Review

Age-related degeneration of articular cartilage in the pathogenesis of osteoarthritis: molecular markers of senescent chondrocytes

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Summary. Aging is a natural process by which every single living organism approaches its twilight of existence in a natural way. However, aging is also linked to the pathogenesis of a number of complex diseases. This is the case for osteoarthritis (OA), where age is considered to be a major risk factor of this important and increasingly common joint disorder. Half of the world's population, aged 65 and older, suffers from OA. Although the relationship between the development of OA and aging has not yet been completely understood, it is thought that age-related changes correlate with other risk factors. The most prominent hypothesis linking aging and OA is that chondrocytes undergo premature aging due to several factors, such as excessive mechanical load or oxidative stress, which induce the so called "stress-induced senescent state", which is ultimately responsible for the onset of OA. This review focuses on molecular markers and mechanisms implicated in chondrocyte aging and the pathogenesis of OA. We discuss the most important age-related morphological and biological changes that affect articular cartilage and chondrocytes. We also identify the main senescence markers that may be used to recognize molecular alterations in the extracellular matrix of cartilage as related to senescence. Since the aging process is strongly associated with the onset of osteoarthritis, we believe that strategies aimed at preventing chondrocyte senescence, as well as the identification of new increasingly sensitive senescent markers, could have a positive impact on the development of new therapies for this severe disease.

Key words: Osteoarthritis, Aging, Cartilage, Chondrocyte, Lubricin, Senescence, Senescence markers

Introduction

Our body is made up of an incredibly large number of cells, around 100 billion, some of them have a rather short life, others such as chondrocytes remain for a lifetime, but at a certain point, the mechanism slows down, cell duplication starts to fail and the cells are no longer replaced by other ones. The cells arrest their cell cycle progression. Each cell has a limited number of possible divisions, which is fixed between 50 and 70. The reason lies in the structure of chromosomes that are duplicated at each division in order to obtain one copy for itself and another for the daughter cell that will play the same role in the body. The division process, however, is not perfect, the cellular mechanism fails to copy the ends of chromosomes, the so-called telomeres (Fig. 1). After each cell division, the telomeres become shorter and shorter and are eventually completely worn out and parts of chromosomes that contain essential genetic information begin to erode. At this point the cell is at the end of its life and it approaches death by

activating specific mechanisms. In order to avoid the aging process, a "magic" molecule able to stretch the ends of chromosomes would be necessary. This normally happens in cells and it is due to telomerase. However, telomerase is active only in embryonic cells and in a few adult cells such as cells of the immune system, in the other ones its activity runs out over time. It is for this reason that we talk about "cell senescence". Cellular senescence is strongly associated with the development of several serious diseases such as cancer, diabetes, cardiovascular and neurodegenerative diseases, and osteoarthritis (OA) (Burnet and Berger, 2014). OA is a degenerative joint disease, which affects especially the articular cartilage, leading to pain and stiffness of the affected joint. OA is one of the most disabling musculoskeletal disorders in the world and it affects mostly the elderly population (Musumeci et al., 2014a).

In this review we discuss how cellular senescence can influence the onset of a complex joint disease such as OA. We will discuss the most important features of cellular senescence and how the age-related changes, which arise at cellular and tissue level, influence the development and progression of OA. Lastly, we will list some of the most important senescence markers used to evidence the senescence of chondrocytes.

Osteoarthritis

OA is the most common form of joint disease and affects mainly hips, knees, hands, and feet, leading to severe disability and loss of quality of life, particularly in the elderly population (Musumeci et al., 2013a). Indeed, half of the world's population, aged 65 and older, suffers from OA. It has been estimated that 9.6% of men
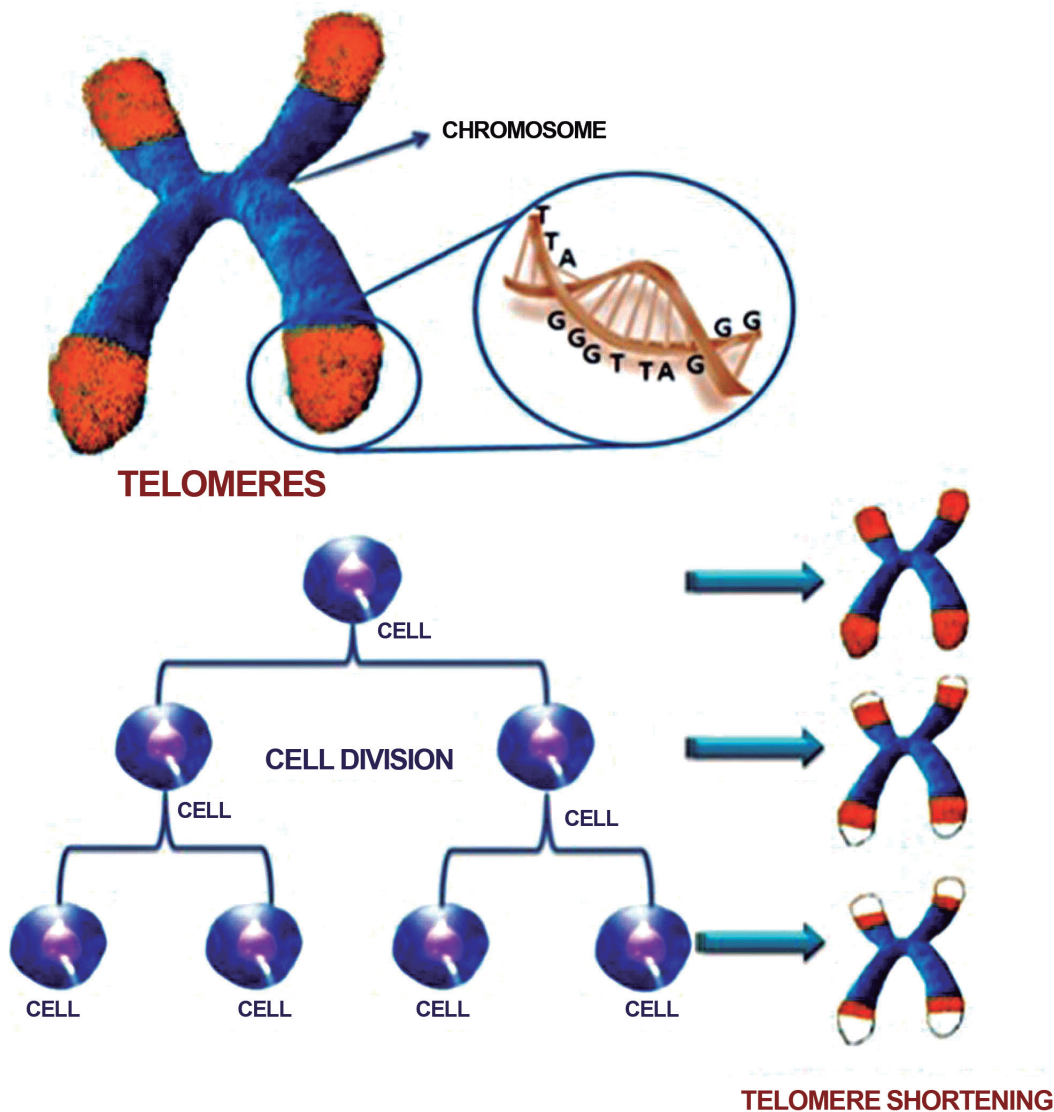

