Cellular senescence in aging and osteoarthritis

Implications for cartilage repair

Wei Seong TOH [1,2], Mats BRITTBERG [3,4], Jack FARR [5], Casper Bindzus FOLDAGER [6], Andreas H GOMOLL [7], James Hoi Po HUI [2,8], James B RICHARDSON [9,10], Sally ROBERTS [9,10], and Myron SPECTOR [11,12]

[1] Faculty of Dentistry, National University of Singapore; [2] Tissue Engineering Program, Life Sciences Institute, National University of Singapore, Singapore; [3] Cartilage Research Unit, University of Gothenburg, Gothenburg; [4] Department of Orthopaedics, Kungsbacka Hospital, Kungsbacka, Sweden; [5] Indiana University School of Medicine, OrthoIndy Cartilage Restoration Center, Indianapolis, IN, USA; [6] Orthopaedic Research Laboratory, Aarhus University Hospital, Aarhus, Denmark; [7] Cartilage Repair Center, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [8] Cartilage Repair Program, Therapeutic Tissue Engineering Laboratory, Department of Orthopaedic Surgery, National University Health System, National University of Singapore, Singapore; [9] Robert Jones and Agnes Hunt Orthopaedic Hospital, Oswestry, Shropshire; [10] Institute for Science andTechnology in Medicine, Keele University, Keele, Staffordshire, UK; [11] Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [12] Tissue Engineering Laboratories, VA Boston Healthcare System, Boston, MA, USA.
Correspondence: dentohws@nus.edu.sg
Submitted 2016-03-14. Accepted 2016-05-16.

Abstract — It is well accepted that age is an important contributing factor to poor cartilage repair following injury, and to the development of osteoarthritis. Cellular senescence, the loss of the ability of cells to divide, has been noted as the major factor contributing to age-related changes in cartilage homeostasis, function, and response to injury. The underlying mechanisms of cellular senescence, while not fully understood, have been associated with telomere erosion, DNA damage, oxidative stress, and inflammation. In this review, we discuss the causes and consequences of cellular senescence, and the associated biological challenges in cartilage repair. In addition, we present novel strategies for modulation of cellular senescence that may help to improve cartilage regeneration in an aging population.

Articular cartilage undergoes substantial changes in matrix structure, molecular composition, metabolic activity, and mechanical properties—and hence functions—with aging. These changes include fibrillation, alteration of proteoglycan structure and composition, decreased anabolic activity, increased collagen cross-linking, and reduced tensile strength and stiffness (Martin and Buckwalter 2001, 2003). These age-related changes can result in impaired efficacy of cartilage repair, with current treatments such as microfracture (Steinwachs et al. 2008, Miller et al. 2010), mosaicplasty (Marcacci et al. 2005), and cell-based therapies (Kon et al. 2011, Kim et al. 2015), and also contribute to an increased incidence of osteoarthritis (OA) (Roos et al. 1995). Studies of OA following joint injuries have shown that the risk of developing posttraumatic OA following an intra-articular fracture of the knee increases by 3–4 fold after 50 years of age (Volpin et al. 1990, Honkonen 1995). The clinical outcomes of current treatment modalities are generally unsatisfactory for most, but not all, older patients. Recent studies have suggested that with advancing age, there is an increasing risk of poor repair and/or treatment failure (Krishnan et al. 2006, Kim et al. 2015).

The incidence of OA rises dramatically with every passing decade (Buckwalter et al. 2001), yet the disease does not affect every individual. Indeed, several studies have suggested that aging increases the risk of OA by compromising the ability of articular cartilage to maintain or restore tissue functioning after injury (Buckwalter et al. 2001). Cell senescence, the loss of the ability of cells to divide, has been noted as the major factor in contributing to aging changes in cartilage homeostasis and function. Cell senescence, which develops during long-term culture of chondrocytes or tissue-derived mesenchymal stem cells (MSCs), is also a major problem in cellular transplantation for cartilage repair (Li and Pei 2012). The underlying mechanisms of cell senescence are not fully understood, but have been increasingly associated with telomere erosion, DNA damage, oxidative stress, and inflammation.

While the cumulative changes over time are by definition "age-related," chronologic age itself may be less important than genetically determined factors modified by additional risk factors ranging from joint alignment to injury, to activity level, to obesity. Given the anticipated "aging epidemic", it is important to address the causes and effects of aging on cartilage degeneration.

© 2016 The Author(s). Published by Taylor & Francis on behalf of the Nordic Orthopedic Federation. This is an Open Access article distributed under the terms of the Creative Commons Attribution-Non-Commercial License (https://creativecommons.org/licenses/by-nc/3.0)
DOI 10.1080/17453674.2016.1235087

Aging and cellular senescence

First described by Hayflick and Moorhead (1961), cellular senescence is a phenomenon of irreversible cell growth arrest after a characteristic number of cell doublings. Both intrinsic and extrinsic mechanisms/pathways are known. Replicative senescence is associated with the replication limit (Hayflick limit) of the cell, and is triggered as a result of exhausted replicative capacity caused by telomere shortening. The other senescence pathway, known as stress-induced premature senescence (SIPS), may be induced by extrinsic stressors such as DNA damage and oxidative stress, giving premature cell cycle arrest. Typical hallmarks of cellular senescence include enlarged cell morphology, reduced telomere length, upregulated p21, p16, and p53 expression, heightened reactive oxygen species (ROS) levels, and elevated senescence-associated β-galactosidase (SA-β-Gal) activity. In addition, senescent cells show altered intracellular protein expression, altered responses to growth factors, and altered secretion profiles such as elevated levels of ROS and pro-inflammatory cytokine production, which contribute further to overall aging and progression of age-related diseases (Loeser 2009, Ashraf et al. 2016).

