressive switch of the expression of phosphorylated Smad2/3 to Smad1/5/8 in old animals.

Loading of articular cartilage is absolutely essential for its physiological maintenance. Reduced joint loading leads to cartilage degeneration, both in humans and animal models, and patients with spinal cord injuries exhibit progressive loss of knee cartilage. Loading of chondrocytes has been shown to activate Smad2/3 signaling in these cells [20]. Compressive loading of bovine cartilage explants upregulates the expression of Smad2/3 response genes bSerpine1, bSmad7, and bAlk5 while the expression of the Smad1/5/8 responsive gene Id1 is downregulated [21]. However, when comparing the responsiveness of young (6–36 months) and old (6–13 years) bovine cartilage, it was found that aged cartilage showed a highly reduced capacity for mechanically mediated activation of Smad2/3P signaling when compared to young cartilage [22]. This reduced responsiveness can be a result of a stiffer matrix of old cartilage which makes it harder to mechanically activate TGF-β signaling, or is the consequence of an age-related reduction in expression of ALK5, as is observed in aged bovine cartilage. It can also be that reduced ALK5 expression itself is the result of a stiffer matrix, since ALK5 expression is regulated by active TGF-β signaling. Irrespective of the underlying cause, it can be expected that an age-related decrease in TGF-β signaling (Smad2/3) might impair cartilage preservation, since intact Smad2/3 signaling is crucial for healthy cartilage. The age-related loss of Smad2/3 signaling can be considered a normal part of the aging process, but this can make articular cartilage more vulnerable to OA development at an advanced age compared to young individuals.

A very interesting observation with regard to TGF-β signaling and OA is the relationship between aging and circadian rhythms. Aging is associated with a loss of the normal circadian rhythm [23]. Recent studies have demonstrated that expression of the core clock transcription factor BMAL1 is disrupted in human OA cartilage and in aged mouse cartilage [24]. Deficiency of BMAL1 in mice reduced the levels of phosphorylated Smad2/3 but increased phosphorylated Smad1/5/8 levels. These results link aging and loss of the circadian rhythm to a shift in the ALK5/ALK1 ratio, which may be related to a loss of articular cartilage homeostasis and OA. All above-described observations point to a role of age-related changes in TGF-β signaling as a driving force of chondrocyte hypertrophy and OA development and progression. Figure 2 summarizes the alterations in TGF-β, ALK1/5, and Smad1/5/8 pathways in chondrosenescence and OA.

Alterations in Protein Kinase and Phosphoprotein Phosphatase Activities in Aging Chondrocytes

Reversible protein phosphorylation on specific Ser/Thr residues regulated by the balanced activities of protein kinases and phosphoprotein phosphatases is a key process that controls all stages and aspects of the chondrocyte life cycle in response to extracellular stimuli [25, 26]. Amongst the key chondrocyte signaling pathways are protein kinase A, protein kinase C, mitogen-activated protein kinases (MAPKs, such as p38, JNK, and ERK), the phosphoinositide-3 kinase (PI3K)/Akt pathway, as well as protein phosphatase 1A (PP1A), PP2A, and calcineurin (PP2B) [27]. Accumulating evidence suggests that some of these signaling pathways are differentially regulated during the aging process in chondrocytes. It is generally accepted that reactive oxygen species (ROS) contribute to senescence not only by causing direct damage to proteins, lipids, and DNA, but also by modulating cell signaling pathways that promote senescence through activation of redox-sensitive kinases and inhibition of redox-sensitive phosphatases [28]. Increased ROS levels can also upregulate proinflammatory cytokines and MMPs, factors that coconspire and mediate cartilage matrix degradation [29]. Given their central role in chondrosenescence [1], age-related alterations in the MAPK pathways are likely to be particularly important. Several stud-