Fig. 1. Telomere shortening. A telomere is a region of repetitive DNA (TTAGGG repeats) at the end of a chromosome, which protects the end of the chromosome from deterioration. Each time cells divide, telomeres shorten, and there is a limit to the number of times a given cell can go on dividing, the so-called "Hayflick limit". When the length of the telomeres is too short cell division stops.

and 18% of women in that age group, have symptomatic OA. Moreover, OA is considered the most important cause of impaired mobility and contributes to 50% of all the musculoskeletal diseases worldwide (Wolf and Pfleger, 2003). The disease is characterized by joint dysfunction due to gradual changes in several structures of the joint such as synovium, subchondral bone and especially articular cartilage (Fig. 2) (Buckwalter and Mankin, 1998a). Progressive wear and tear on articular cartilage can lead to a progressive cartilage tissue loss, further exposing the bony ends, leaving them without protection (Buckwalter and Mankin, 1998b). This finally deteriorates into the most common form of arthritis, or rather moderate OA at early stage (Fig. 3) and severe OA at advanced stage (Fig. 4) (Musumeci et al., 2014a).

The degradation, consequent loss of articular cartilage and formation of osteophytes lead to chronic pain and functional restrictions in the affected joint. Unfortunately, articular cartilage has a limited regenerative capacity (Iwamoto et al., 2013; Musumeci et al., 2013b). Consequently, once injured, cartilage is much more difficult to self-heal and the only way to improve the patient's condition is therapeutic intervention. Different factors can be involved in the development of OA, such as joint injury, genetic predisposition, defective position of joints, obesity, malnutrition and excessive mechanical load, which all lead to similar alterations of the articular cartilage (Goldring and Goldring, 2007; Lee et al., 2013; Musumeci et al., 2014b,c). However, the prevalence of OA rises directly with age, which represents the most prominent risk factor for the initiation and progression of primary OA, but it is important to underline that OA is not a simple "wearing out in time" of the joints and the degenerative changes related to age can be distinguished from those due to the disease (Musumeci et al., 2013c). The relationship between the development of OA and aging is not completely understood and it is thought that the age-related changes are correlated to other risk factors, which may occur concurrently or in conjunction with it. In reality, not all older adults develop OA and OA-like changes can also develop without a significant contribution of aging. Thus, aging and OA are inter-related but not inter-dependent (Loeser, 2004). However, there is also a possibility that the chondrocytes undergo premature aging due to several factors, such as excessive mechanical load or oxidative stress. In the latter case, aging and the development of OA are both inter-related and inter-dependent (Martin et al., 2004a; Akagi, 2010).