Age-dependent changes

Accumulation of senescent cells with age has been observed in most tissues, including cartilage and bone (Campisi 2011). Cell yields of chondrocytes from articular cartilage were found to be 2-fold lower in older human donors (> 40 years of age) than in younger donors (Barbero et al. 2004). Cell proliferation rates and matrix biosynthetic activities (aggrecan and type-II collagen) of chondrocytes also appear to decline after the age of 20 (Pestka et al. 2011). Similarly, Adkisson et al. (2010) reported a dramatic difference in chondrogenic potential between chondrocytes from juvenile donors (< 13 years old) and those from adult donors, with 100 times more proteoglycan in neocartilage produced by juvenile chondrocytes. Recently, Liu et al. (2013) compared the biological properties of juvenile and adult bovine articular cartilage. In that study, juvenile bovine cartilage showed a greater cell density, a higher cell proliferation rate, increased cell outgrowth, elevated glycosaminoglycan (GAG) content, and enhanced matrix metalloproteinase (MMP)-2 activity. These physiological age-dependent changes may partly explain the inferior clinical outcomes of autologous chondrocyte implantation (ACI) and mosaicplasty performed in older patients (Marcacci et al. 2005, Kon et al. 2011). MSCs are a source of chondrocytes and osteoblasts that make up cartilage and bone. MSCs are emerging as a promising alternative source of cells for treatment of cartilage defects (Nejadnik et al. 2010) and OA (Kim et al. 2015). A number of studies that have examined the differences in proliferation and differentiation potentials of animal and human MSCs isolated from young and aged donors have given conflicting results (Stolzing et al. 2008, Zhou et al. 2008, Choudhery et al. 2014), which may be attributable to the different sources of MSCs and culture conditions used.

Unlike bone marrow MSCs from young donors, bone marrow MSC cultures from aged donors have been found to consist mainly of cells showing signs of cellular aging—including enlarged cell morphology, accumulation of ROS levels, upregulated p21 and p53 expression, and increased SA-β-Gal activity (Stolzing et al. 2008, Zhou et al. 2008). Accordingly, there was an age-dependent decrease in functional colony forming ability, viability, proliferation, and differentiation capabilities, revealing intrinsic alterations in bone marrow MSCs with aging and also their contribution to the overall process of skeletal aging (Baxter et al. 2004, Zhou et al. 2008). These age-dependent changes have important implications when using autologous MSCs for treatment of cartilage lesions and OA. In a recent study, Kim et al. (2015) evaluated the efficacy of autologous adipose MSC therapy in treatment of OA in 49 patients (55 knees), with a mean follow-up period of 2 years. They found that adipose MSC treatment led to promising clinical outcomes. However, age was identified as a major independent predictor of clinical failure after MSC implantation, with the age of 60 years being the cutoff value for obtaining encouraging outcomes after implantation.

Collectively, age-related changes not only affect the structural and matrix composition of articular cartilage but also the properties and functions of chondrocytes and MSCs, with serious implications for the success of an autologous cellular treatment and for the clinical outcome in the elderly population.

Senescent cells and cartilage degeneration

The age-dependent differences observed in chondrocytes and MSCs harvested from young and aged donors raise concerns that senescent cells including chondrocytes and MSCs accumulate in vivo with age, and that they may contribute to alterations in cartilage maintenance and homeostasis, leading to cartilage degeneration (Martin and Buckwalter 2001). In a seminal study in mice, Baker et al. (2011) showed that the onset of age-related pathologies of at least the adipose tissue, skeletal muscle, and eye could be delayed by selective clearance of $p16^{INK4a}$-positive senescent cells, a typical marker for cellular senescence. Importantly, this study suggested that senescent cells have a role in aging and progression of age-related diseases.

Molecular mechanisms of cellular senescence

In recent years, we have begun to understand the molecular mechanisms underlying the contribution of cell senescence to the initiation and progression of OA. However, the causal relationship is often difficult to establish, and many questions remain. Does aging induce oxidative stress and/

or inflammation, or does some other factor induce oxidative stress and/or inflammation, which in turn drive aging? What are the underlying mechanisms that determine normal (i.e. disease- and injury-free) articular cartilage aging as opposed to articular cartilage degeneration that leads to OA? There are as yet no definitive answers to these questions. Here, we will summarize our current understanding of the mediators of cellular senescence and their implications for aging and OA development.

Telomeres

Several attempts have been made to investigate the roles of telomeres in chondrocyte and MSC senescence (Martin and Buckwalter 2003, Baxter et al. 2004). Primitive cell sources such as human embryonic and fetal MSCs have been reported to have longer telomeres and telomerase activity, and senesce later in culture than their adult counterparts (Guillot et al. 2007). The telomere length of articular chondrocytes in 1- to 72-year-old donors ranges from 9 to 11 kbp in > 55-year-old individuals to 12 kbp in individuals less than 20 years old (Martin and Buckwalter 2003). Similarly, the telomere length of human MSCs ranges from 10 to 11 kbp in fetal sources to as low as 7 kbp in adult bone marrow MSCs (Guillot et al. 2007). Baxter et al. (2004) further confirmed that telomeres shorten in vivo by an average of 17 bp per year in postnatal human bone marrow MSCs. In a study by Parsch et al. (2004), the telomere length of expanded human bone marrow MSCs was found to be shorter than those from expanded chondrocytes from the same donor (11 kbp as opposed to 13 kbp), and it tended to remain shorter after differentiation in chondrogenic spheroids. These subtle inconsistencies regarding telomere length could, however, be attributed to the different techniques used in measurement of telomere length. Even so, it is commonly agreed that cells undergo gradual erosion/shortening of the telomeres as a function of replication during aging—both in vitro and in vivo (Harley et al 1990).

Telomere shortening may be induced and accelerated by oxidative stress and DNA damage. Recent studies have found accelerated telomere attrition and senescence in human chondrocytes (Brandl et al. 2011a) and MSCs (Brandl et al 2011b) when they were cultured under sub-lethal and prolonged treatment with low doses of oxidative stress. Furthermore, telomere dysfunction is linked to a decline in mitochondrial biogenesis/function through activation of p53 and repression of PGC-1α/β (peroxisome proliferator-activated receptor gamma, coactivator 1 alpha and beta), and a consequent decrease in mitochondrial mass and energy production (Sahin et al. 2011).