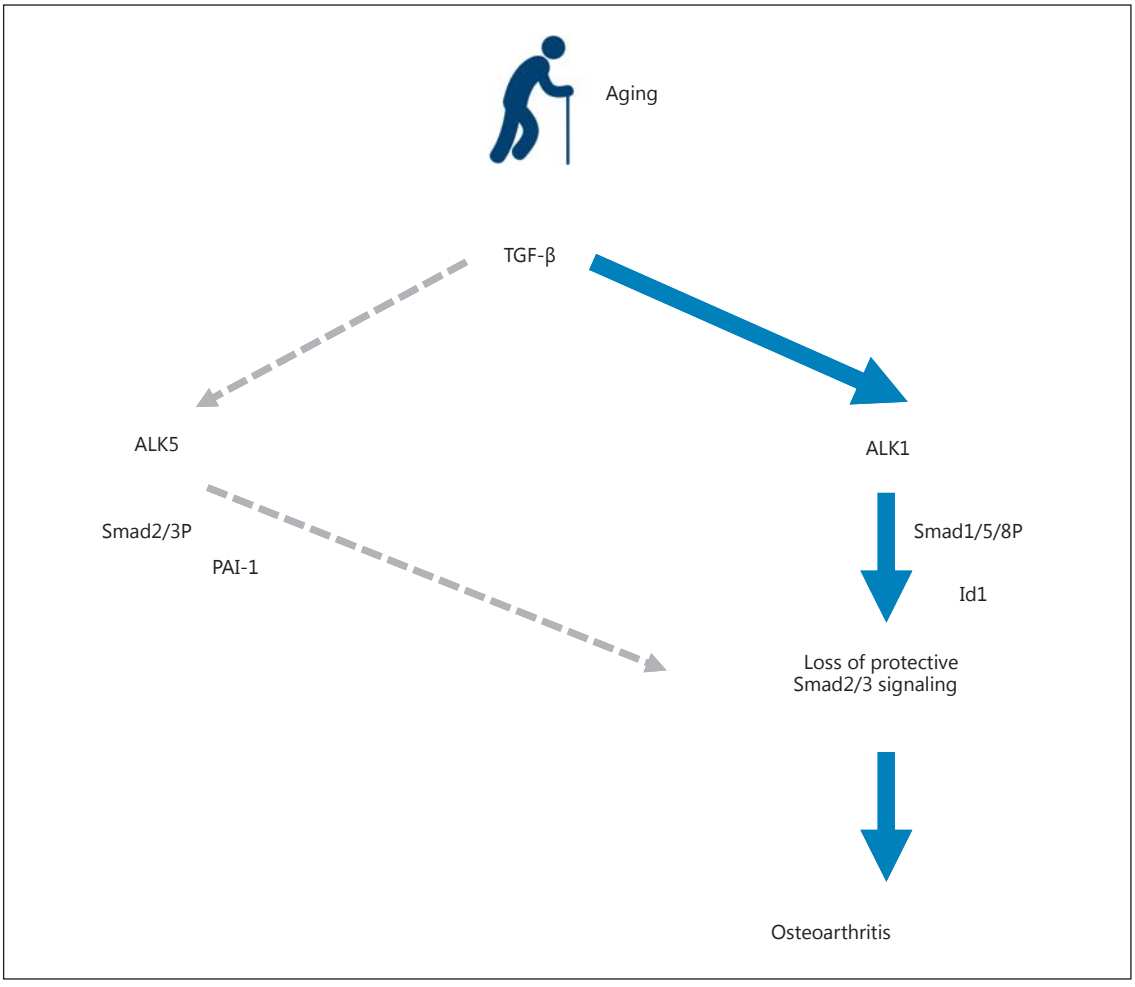

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Fig. 2. Alterations in TGF- β, ALK1/5, and Smad1/5/8 pathways in chondrocyte aging. Chondrosenescence results in a switch from the ALK5 to ALK1 pathway leading to the loss of protective Smad2/3 signaling in OA.

ies have suggested the involvement of MAPKs in mediating chondrosenescence. Caveolin-1, the integral membrane protein that serves as a scaffold and can regulate cell signaling pathways, is involved in chondrosenescence; it has been reported to play a role in premature chondrocyte senescence induced by IL-1β and oxidative stress through the activation of p38 MAPK in human and rat models [30]. Elevated levels of ROS can also contribute to reduced proteoglycan synthesis by causing an imbalance in the activity of the PI3K/Akt and the ERK MAPK pathway in human chondrocytes, which have opposing roles in cartilage matrix production [31]. Since the PI3K/Akt pathway plays important roles in regulating the chondrocyte cell cycle, proliferation, differentiation and apoptosis [32], and its activation increases proteoglycan synthesis in human chondrocytes [33], the age-related modulation of its activity may be an important contributor to chondrosenescence. Excessive ROS may also inhibit the insulin signaling pathway through activation of the stress-induced JNK in a human in vitro model [34].

Senescent cells are known to exhibit altered activity and expression of regulatory proteins that control the cell cycle, including p53 and the cyclin-dependent kinase inhibitors $p21^{CIP1}$ and $p16^{INK4A}$ [35]. Oxidative stress-induced PKCδ activation was reported to cooperate with the $p16^{INK4A}$ retinoblastoma protein pathway in human cells to promote senescence [36]. Oxidative stress is also known to reduce the activity of the calcium-calmodulin-dependent phosphoprotein phosphatase calcineurin, which interferes with the ERK pathway and Sox9 phosphorylation in a chicken micromass model [37]. AMP-activated protein kinase (AMPK), a highly conserved sensor of in-