Cellular aging

Cellular aging, or cell senescence, refers to the limited capacity of mitotic cells to further multiply in time (over 30-40 divisions). This limit is known as the "Hayflick limit" (Hayflick, 1984). This form of
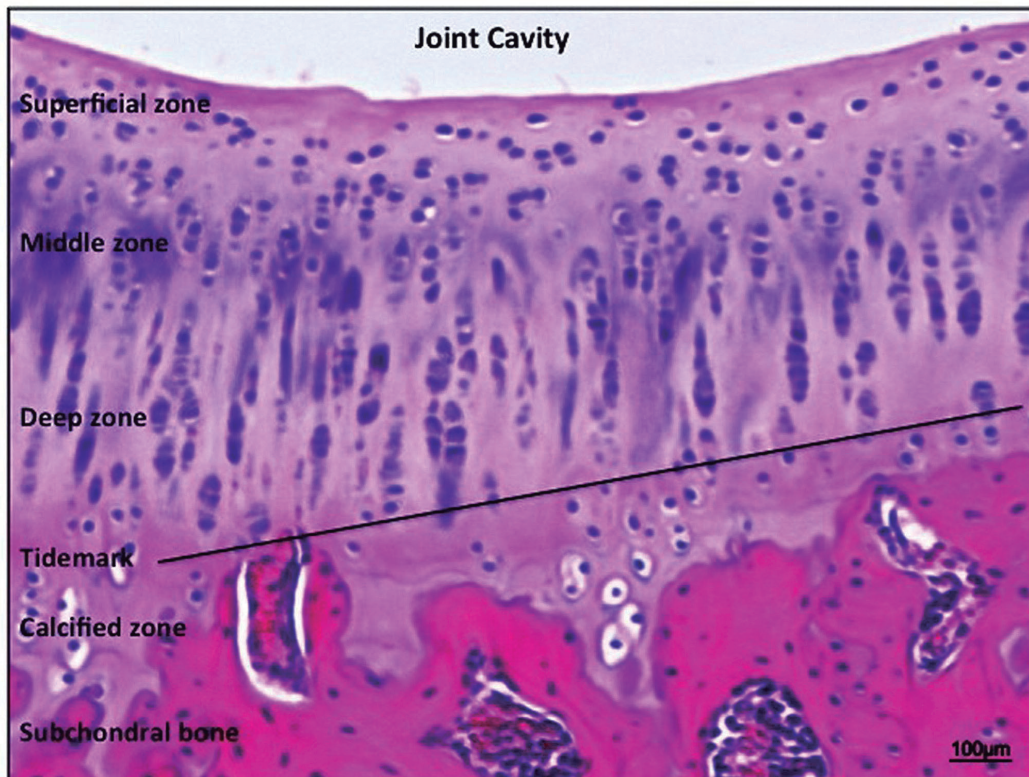

Fig. 2. Healthy articular knee cartilage from rat. Hematoxylin and Eosin staining. Normal articular knee hyaline cartilage layers: superficial zone, intermediate (or middle zone), radial zone (or deep zone) and calcified cartilage (or calcified zone). Tidemark, the border between non-calcified and calcified cartilage. In the superficial zone, cells are flat and small; in the middle and deep zone, cells are organized in columns; the tidemark is evident. Scale bars: 100 *μ*m.

senescence is called "replicative senescence", also known as intrinsic senescence, which results from an arrest in cell-cycle progression. Some of the changes exhibited by cells, which have undergone replicative senescence can be found in cells in older adults, such as shortened telomeres, formation of senescence-associated (SA) heterochromatin (Muller, 2009) and changes of phenotype with an alteration in gene expression (Bodnar et al., 1998). It has been hypothesized that the telomere length could be considered as a marker for replicative senescence. Telomeres cannot be completely replicated in primary cells and become shorter with each round of cell division. Telomeres are nucleoprotein structures (TTAGGG repeats) that cap the ends of the linear eukaryotic chromosomes and thereby protect their stability and integrity during replication by protecting chromosome ends against exonucleases (Fig. 1). Telomeres are replicated by a special reverse transcriptase called telomerase, in a complex mechanism that is coordinated with the genome's replication. Telomerase is an RNA-dependent DNA polymerase that synthesizes telomeric DNA sequences and comprises two essential components. One is the functional RNA component (in humans called hTERC), which serves as a template for telomeric DNA synthesis. The other is a catalytic protein (hTERT) with reverse transcriptase activity and the primary determinant for the enzyme