The role of telomere shortening in OA is less clear. Harbo et al. (2012) reported an association between the presence of ultra-short telomeres and mean telomere length on the one hand and proximity to the lesion, severity of OA, and the level of senescence on the other. However, in another study by Rose et al. (2012), OA cartilage and normal cartilage from autopsies were compared. In that study, a higher degree of genomic DNA damage was detected in OA compared to normal chondrocytes, but there was no evidence of critical telomere shortening.

p53, p16, and p21

The expression of senescence-related genes including p53, p21, and p16 increases in senescent cells, and results in cell cycle arrest through inhibition of several cyclin-dependent kinases. Quantification of these cell cycle inhibitors in chondrocytes (Loeser 2009) and MSCs (Stewart et al. 2003, Park et al. 2005) revealed a concomitant increase in expression of p53, $p16^{INK4a}$, and $p21^{Cip1}$ proteins with cell senescence, and a positive correlation with the presence of SA-β-Gal. Accordingly, these senescence-related proteins mediate cellular senescence by phosphorylation of retinoblastoma (Rb) through the p53-p21-pRb pathway and/or the p16-pRB pathway (Takahashi et al. 2006). It has been reported that oxidative stress induced by treatment with hydrogen peroxide and inflammation induced by treatment with IL-1β mediate chondrocyte senescence via the p53-p21-pRb pathway, with induction of caveolin 1 and activation of p38 mitogen-activated protein kinase (MAPK), resulting in cell senescence and apoptosis. Caveolin 1 is the principal structural component of caveolae, and has been positively associated with articular cartilage degeneration in human and rat OA (Dai et al. 2006). Furthermore, overexpression of caveolin 1 induced p38 MAPK activation and impaired the ability of chondrocytes to produce type-II collagen and aggrecan (Dai et al. 2006).

To date, $p16^{INK4a}$ appears to be the most prominent marker and mediator of cell senescence via the p16-pRB pathway in both chondrocytes (Zhou et al. 2004) and MSCs (Shibata et al. 2007), and knockdown of the gene has been shown to rescue OA chondrocytes (Zhou et al. 2004) and MSCs (Shibata et al. 2007) to normal functioning. Interestingly, Philipot et al. (2014) showed that $p16^{INK4a}$ accumulates not only in response to inflammatory stimuli but also during MSC chondrogenesis. Similarly, it has been established that the decline in anabolic functions of articular chondrocytes is associated with the accumulation of $p16^{INK4a}$-positive chondrocytes with short telomeres and features of hypertrophy (Loeser 2009). Furthermore, $p16^{INK4a}$ has been found at higher levels in OA chondrocytes relative to levels in age-matched normal tissue, which in turn had higher levels than in fetal tissue (Zhou et al. 2004).

Oxidative stress

Oxidative stress is commonly believed to be the major inducer of DNA damage and cell senescence (Loeser 2011). Studies have found that increased oxidative stress with aging reduces chondrocyte survival and the response to growth factors (Carlo and Loeser 2003, Loeser et al. 2014). This has subsequently been linked to the development of OA, where OA cartilage showed extensive staining of a marker of oxidative damage—nitrotyrosine—in the degenerating regions of OA cartilage as compared to the intact regions of the same explants (Yudoh et al. 2005). Interestingly, the degree of nitrotyrosine staining

paralleled the severity of histological changes in OA cartilage, suggesting a correlation between oxidative damage and articular cartilage degeneration (Yudoh et al. 2005).

Further studies have confirmed that ROS activates several genes and downstream signaling pathways that induce senescence, dysfunction, and apoptosis (Ashraf et al. 2016). Oxidative stress induces chondrocyte senescence mainly by upregulating expression of p53 and p21, and also by activating p38 MAPK and phosphatidylinositol 3-kinase/Akt (PI3K/Akt) signaling pathways (Dai et al. 2006, Yu and Kim 2013). In addition, ROS results in telomere-related genomic instability, matrix loss, premature senescence, mitochondrial dysfunction, and apoptosis of chondrocytes and MSCs (Martin et al. 2004b, Yudoh et al. 2005, Estrada et al. 2013, Li et al. 2015a, 2015b, Sakata et al. 2015).

The involvement of ROS in MSC senescence is further supported by studies that have shown marked improvements in cell proliferation and replicative lifespan upon treatment with antioxidants such as N-acetylcysteine (NAC) (Lin et al. 2005) and ascorbic acid (Lin et al. 2005, Choi et al. 2008). Indirect evidence from other studies has also demonstrated enhanced proliferation and/or differentiation capacities of chondrocytes (Foldager et al. 2011) and MSCs (Estrada et al. 2013, Munir et al. 2014) when cultured under physiological oxygen concentrations (3–5%) instead of the oxygen concentration of ~20% in ambient air usually used in laboratory practice.

Pro-inflammatory cytokines

With aging, it has been observed that there is a systemic increase in levels of pro-inflammatory cytokines including C-reactive protein (CRP), IL-6, and tumor necrosis factor (TNF)-α, resulting in a chronic low-grade state of inflammation that has been implicated in the development of several chronic diseases of aging including OA (Greene and Loeser 2015). Franceschi et al. (2000) coined the term “inflamm-aging” to describe the pro-inflammatory state that occurs with increasing age. Indeed, epidemiological studies have indicated that there are strong links between inflammation and OA, as elevated levels of CRP, IL-6, and TNF-α were detected in people with knee OA, and levels of these pro-inflammatory markers were found to correlate with risk of disease progression (Spector et al. 1997, Livshits et al. 2009) as well as pain and joint dysfunction (Stannus et al. 2013). Locally in the joint, chondrocytes, meniscal cells, and infrapatellar fat pad-derived cells can be the local source of inflammatory mediators that increase with aging and contribute to OA (Greene and Loeser 2015). Senescent cells not only show features of growth arrest but also of senescence-associated secretory phenotype (SASP), which produces pro-inflammatory cytokines and matrix-degrading enzymes involved in joint tissue destruction (Loeser 2011, Philipot et al. 2014). Freund et al. (2010) composed a list of SASP factors implicated as inducers of cellular senescence; many of these are produced at high levels and are also present in OA tissues and/or synovial fluid. These include granulocyte macrophage colony stimulating factor (GM-CSF), growth regulated oncogene (GRO)α,β,γ, insulin-like growth factor-binding protein (IGFBP)-7, IL-1α, IL-6, IL-7, IL-8, monocyte chemoattractant protein (MCP)-1, MCP-2, macrophage inflammatory protein (MIP)1α, MMP-1, MMP-10, and MMP-3 (Greene and Loeser 2015). These findings agree with the results of early studies in which human articular chondrocytes from older donors were found to secrete elevated amounts of catabolic MMPs such as MMP-13 (Forsyth et al. 2005) and cytokines including interleukin (IL)-1 and IL-7, with the ability to induce more MMP production (Long et al. 2008)—resulting in cartilage degeneration. The underlying mechanisms for SASP in cell senescence are still being elucidated, but have been associated with DNA damage (Freund et al. 2011) and oxidative stress (Salminen et al. 2012). Furthermore, inflammation stimulated by IL-1β treatment has been shown to induce p16^{INK4a} expression, which in turn induces the production of MMPs (MMP-1 and MMP-13), thus linking inflammation to senescence and OA pathogenesis (Philipot et al. 2014).