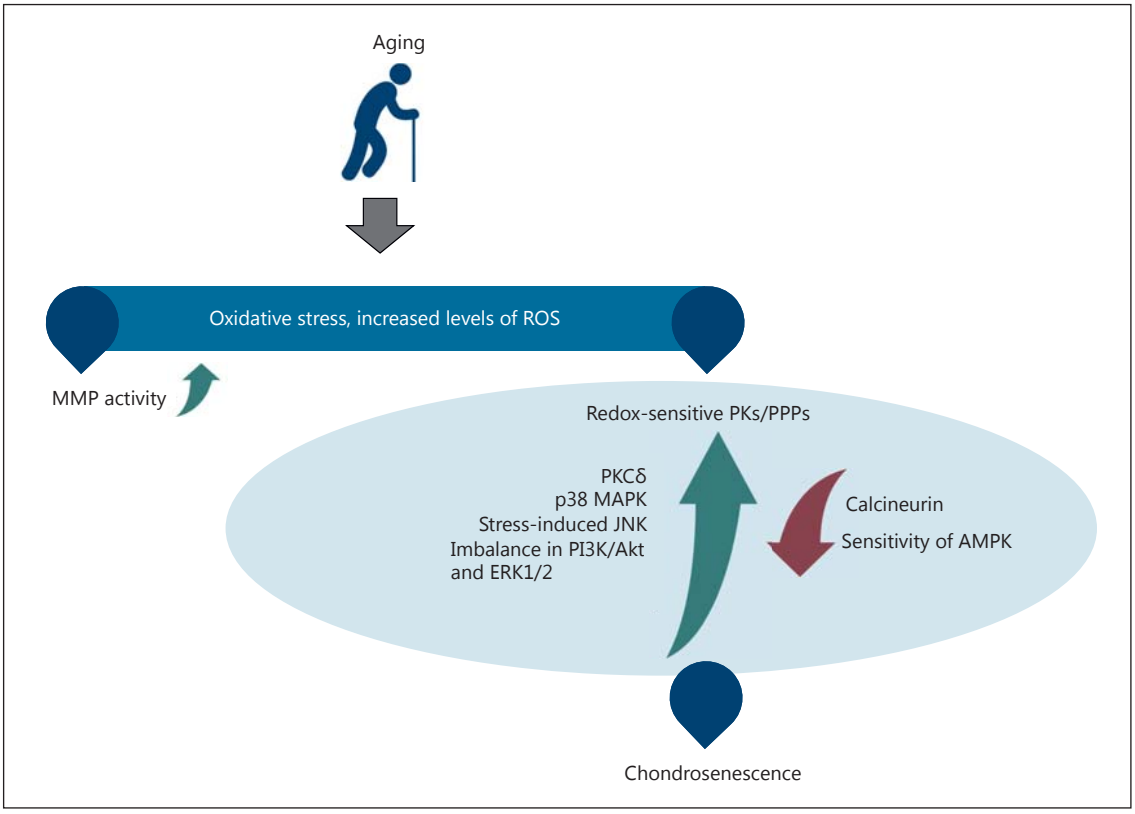

Fig. 3. Effects of chondrocyte aging on redox-sensitive protein kinase and phosphoprotein phosphatase signaling pathways. Oxidative stress and increased levels of ROS stimulate inflammatory and catabolic pathways, upregulate MMPs, and alter the activity of signaling pathways in chondrocytes. PPPs = Phosphoprotein phosphatases.

creased levels of AMP and ADP originating from ATP depletion [38], also has a role in aging as its sensitivity is reduced in aging tissues [39]. Since a number of upstream protein kinases and phosphoprotein phosphatases are involved in its regulation, it is possible that there are age-related changes in the function of protein phosphatases, i.e. PP2A or PP2Cα, which could suppress AMPK activation with aging [39]. Given its key role as an energy sensor in chondrocytes [40], AMPK is an emerging new target for preventing chondrosenescence and OA. Figure 3 summarizes our current understanding of the effects of chondrocyte aging on redox-sensitive protein kinase and phosphoprotein phosphatase signaling pathways.

Conclusions and Future Directions

The incidence of OA is steadily rising across the world as the aging population grows. By 2037, it is estimated that the proportion of the population aged 70–90 years and beyond will expand significantly. Indeed, by the middle of this century, the proportion of centenarians in the United Kingdom and Europe will grow by more than 10-fold. Therefore, health and social care systems across the globe need to prepare for the oncoming 'tsunami' of OA cases in the coming decades. Although aging is a non-modifiable risk factor for the development of OA, there is clinical evidence to suggest that the risk for developing OA can be mitigated and reduced by lifestyle changes that involve weight management (i.e. weight loss and calorie restriction, avoiding obesity/overweight) and maintaining high levels of mobility, thus avoiding the sedentary lifestyles that have become the root cause of most of the world's healthcare issues. At the present time, there are no pharmacological agents available for the treatment of OA. A better understanding of the basic cell signaling mechanisms underlying aging in articular chondrocytes and other cell types in the joint is likely to facilitate the development of new therapeutic strategies for OA, specifically the development of disease-modifying osteoar-

thritis drugs. In this regard, research on TGF-β signaling is more advanced than work on protein kinase and phosphoprotein phosphatase pathways in chondrocytes, and much more research is needed using both human and animal models to understand how calcium signaling and kinase/phosphatase activities are implicated in chondrocyte senescence in the context of OA pathogenesis and progression. Understanding deregulated cell signaling mechanisms in chondrocyte aging may also reveal therapeutic targets and pathways for a range of other age-related osteoarticular disorders. Aging is inevitable, but age-related diseases may be modifiable as long as we have a firm understanding of the molecular gerontology of chondrosenescence in OA. Future research should focus on humans and suitable animal models in order to develop a unified 'one health' therapeutic solution to OA.

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