activity (Bryan and Cech, 1999; Kupiec, 2014). The level of telomerase in normal human somatic tissues is insufficient to prevent telomere shortening. Telomeres can be lengthened through increasing telomerase activity by exogenous expression of hTERT or hTR (the RNA template) (Greider, 1998). As proof of this concept the chondrocytes transduced with hTERT proved to be able to increase telomere length and therefore to prolong cell lifespan, increasing in this way the efficacy of cartilage repair (Martin and Buckwalter, 2003). Another type of senescence is "stress-induced senescence", also known as extrinsic senescence, which is independent of telomere length. In quiescent cells such as chondrocytes, this type of senescence may be more important than the replicative version, because progressive telomere shortening cannot completely explain senescence in these post-mitotic cells (Ben-Porath and Weinberg, 2005; Chen and Goligorsky, 2006). The various types of stress responsible for this kind of senescence include DNA damage, oxidative stress, oncogene activity, ultraviolet radiation and chronic inflammation (Itahana et al., 2004; Campisi, 2005). Oxidative stress is thought to play a major role as a stressor. It results when the amount of reactive oxygen species (ROS) exceeds the anti-oxidant capacity of the cell. ROS are generated by intracellular enzymes such as nicotine amide adenine dinucleotide phosphate (NADPH) oxidase and 5-
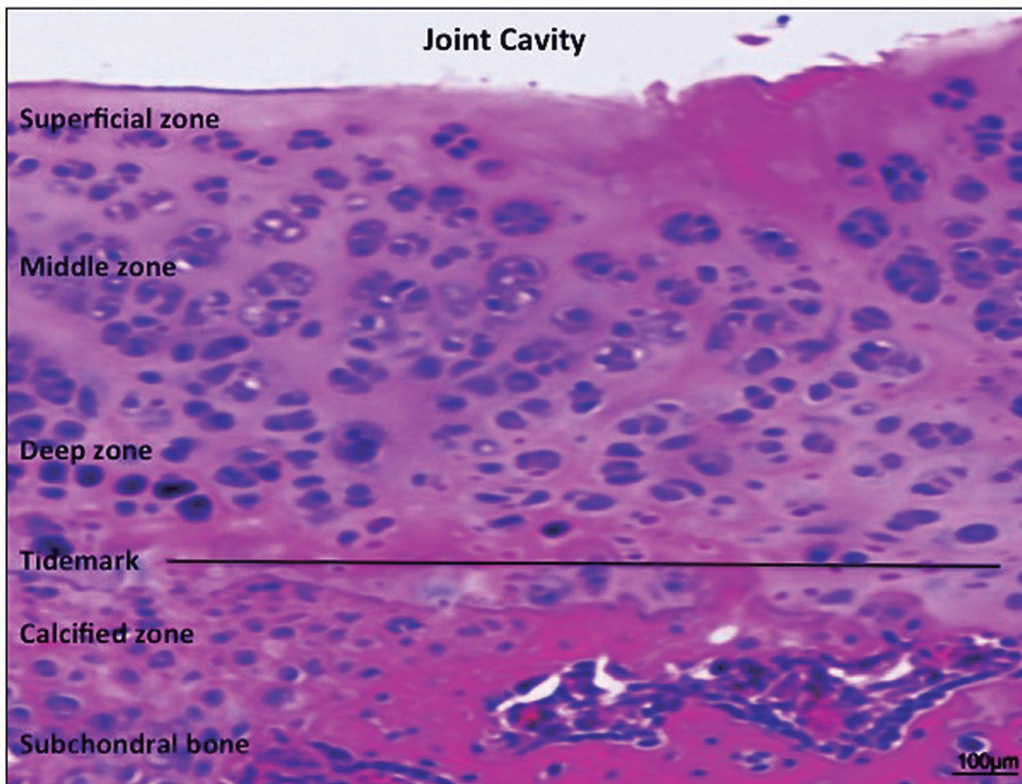

Fig. 3. Moderate OA articular knee cartilage from rat. Hematoxylin and Eosin staining. Articular knee hyaline cartilage layers at early OA stage: superficial zone, intermediate (or middle zone), radial zone (or deep zone) and calcified cartilage (or calcified zone). Tidemark, the border between non-calcified and calcified cartilage. Clear deep fissures in the articular surface, the cells from the superficial, intermediate and deep zone, where chondrocytes are not arranged in columns. The tidemark is not intact in all its extension and the subchondral bone shows little fibrillation. Scale bars: 100 μm.

lipoxygenase in response to activation of specific cell signaling pathways (Kamata and Hirata, 1999; Finkel and Holbrook, 2000). A direct role for increased ROS levels in promoting cell senescence is a positive feedback activation of the ROS-protein kinase C delta (PKCδ) signaling pathway, which cooperates with the p16^{INK4A}-retinoblastoma protein (Rb) pathway, which plays an important role in the control of cell-cycle progression (Takahashi et al., 2006). Telomere shortening is also observed in stress-induced senescence and it is due to oxidative damage to DNA caused by ROS. The ends of chromosomes are particularly sensitive to oxidative damage, which causes telomere erosion similar to that seen with replicative senescence (Yudoh et al., 2005). Also, the ROS generated from excessive mechanical loading and stimulation of cytokines contribute to DNA damage, which subsequently results in telomere shortening (Tomiyama et al., 2007; Davies et al., 2008). Cellular senescence, as well as apoptosis, can be viewed as a powerful tumor-suppressor mechanism that withdraws cells with irreparable DNA damage from the cell cycle (de Lange and Jacks, 1999; Artandi and DePinho, 2000; Puzzo et al., 2014) through the intrinsic or mitochondrial (Loreto et al., 2011a; Caltabiano et al., 2013) and extrinsic apoptosis pathway (Loreto et al., 2011b; Cardile et al., 2013). Several recent studies report that cartilage degeneration also coincides with increased apoptotic