Possible anti-aging strategies for cartilage regeneration

Emerging cartilage regeneration strategies aim for long-lasting replacement of damaged tissue, with functional improvements in pain and mobility. With the aging population, effective strategies for cartilage regeneration would need to address and overcome the pertinent issues of cellular senescence and attendant age-related changes, so as to bring about long-lasting functional cartilage regeneration. Most of these alternative/adjuvant strategies are still in the realm of laboratory-based in vitro experimentation and pre-clinical evaluation in animals, but they offer exciting insights and perspectives for future treatment in the aging population (Diagram).

Sources of cells

Various cell sources have been studied for cartilage tissue engineering and regeneration, including juvenile or adult chondrocytes (Brittberg et al. 1994, Adkisson et al. 2010) and adult stem cells (Lee et al. 2007, Toh et al. 2010). Several studies have found that the primitive sources of chondrocytes (Adkisson et al. 2010, Choi et al. 2014) and MSCs (Guillot et al. 2007) have greater proliferation and differentiation capabilities. In a recent study by Choi et al. (2016), chondrocytes harvested from fetal cartilage at 12 weeks post-gestation showed better proliferation and differentiation potential than chondrocytes and MSCs harvested from young donors (8–25 years old). Similarly, it has been shown that the primitive human umbilical cord MSCs have better proliferation and differentiation capacities than adult adipose and bone marrow MSCs (Zhang et al. 2011, Jin et al. 2013). These differences are probably related to the intrinsic

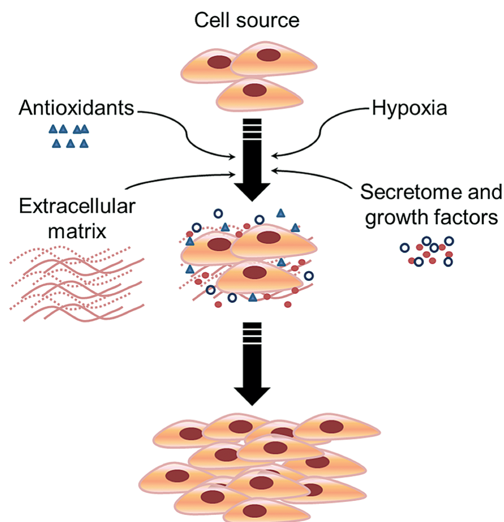

Diagram summarizing the strategies that may be used to overcome the problem of cellular senescence.

expression of cell senescence markers, telomerase activity, and pluripotency genes. The potency of cells in relation to their developmental origin has been further demonstrated in overexpression studies (Liu et al. 2009, Huang et al. 2014), where adult MSCs overexpressing pluripotency genes including OCT4 and NANOG showed marked improvements in proliferation, colony formation, and chondrogenesis. Apart from differences in proliferation and differentiation potential, the growth factor responsiveness, secretion of growth factors, and signaling pathways during chondrogenesis most likely differ depending on the anatomical locus (Afizah et al. 2007) and the developmental origin of these cells (Zhang et al. 2011, Brady et al. 2014, Toh et al. 2014a).

Secretome and growth factors

It is well established that TGF-β/Smad signaling has important roles in various stages of chondrogenesis, from mesenchymal condensation and chondrocyte proliferation to extracellular matrix (ECM) deposition—and finally terminal differentiation (van der Kraan et al. 2009). However, it also appeared that during TGF-β3-induced chondrogenic differentiation of bone marrow MSCs, there was upregulation of $p16^{INK4a}$—which was concomitant with expression of both type-IIB collagen and MMP13, a terminal differentiation marker (Philipot et al. 2014). This implicates the role of TGF-β/Smad signaling in cellular senescence during chondrogenesis and OA development, although the underlying mechanisms remain to be fully determined.

Chondrogenesis is regulated at various stages by several different growth factors. As mentioned, MSCs derived from different tissue sources are likely to express distinct ranges of growth factors and receptors that determine differentiation capabilities and growth factor responsiveness (Toh et al. 2014a). Several groups have explored the combination or sequential addition of growth factors and small molecules to steer chondrogenesis towards stable cartilage formation (Yang et al. 2011, Handorf and Li 2014). Others have started to look into the factors secreted by cells as a means of understanding the paracrine functions of chondrocytes and MSCs in chondrogenesis and cartilage regeneration (Gelse et al. 2009, Wu et al. 2011, Zhang et al. 2016a, 2016b). These secretome factors—including growth factors, cytokines, and microvesicles—have diverse functions that have yet to be fully uncovered (da Silva et al. 2009, Toh et al. 2014a, Zhang et al. 2016a, 2016b). The secretome is complex in composition and may differ among different cell types, depending on their somatic function, developmental origin, and differentiation (Bara et al. 2013, Toh et al. 2014a).