chondrocytes (Musumeci et al., 2011a,b; Galanti et al., 2013). Therefore, the senescence signals, that is, a telomere-based one or a stress-based one, trigger a DNA damage response and this response shares a common signaling pathway that converges on either or both of the well-established two tumor- suppressor proteins, p53 (the p53-p21-pRb pathway) (Martin and Buckwalter, 2003; Herbig and Sedivy, 2006) and RB and pRb proteins (the p16-pRb pathway) (Musumeci et al., 2010, 2011c). In the p53-p21-pRb pathway, senescence stimuli activate the p53, which then can induce senescence by activating pRb through p21, which is a transcriptional target of p53. This senescence can be reversed upon subsequent inactivation of p53. In the p16-pRb pathway, senescence stimuli induce p16, which activates pRb. Once the pRb pathway is engaged by p16, the senescence cannot be reversed by subsequent inactivation of p53, silencing of p16 or inactivation of pRb (Beauséjour et al., 2003). The difference between these two pathways is that the p53-p21-pRb pathway mediates the senescence due to telomere shortening and the p16-pRB pathway is thought to mediate premature senescence (Beauséjour et al., 2003). Once cells have entered senescence, they are arrested in the G1 phase of the cell cycle and display a characteristic morphology (vacuolated, flattened cells) and altered gene expression (Cristofalo et al., 2004). The senescent cells exhibit the so-called "senescent secretory phenotype" (SSP), which
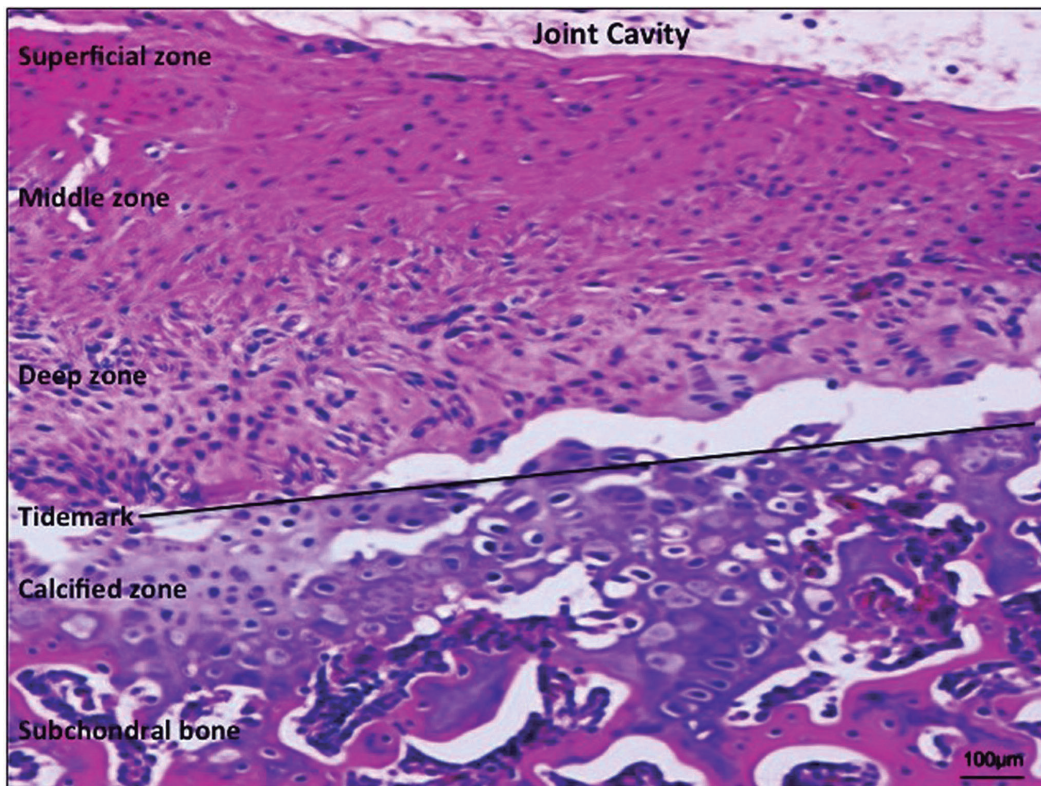

Fig. 4. Severe OA articular knee cartilage from rat. Hematoxylin and Eosin staining. Articular knee hyaline cartilage layers at advanced OA stage, due to aging: superficial zone, intermediate (or middle zone), radial zone (or deep zone) and calcified cartilage (or calcified zone). Tidemark, the border between non-calcified and calcified cartilage. Cells are arranged in clusters especially around fissures or disappear completely as the disease progresses. The organization of cartilage is completely disordered and replaced by fibrocartilaginous, scar-like tissue with fibroblast like cells. Scale bars: 100 *μ*m.

could be also correlated with the development of OA. It is interesting to note that the senescent cells, which are mitotically inactive, are biologically active (Campisi, 2005). These cells are able to increase the expression of genes that inhibit proliferation and to increase the secretion of several proteins, including inflammatory cytokines such as interleukin-6 (IL-6) and interleukin-1β (IL-1β), degradative enzymes such as metalloproteinases (MMPs) and growth factors such as epidermal growth factor (EGF) that regulate cell proliferation and all of which may stimulate tissue aging and tumorigenesis (Zhang et al., 2003). Recent studies reported that the increased expression of the IL-8 receptor CXCR2 and insulin-like growth factor binding protein 7 (IGFBP7) could also contribute to cell aging (Acosta et al., 2008; Wajapeyee et al., 2008). The accumulation of cells expressing the SSP can also contribute to tissue senescence by impairing the extracellular matrix due to the increased secretion of degradative enzymes (Campisi and d'Adda di Fagagna, 2007). Another important feature of senescent cells is represented by the epigenetic changes related to the formation of foci of heterochromatin, referred to as senescence-associated heterochromatin foci (SAHFs), which include histone variants such as the macro-H2A (Zhang and Adams, 2007).

Age-related articular cartilage degeneration

Articular cartilage matrix undergoes several changes with age, including structural, molecular and mechanical ones, surface fibrillation, alterations in structure and composition of proteoglycans, increased collagen cross-linking and decreased tensile strength and stiffness (Hollander et al., 1995; Squires et al., 2003) (Fig. 5). Deterioration in chondrocyte function accompanies these changes also in the extracellular matrix (Aurich et al., 2002). Several reports revealed that chondrocyte senescence contributes to the risk for cartilage degeneration by the decreased ability of chondrocytes to maintain and repair the articular cartilage tissue (Martin and Buckwalter, 2001a; Aigner et al., 2002). There is clinical evidence from Magnetic Resonance Imaging (MRI) studies that the articular cartilage in the knee thins with aging, especially at the patella and at the femoral side of the joint (Hudelmaier et al., 2001; Ding et al., 2005). The progressive articular cartilage thinning with age is related to gradual loss of cartilage matrix and decrease in cartilage hydration and cellularity. This kind of damage stimulates a chondrocyte specific synthetic and proliferative response that may maintain or even restore the articular cartilage. This response may continue for years. However, in instances of progressive joint degeneration the anabolic response eventually declines and the imbalance between chondrocyte synthetic activity and degradative activity leads to progressive thinning of articular cartilage. These alterations may further accelerate the loss of articular cartilage (Buckwalter et al., 2000). Different changes

observed in articular cartilage with aging are probably due to chondrocyte senescence, which results in the progressive decrease in cell function. In fact, the mitotic and synthetic activity of human chondrocytes decline with age. They become less responsive to anabolic mechanical stimuli, to anabolic cytokine and to insulin-like growth factor I (IGF-I). The cells synthesize smaller aggrecans and less functional link proteins leading to the formation of smaller and more irregular proteoglycan aggregates. The latter is the most striking change in articular cartilage matrix related with age. Aggrecans are the molecules that give articular cartilage its stiffness to compression, resilience and durability, thus their alteration makes the tissue more vulnerable to injury and development of progressive degeneration (Buckwalter et al., 1986; Buckwalter and Rosenberg, 1988; Bolton et al., 1999; Martin and Buckwalter, 2000). Moreover, the senescent cartilage matrix appears more susceptible to the accumulation of advanced glycation end-products (AGEs) in cartilage collagen, which results in increased cross-linking and in subsequent increased stiffness, making the cartilage more susceptible to fatigue failure (Verzijl et al., 2002). In addition, the increased levels of AGEs in articular cartilage may also affect chondrocyte function by decreasing its anabolic activity. The mechanism proposed to be responsible for this alteration is the expression of the receptor for the advanced glycation end-products (RAGE) by chondrocytes, which proves to be increased both with aging and in development of OA (DeGroot et al., 1999; Loeser et al., 2005). Stimulation of chondrocyte RAGE results in increased production of MMPs and in modulation of the chondrocyte phenotype to hypertrophy, which represent two hallmarks of OA (Cecil et al., 2005; Yammani et al., 2006). Furthermore, RAGE signaling is also associated with increased levels of ROS, providing another link between oxidative stress, aging and OA (Loeser, 2004). Another important feature of the aged articular cartilage is its increased calcification, as demonstrated radiographically (Felson et al., 1989). This could be associated with the increased activity of transglutaminase, involved in the biomineralization process (Rosenthal et al., 1997) and with increased production of the inorganic pyrophosphate in response to transforming growth factor β (TGF-β) stimulation (Felson et al., 1989). Chondrocalcinosis is strongly associated with OA, but there is evidence of older people with asymptomatic chondrocalcinosis, thus it proves not to be inter-dependent with the development of OA and its role is not completely clear (Doherty and Dieppe, 1988; Rosen et al., 1997).

Chondrocyte senescence

Chondrocytes from older adults exhibit many changes, typical of cell senescence, when compared with cells isolated from young adults. The most evident change is represented by telomere shortening, characteristic of replicative senescence. This evidence is

controversial as adult articular chondrocytes rarely, if ever, divide in normal tissue *in vivo*. The lack of cell division in normal adult articular cartilage suggests that the chondrocytes present in the cartilage of an older adult are likely to be the same cells that were present decades earlier. This fact makes these cells more susceptible to the accumulation of changes from both aging and extrinsic stress. In fact, it is most likely that chondrocyte senescence is the extrinsic type, induced by different stressors. The telomere shortening in adult chondrocytes could be due to DNA damage from ROS as discussed further above (Mankin, 1963; Martin and Buckwalter, 2001b; Martin et al., 2004b). The increased ROS levels could be both age-related (Del Carlo and Loeser, 2003) and generated from excessive mechanical loading and/or stimulation by cytokines (Kurz et al., 2005; Davies et al., 2008). There is also evidence for reduced levels of antioxidant enzymes in cartilage with aging and in OA that would contribute to chondrocyte senescence and oxidative damage. In human articular chondrocytes, decreased levels of mitochondrial superoxide dismutase were found both with aging and in OA cells (Finkel and Holbrook, 2000). Moreover, it has been hypothesized that joint injury accelerates chondrocyte senescence and that this acceleration plays a role in the joint degeneration responsible for post-