In recent years, there has been a surge of interest in exploring the use of conditioned medium (CM) from embryonic stem cells (ESCs) and its related secretome as an aging intervention to inhibit cellular senescence (Conboy et al. 2011, Bae et al. 2016). Bae et al. (2016) found that treatment of senescent human dermal fibroblasts with CM from mouse ESCs showed an altered senescence phenotype, including reduced SA-β-Gal activity and reduced expression of both p53 and p21. In that study, platelet-derived growth factor (PDGF)-BB in the ESC-CM was found to play a critical role in anti-senescence through upregulation of fibroblast growth factor (FGF)-2. Collectively, secretome research is likely to shed light on the mechanisms of cartilage repair and offer opportunities for development of possible anti-aging strategies for effective cartilage regeneration.

Antioxidants

Several antioxidants have been reported to have protective effects on chondrocytes and MSCs against oxidative stress and inflammation-induced cellular senescence and apoptosis (Dave et al. 2008, Li et al. 2015a, 2015b, Sakata et al. 2015). For instance, NAC has been reported to protect chondrocytes (Yu and Kim 2013) and MSCs (Li et al. 2015a) from oxidative stress-induced apoptosis, and has been shown to enhance the proliferation and replicative lifespan of MSCs (Lin et al. 2005). Other studies have demonstrated the potent antioxidant and anti-inflammatory effects of plant-derived polyphenol resveratrol against IL-1-induced inflammation in human OA chondrocytes (Liu et al. 2014), and in reducing progression of OA in a mouse model (Li et al. 2015b). There is also interest in developing biomaterial scaffolds to incorporate these antioxidants, for their beneficial antioxidant and anti-inflammatory properties in cartilage repair (Toh et al. 2014b). In a recent study, Wang et al. (2014) demonstrated the efficacy of a collagen/resveratrol (Col/Res) hydrogel in treating osteochondral defects in a rabbit model. The cell-free Col/Res scaffold was able to downregulate the expression of inflammation-related genes including IL-1β, MMP13, and COX-2, while promoting complete cartilage regeneration by the end of 12 weeks. It would be of interest to investigate the

effects of aging on the reparative response to these therapeutic antioxidants, to further confirm the anti-aging benefits of this strategy.

Hypoxia

It is well-established that chondrocytes and MSCs reside under physiological oxygen concentrations (hypoxic conditions). Early studies found that an oxygen concentration of 21% attenuated the growth of human articular chondrocytes and MSCs, and this was associated with oxidative damage as a result of increased oxidant production (Moussavi-Harami et al. 2004, Martin et al. 2004b). Subsequently, it was found that hypoxia during cell expansion was effective in maintaining the chondrogenic potential of human articular chondrocytes (Egli et al. 2008). However, hypoxia during cell expansion was ineffective in enhancing the chondrogenic potential of osteoarthritic (OA) human chondrocytes for subsequent cartilage formation in vitro (Schrobback et al. 2012).

Human MSCs cultured under hypoxic conditions have been reported to maintain their stemness properties and delay senescence better than cells cultured under normoxia (Choi et al. 2014). The molecular mechanisms by which hypoxia regulates premature senescence are not fully understood, but hypoxia has been associated with activation of AKT signaling (Palumbo et al. 2013), expression of TWIST (Tsai et al. 2011), and downregulation of extracellular signal-regulated kinase (ERK) signaling (Jin et al. 2010), which inhibited senescence-associated upregulation of the p16 and p21 genes (Jin et al. 2010, Tsai et al. 2011, Palumbo et al. 2013). More importantly, human MSCs expanded under hypoxic conditions showed enhanced proliferative capacity with intact genomic integrity (Tsai et al. 2011) and improved chondrogenic potential (Xu et al. 2007, Adesida et al. 2012).

Extracellular matrix

The ECM is a complex network of proteins and glycosaminoglycans (GAGs) that surrounds the cells and is critical in directing cell fate and functions (Toh et al. 2015). It is becoming clear that there are changes to ECM structure and composition during development, aging, and/or disease that suggest that the ECM has an important role in various cellular processes including proliferation and differentiation, and in overall cartilage hemostasis (Kvist et al. 2008, Toh et al. 2013). During OA, cartilage tissues not only show selective loss of territorial matrix proteins including type-II collagen and GAGs, but also changes in distribution of the pericellular matrix proteins including type-VI collagen, perlecan, type-IV collagen, laminins, nidogens, and matrilins (Söder et al. 2002, Kruegel et al. 2008, Zhang et al. 2014, Foldager et al. 2014, 2016). For example, levels of matrilin-2/3 have been reported to be highly expressed in OA tissues (Pullig et al. 2002, Zhang et al. 2014), while nidogen-1 but not nidogen-2 was found to be reduced in amount around diseased chondrocytes (Kruegel et al. 2008). Other studies on type-VI collagen (Peters et al. 2011), perlecan (Srinivasan et al. 2012), and laminin (Schminke et al. 2016) have also suggested some potential of these pericellular matrix proteins as therapeutic candidates for engineering and repair of cartilage tissue. Type-VI collagen has been demonstrated in in vitro studies to enhance the growth of adult and osteoarthritic chondrocytes (Smeriglio et al. 2015), and to protect chondrocytes from monoiodoacetate-induced cell death (Peters et al. 2011).

In recent years, there has been a surge of interest in exploring the use of decellularized stem cell ECMs to recapitulate the stem cell "niche", not only to influence the fate and functions of stem cells, but also to rejuvenate and enhance the replicative lifespans and differentiation capacities of MSCs and chondrocytes (Pei and He 2012, He and Pei 2013, Ng et al. 2014). Notably, decellularized stem cell matrices derived from synovium MSCs have been demonstrated to enhance the growth and replicative lifespan of chondrocytes, with better maintenance of phenotype and subsequent (re)differentiation capacity (Pei and He 2012). When cultured on decellularized stem cell matrices from either adipose tissue MSCs or synovium MSCs, adipose tissue MSCs showed improved proliferation and a lower level of intracellular ROS compared to those grown on non-coated flasks (He and Pei 2013).

Conclusion

With an increase in the aging world population, the number of cases of OA can be expected to increase globally. The underlying mechanisms that determine normal (disease- and injury-free) articular cartilage aging—rather than articular cartilage degeneration leading to OA—are unclear. A better understanding of the basic mechanisms underlying cellular senescence and how this process could be modified would possibly provide new strategies for treatment of cartilage lesions and OA in an increasingly aging population. Current efforts in cell sourcing and in using hypoxia, growth factors, secretome, and ECM proteins have shown promise in alleviating cellular senescence, but they require further studies in order to translate into clinical applications.

We thank the organizing committee of First Aarhus Regenerative Orthopaedics Symposium, 2015. We appreciate the meaningful interaction and discussion during the symposium that has inspired the writing of this review.

No competing interests declared.

Adesida A, Mulet-Sierra A, Jomha N. Hypoxia mediated isolation and expansion enhances the chondrogenic capacity of bone marrow mesenchymal stromal cells. Stem Cell Res Ther 2012; 3 (2): 9.

Adkisson H D, Martin J A, Amendola R L, Milliman C, Mauch K A, Katwal A B, et al. The potential of human allogeneic juvenile chondrocytes for restoration of articular cartilage. Am J Sports Med 2010; 38 (7): 1324-33.

Afizah H, Yang Z, Hui J H P, Ouyang H-W, Lee E-H. A comparison between the chondrogenic potential of human bone marrow stem cells (BMSCs) and adipose-derived stem cells (ADSCs) taken from the same donors. Tissue Eng 2007; 13 (4): 659-66.

Ashraf S, Cha B H, Kim J S, Ahn J, Han I, Park H, et al. Regulation of senescence associated signaling mechanisms in chondrocytes for cartilage tissue regeneration. Osteoarthritis Cartilage 2016; 24 (2): 196-205.

Bae Y-U, Choi J-H, Nagy A, Sung H-K, Kim J-R. Antisenescence effect of mouse embryonic stem cell conditioned medium through a PDGF/FGF pathway. FASEB J 2016; 30 (3): 1276-86.

Baker D J, Wijshake T, Tchkonia T, LeBrasseur N K, Childs B G, van de Sluis B, et al. Clearance of $p16^{Ink4a}$-positive senescent cells delays ageing-associated disorders. Nature 2011; 479 (7372): 232-6.

Bara J J, McCarthy H E, Humphrey E, Johnson W E B, Roberts S. Bone marrow-derived mesenchymal stem cells become antiangiogenic when chondrogenically or osteogenically differentiated: implications for bone and cartilage tissue engineering. Tissue Eng Part A 2013; 20 (1-2): 147-59.

Barbero A, Grogan S, Schäfer D, Heberer M, Mainil-Varlet P, Martin I. Age related changes in human articular chondrocyte yield, proliferation and post-expansion chondrogenic capacity. Osteoarthritis Cartilage 2004; 12 (6): 476-84.

Baxter M A, Wynn R F, Jowitt S N, Wraith J E, Fairbairn L J, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion. STEM CELLS 2004; 22 (5): 675-82.

Brandl A, Hartmann A, Bechmann V, Graf B, Nerlich M, Angele P. Oxidative stress induces senescence in chondrocytes. J Orthop Res 2011a; 29 (7): 1114-20.

Brandl A, Meyer M, Bechmann V, Nerlich M, Angele P. Oxidative stress induces senescence in human mesenchymal stem cells. Exp Cell Res 2011b; 317 (11): 1541-7.

Brittberg M, Lindahl A, Nilsson A, Ohlsson C, Isaksson O, Peterson L. Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation. New Eng J Med 1994; 331 (14): 889-95.

Campisi J. Cellular senescence: putting the paradoxes in perspective. Curr Opin Genet Dev 2011; 21 (1): 107-12.

Carlo M D, Loeser R F. Increased oxidative stress with aging reduces chondrocyte survival: Correlation with intracellular glutathione levels. Arthritis Rheum 2003; 48 (12): 3419-30.

Choi K-M, Seo Y-K, Yoon H-H, Song K-Y, Kwon S-Y, Lee H-S, et al. Effect of ascorbic acid on bone marrow-derived mesenchymal stem cell proliferation and differentiation. J Biosci Bioeng 2008; 105 (6): 586-94.

Choi J R, Pingguan-Murphy B, Wan Abas W A B, Noor Azmi M A, Omar S Z, Chua K H, et al. Impact of low oxygen tension on stemness, proliferation and differentiation potential of human adipose-derived stem cells. Biochem Biophys Res Commun 2014; 448 (2): 218-24.

Choi W H, Kim H R, Lee S J, Jeong N, Park S R, Choi B H, et al. Fetal cartilage-derived cells have stem cell properties and are a highly potent cell source for cartilage regeneration. Cell Transplant 2016; 25 (3): 449-61.

Choudhery M, Badowski M, Muise A, Pierce J, Harris D. Donor age negatively impacts adipose tissue-derived mesenchymal stem cell expansion and differentiation. J Transl Med 2014; 12 (1): 8.

Conboy I M, Yousef H, Conboy M J. Embryonic anti-aging niche. Aging (Albany NY) 2011; 3 (5): 555-63.

da Silva Meirelles L, Fontes A M, Covas D T, Caplan A I. Mechanisms involved in the therapeutic properties of mesenchymal stem cells. Cytokine Growth Factor Rev 2009; 20 (5–6): 419-27.

Dai S-M, Shan Z-Z, Nakamura H, Masuko-Hongo K, Kato T, Nishioka K, et al. Catabolic stress induces features of chondrocyte senescence through overexpression of caveolin 1: Possible involvement of caveolin 1–induced down-regulation of articular chondrocytes in the pathogenesis of osteoarthritis. Arthritis Rheum 2006; 54 (3): 818-31.

Dave M, Attur M, Palmer G, Al-Mussawir H E, Kennish L, Patel J, et al. The antioxidant resveratrol protects against chondrocyte apoptosis via effects on mitochondrial polarization and ATP production. Arthritis Rheum 2008; 58 (9): 2786-97.