traumatic OA. Indeed, excessive loading of articular surfaces due to acute joint trauma or post-traumatic joint instability, incongruity or mal-alignment increases release of ROS, and the increased oxidative stress on chondrocytes accelerates chondrocyte senescence (James et al., 2004). Other important features of chondrocyte senescence are the exhibition of SSP, which has important implications in development and progression of OA and the decline in the proliferative and anabolic response to growth factors, as well as their reduction in cartilage. It has been noted that senescent chondrocytes lose the ability in response to: IGF-I, which is known to be an important autocrine survival factor that stimulates cartilage matrix synthesis (Martin et al., 1997); TGF-β, an important cartilage anabolic factor (Scharstuhl et al., 2002) and to bone morphogenetic protein 6 (BMP-6), known to stimulate proteoglycan synthesis (Bobacz et al., 2003). Chondrocyte senescence also contributes to the decline in the cell number within the cartilaginous tissue, due to increased cell death. Several studies demonstrated the loss of cellular density in cartilage with aging or/and in OA (Vignon et al., 1976; Adams and Horton, 1998; Horton et al., 1998; Aigner et al., 2004a; Kuhn et al., 2004). These findings provide evidence to support the concept that chondrocyte senescence may be involved in the progression of cartilage degeneration,
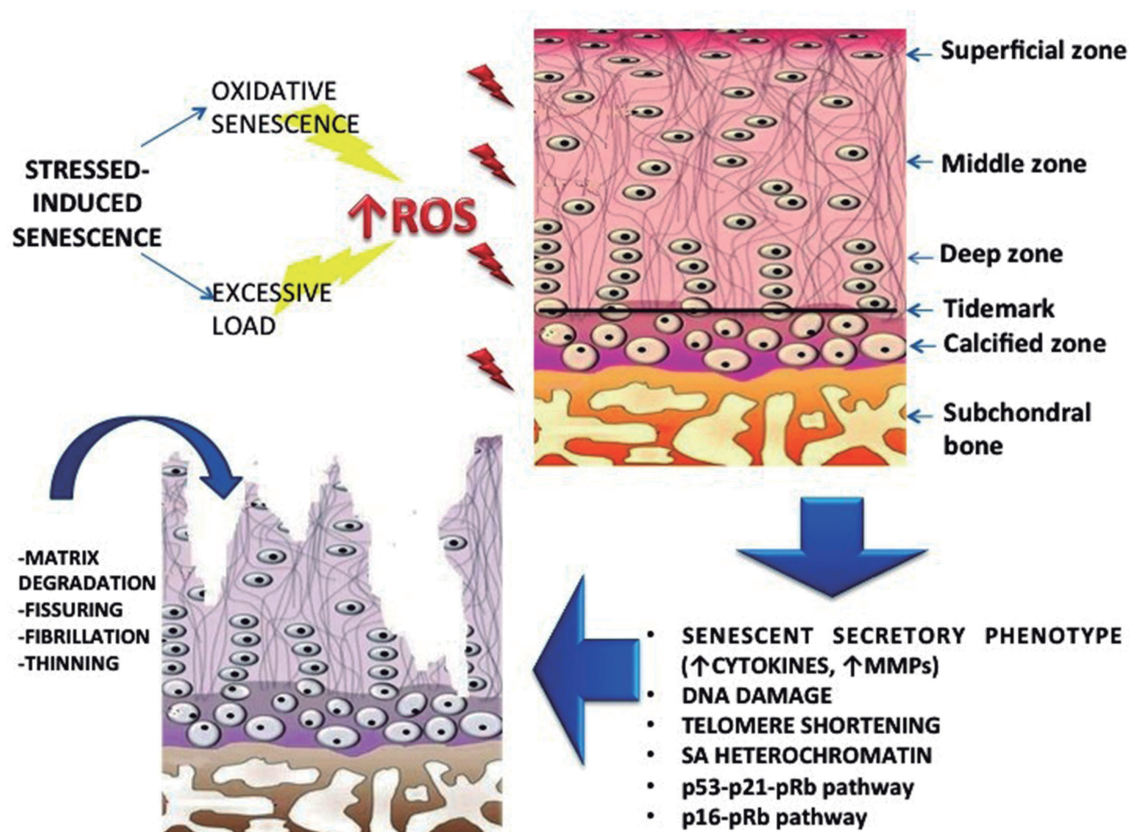

Fig. 5. Stress-induced senescence and Osteoarthritis. The telomere shortening process in senescent chondrocytes is more probably due to the stress-induced type of senescence. Oxidative stress and excessive mechanical loading are thought to be the major stressors that induce the increased production of ROS, which are responsible for DNA damage and for the subsequent senescence of the cells. Once cells have entered senescence, they are arrested in the G1 phase of the cell cycle and they display a characteristic gene expression called "senescent secretory phenotype", which is strongly correlated with the development of OA.

because of their decreased ability to maintain or restore the articular cartilage (Fig. 5).

Chondrocyte senescence markers

An altered gene expression pattern on the cellular level appears to be one potentially important facet of chondrocyte behavior in OA cartilage (Aigner et al., 2004b, 2007). The diversification of gene expression in senescent chondrocytes is due to stochastic DNA damage, which represents a core mechanism in cellular aging in general and in OA cartilage degeneration in particular. The evidence of senescence in chondrocytes can be investigated using several senescence markers, such as senescent-associated-β-galactosidase (SA-βgal), highly condensed domains of facultative heterochromatin SAHF, increased p53, p21, pRb and p16^{INK4a} (Fig. 5). Staining for SA-βgal has been shown to be present in articular chondrocytes from older adults and in OA chondrocytes (Martin and Buckwalter, 2001b; Price et al., 2002). SA-βgal is related to the detection of increased levels of the lysosomal enzyme β-galactosidase at pH 6.0 rather than at the normal pH 4.5. Detection of its activity at pH 6.0 is thought to be due to an increase in lysosomal mass (Itahana et al., 2007). Chondrocyte SA-βgal staining, as well as telomere shortening, has also been noted after treatment *in vitro* with IL-1β or H_2O_2 consistent with stress-induced senescence (Dai et al., 2006). Although SA-βgal is a useful senescence marker, its activity is critically dependent on the detection conditions, and SA-βgal is also expressed in the non-senescent cells that have a high lysosomal content (Kurz et al., 2000; Matthews et al., 2006). Multiple markers of senescence are therefore recommended to demonstrate senescence *in vivo*. SAHF are thought to repress expression of proliferation-promoting genes, thereby contributing to senescence-associated proliferation arrest. Inclusion of proliferation-promoting genes, such as cyclin A, into these compact chromatin foci is thought to silence expression of those genes, which are associated with cell cycle arrest (Adams, 2007). *Ink4a* encodes an archetypical cyclin-dependent inhibitor (CKI) associated with senescence.