Estrada J C, Torres Y, Benguria A, Dopazo A, Roche E, Carrera-Quintanar L, et al. Human mesenchymal stem cell-replicative senescence and oxidative stress are closely linked to aneuploidy. Cell Death Dis 2013; 4: e691.

Foldager C B, Nielsen A B, Munir S, Ulrich-Vinther M, Søballe K, Bünger C, et al. Combined 3D and hypoxic culture improves cartilage-specific gene expression in human chondrocytes. Acta Orthop 2011; 82 (2): 234-40.

Foldager C B, Toh W S, Gomoll A H, Olsen B R, Spector M. Distribution of basement membrane molecules, laminin and collagen type IV, in normal and degenerated cartilage tissues. Cartilage 2014; 5 (2): 123-32.

Foldager C B, Toh W S, Christensen B B, Lind M, Gomoll A H, Spector M. Collagen type IV and laminin expressions during cartilage repair and in late clinically failed repair tissues from human subjects. Cartilage 2016; 7(1): 52-61.

Forsyth C B, Cole A, Murphy G, Bienias J L, Im H-J, Loeser R F. Increased matrix metalloproteinase-13 production with aging by human articular chondrocytes in response to catabolic stimuli. J Gerontol A Biol Sci Med Sci 2005; 60 (9): 1118-24.

Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging: an evolutionary perspective on immunosenescence. Ann N Y Acad Sci 2000; 908 (1): 244-54.

Freund A, Orjalo A V, Desprez P-Y, Campisi J. Inflammatory networks during cellular senescence: causes and consequences. Trends Mol Med 2010; 16 (5): 238-46.

Freund A, Patil C K, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. EMBO J 2011; 30 (8): 1536-48.

Gelse K, Brem M, Klinger P, Hess A, Swoboda B, Hennig F, et al. Paracrine effect of transplanted rib chondrocyte spheroids supports formation of secondary cartilage repair tissue. J Orthop Res 2009; 27 (9): 1216-25.

Greene M A, Loeser R F. Aging-related inflammation in osteoarthritis. Osteoarthritis Cartilage 2015; 23 (11): 1966-71.

Guillot P V, Gotherstrom C, Chan J, Kurata H, Fisk N M. Human first-trimester fetal MSC express pluripotency markers and grow faster and have longer telomeres than adult MSC. STEM CELLS 2007; 25 (3): 646-54.

Handorf A M, Li W-J. Induction of mesenchymal stem cell chondrogenesis through sequential administration of growth factors within specific temporal windows. J Cell Physiol 2014; 229 (2): 162-71.

Harbo M, Bendix L, Bay-Jensen A-C, Graakjaer J, Soe K, Andersen T, et al. The distribution pattern of critically short telomeres in human osteoarthritic knees. Arthritis Res Ther 2012; 14 (1): R12.

Hayflick L, Moorhead P S. The serial cultivation of human diploid cell strains. Exp Cell Res 1961; 25 (3): 585-621.

He F, Pei M. Extracellular matrix enhances differentiation of adipose stem cells from infrapatellar fat pad toward chondrogenesis. J Tissue Eng Regen Med 2013; 7 (1): 73-84.

Honkonen S E. Degenerative arthritis after tibial plateau fractures. J Orthop Trauma 1995; 9 (4): 273-7.

Huang C E, Hu F W, Yu C H, Tsai L L, Lee T H, Chou M Y, et al. Concurrent expression of Oct4 and Nanog maintains mesenchymal stem-like property of human dental pulp cells. Int J Mol Sci 2014; 15(10): 18623-39.

Jin Y, Kato T, Furu M, Nasu A, Kajita Y, Mitsui H, et al. Mesenchymal stem cells cultured under hypoxia escape from senescence via down-regulation of p16 and extracellular signal regulated kinase. Biochem Biophys Res Commun 2010; 391 (3): 1471-6.

Jin H, Bae Y, Kim M, Kwon S-J, Jeon H, Choi S, et al. Comparative analysis of human mesenchymal stem cells from bone marrow, adipose tissue, and umbilical cord blood as sources of cell therapy. Int J Mol Sci 2013; 14 (9): 17986.

Kim Y S, Choi Y J, Koh Y G. Mesenchymal stem cell implantation in knee osteoarthritis: an assessment of the factors influencing clinical outcomes. Am J Sports Med 2015; 43 (9): 2293-301.

Kon E, Filardo G, Condello V, Collarile M, Di Martino A, Zorzi C, et al. Second-generation autologous chondrocyte implantation: results in patients older than 40 years. Am J Sports Med 2011; 39 (8): 1668-75.

Krishnan S P, Skinner J A, Bartlett W, Carrington R W J, Flanagan A M, Briggs T W R, et al. Who is the ideal candidate for autologous chondrocyte implantation? J Bone Joint Surg (Br) 2006; 88-B (1): 61-4.

Kruegel J, Sadowski B, Miosge N. Nidogen-1 and nidogen-2 in healthy human cartilage and in late-stage osteoarthritis cartilage. Arthritis Rheum 2008; 58 (5): 1422-32.

Kvist A J, Nyström A, Hultenby K, Sasaki T, Talts J F, Aspberg A. The major basement membrane components localize to the chondrocyte pericellular matrix — A cartilage basement membrane equivalent? Matrix Biol 2008; 27 (1): 22-33.

Li J, Pei M. Cell Senescence: A Challenge in Cartilage Engineering and Regeneration. Tissue Eng Part B Rev 2012; 18 (4): 270-87.

Li C-J, Sun L-Y, Pang C-Y. Synergistic Protection of N-acetylcysteine and ascorbic acid 2-phosphate on human mesenchymal stem cells against mitoptosis, necroptosis and apoptosis. Sci Rep 2015a; 5: 9819.

Li W, Cai L, Zhang Y, Cui L, Shen G. Intra-articular resveratrol injection prevents osteoarthritis progression in a mouse model by activating SIRT1 and thereby silencing HIF-2α. J Orthop Res 2015b; 33 (7): 1061-70.

Lin T-M, Tsai J-L, Lin S-D, Lai C-S, Chang C-C. Accelerated growth and prolonged lifespan of adipose tissue-derived human mesenchymal stem cells in a medium using reduced calcium and antioxidants. Stem Cells Dev 2005; 14 (1): 92-102.