Indeed, the over-expression of p16^{INK4a} in chondrocytes is associated with SSP, which includes increased production of pro-inflammatory cytokines (such as IL-6, IL-8, IL-1β) and matrix remodeling regulatory metalloproteinases (such as MMP1, MMP13, etc) (Leonardi et al., 2008; Loreto et al., 2013). As mentioned above, all these factors are deleterious for cartilage integrity. According to this finding, the repressed levels of miR-24, a negative regulator of p16^{INK4a}, was also found in OA cartilage (Philipot et al., 2014). Recently, the expression of Caveolin1, a protein that participates in premature cellular senescence, was also investigated in human OA cartilage. It was observed that the treatments with catabolic factors of oxidative stress (H_2O_2) and IL-1β, which simulate the OA environment, was able to up-regulate the expression of caveolin1. The over-expression of caveolin1 is associated with cartilage degeneration and the mediation of the premature senescence in OA chondrocytes by activating p38 MA0PK, which impair the ability of chondrocytes to produce type II collagen and aggrecan (Dai et al., 2006). Other important senescence markers are represented by telomere length and telomerase activity. As discussed in detail above, telomere shortening is the most representative feature of cellular aging, and it is due to the decreased expression of telomerase with time which leads to telomere instability. Telomere length can be measured by using the Single Telomere Length Assay (STELA), Southern blot analysis, Q-PCR and the more recent Quantitative-Peptide Nucleic Acid-Fluorescent *in situ* Hybridization assay (Q-PNA-FISH) (Cukusic et al., 2014). Telomerase activity can be measured for example by using a Telomere Repeat Amplification Protocol (TRAP) (Zhou and Xing, 2012) (Table 1). Recently, several studies have been focused on the expression of lubricin, also known as proteoglycan 4 (PRG4) or superficial zone protein (SZP), in different experimental conditions, in particular in conjunction with physical activity (Musumeci et al., 2013d). Lubricin is a chondroprotective glycoprotein that serves as a critical boundary lubricant between opposing cartilage surfaces (Musumeci et al., 2013e; Leonardi et al., 2011). It has a major protective role in

Table 1. An overview of the key markers for the senescent chondrocytes and their functions.

CHONDROCYTE SENESCENCE MARKERS

Senescence markers	Function
SA-βgal	Hydrolase enzyme that catalyzes the hydrolysis of β-galactosides into monosaccharides only in senescent cells.
SAHF	Packaging of proliferation-promoting genes in cellular senescence.
p53	Transcription factor involved in cell-cycle control, DNA repair, apoptosis, cellular stress responses and modulation of cellular senescence.
p21	Universal inhibitor of the cyclin-dependent kinases 1 required to arrest cells at the G1 and G2 checkpoints of the cell cycle after DNA damage.
pRb	Potent repressor of genes that functions during DNA replication and thus causes cell cycle arrest.
p16	Tumor suppressor protein that plays an important role in cell cycle regulation by decelerating cell progression from G1 phase to S phase.
Caveolin 1	Protein that induces p38 MAPK activation and impairs the ability of chondrocytes to produce type II collagen and aggrecan.
Telomere length	Telomere length shortens with each cell replication and characterizes cellular senescence.
Telomerase activity	Telomerase activity diminishes with age and loses its ability to stabilize the shortened telomeres.

preventing cartilage wear and synovial cell adhesion, proliferation, and in reducing the coefficient of friction of the articular cartilage surface (Musumeci et al., 2013f; Leonardi et al., 2012a,b). Since lubricin has a fundamental role in maintaining the homeostasis of the articular cartilage and in preventing its degeneration, we hypothesized that its expression would decrease in senescent chondrocytes and that it could be evaluated as a new specific chondrocyte senescence marker. These data have been confirmed in our recent and interesting study in which we demonstrated the decreased expression of lubricin is associated with chondrocytes senescence as well as with OA (Musumeci et al., 2014d).

Conclusions

Although the direct relationship between the aging process and the development of OA is not completely understood, we may surmise that chondrocyte senescence contributes to cartilage degeneration by impairing the ability of these cells to maintain and repair the cartilage tissue. Moreover, we have also seen that these two processes (aging and OA) could be inter-dependent. There are several lines of evidence that suggest chondrocytes exposed to the "osteoarthritic environment" are characterized by oxidative stress and production of cytokines, and this induces the so-called stress-induced senescent state, which may contribute to cartilage degeneration as we have discussed above. All these observations suggest that a better understanding of the changes arising with age in articular cartilage and how they influence the response of the tissue to different stressors, as well as the identification of new increasingly sensitive senescence markers, would be very useful in the preventive detection of the disease and in its consequent treatment. Further research is required to unravel the detailed mechanisms of senescence related to the pathogenesis of OA. Strategies aimed at preventing chondrocyte senescence could have a positive impact on the development of new therapies for OA and on halting the progression of this severe disease.

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