Liu T M, Wu Y N, Guo X M, Hui J H, Lee E H, Lim B. Effects of ectopic Nanog and Oct4 overexpression on mesenchymal stem cells. Stem Cells Dev 2009; 18(7): 1013-22.

Liu H, Zhao Z, Clarke R B, Gao J, Garrett I R, Margerrison E E C. Enhanced tissue regeneration potential of juvenile articular cartilage. Am J Sports Med 2013; 41 (11): 2658-67.

Liu L, Gu H, Liu H, Jiao Y, Li K, Zhao Y, et al. Protective effect of resveratrol against IL-1β-induced inflammatory response on human osteoarthritic chondrocytes partly via the TLR4/MyD88/NF-κB signaling pathway: an "in vitro study". Int J Mol Sci 2014; 15 (4): 6925.

Livshits G, Zhai G, Hart D J, Kato B S, Wang H, Williams F M K, et al. Interleukin-6 is a significant predictor of radiographic knee osteoarthritis: The Chingford study. Arthritis Rheum 2009; 60 (7): 2037-45.

Loeser R F. Aging and osteoarthritis: the role of chondrocyte senescence and aging changes in the cartilage matrix. Osteoarthritis Cartilage 2009; 17 (8): 971-9.

Loeser R F. Aging and osteoarthritis. Curr Opin Rheumatol 2011; 23 (5): 492-6.

Loeser R F, Gandhi U, Long D L, Yin W, Chubinskaya S. Aging and oxidative stress reduce the response of human articular chondrocytes to insulin-like growth factor 1 and osteogenic protein 1. Arthritis Rheumatol 2014; 66 (8): 2201-9.

Long D, Blake S, Song X-Y, Lark M, Loeser R. Human articular chondrocytes produce IL-7 and respond to IL-7 with increased production of matrix metalloproteinase-13. Arthritis Res Ther 2008; 10 (1): R23.

Marcacci M, Kon E, Zaffagnini S, Iacono F, Neri M P, Vascellari A, et al. Multiple osteochondral arthroscopic grafting (mosaicplasty) for cartilage defects of the knee: Prospective study results at 2-year follow-up. Arthroscopy 2005; 21 (4): 462-70.

Martin J A, Buckwalter J A. Telomere erosion and senescence in human articular cartilage chondrocytes. J Gerontol A Biol Sci Med Sci 2001; 56 (4): B172-B9.

Martin J A, Buckwalter J A. The role of chondrocyte senescence in the pathogenesis of osteoarthritis and in limiting cartilage repair. J Bone Joint Surg 2003; 85 (suppl 2): 106-10.

Martin J A, Brown T, Heiner A, Buckwalter J A. Post-traumatic osteoarthritis: The role of accelerated chondrocyte senescence. Post-traumatic osteoarthritis: The role of accelerated chondrocyte senescence. Biorheology 2004a; 41(3-4): 479-91.

Martin J A, Klingelhutz A J, Moussavi-Harami F, Buckwalter J A. Effects of oxidative damage and telomerase activity on human articular cartilage chondrocyte senescence. J Gerontol A Biol Sci Med Sci 2004b; 59 (4): B324-B36.

Miller B S, Briggs K K, Downie B, Steadman J R. Clinical outcomes following the microfracture procedure for chondral defects of the knee: a longitudinal data analysis. Cartilage 2010; 1 (2): 108-12.

Moussavi-Harami F, Duwayri Y, Martin J A, Moussavi-Harami F, Buckwalter J A. Oxygen effects on senescence in chondrocytes and mesenchymal stem cells: consequences for tissue engineering. Iowa Orthop J 2004; 24: 15-20.

Munir S, Foldager C, Lind M, Zachar V, Søballe K, Koch T. Hypoxia enhances chondrogenic differentiation of human adipose tissue-derived stromal cells in scaffold-free and scaffold systems. Cell Tissue Res 2014; 355 (1): 89-102.

Nejadnik H, Hui J H, Feng Choong E P, Tai B-C, Lee E H. Autologous bone marrow-derived mesenchymal stem cells versus autologous chondrocyte implantation: an observational cohort study. Am J Sports Med 2010; 38 (6): 1110-6.

Ng C P, Mohamed Sharif A R, Heath D E, Chow J W, Zhang C B Y, Chan-Park M B, et al. Enhanced ex vivo expansion of adult mesenchymal stem cells by fetal mesenchymal stem cell ECM. Biomaterials 2014; 35 (13): 4046-57.

Srinivasan P P, McCoy S Y, Jha A K, Yang W, Jia X, Farach-Carson M C, et al. Injectable perlecan domain 1-hyaluronan microgels potentiate the cartilage repair effect of BMP2 in a murine model of early osteoarthritis. Biomed Mater 2012; 7 (2): 024109.

Palumbo S, Tsai T-L, Li W-J. Macrophage migration inhibitory factor regulates AKT signaling in hypoxic culture to modulate senescence of human mesenchymal stem cells. Stem Cells Dev 2013; 23 (8): 852-65.

Parsch D, Fellenberg J, Brümmendorf T, Eschlbeck A-M, Richter W. Telomere length and telomerase activity during expansion and differentiation of human mesenchymal stem cells and chondrocytes. J Mol Med 2004; 82 (1): 49-55.

Pei M, He F. Extracellular matrix deposited by synovium-derived stem cells delays replicative senescent chondrocyte dedifferentiation and enhances redifferentiation. J Cell Physiol 2012; 227 (5): 2163-74.

Pestka J, Schmal H, Salzmann G, Hecky J, Südkamp N, Niemeyer P. In vitro cell quality of articular chondrocytes assigned for autologous implantation in dependence of specific patient characteristics. Arch Orthop Trauma Surg 2011; 131 (6): 779-89.

Peters H C, Otto T J, Enders J T, Jin W, Moed B R, Zhang Z. The protective role of the pericellular matrix in chondrocyte apoptosis. Tissue Eng Part A 2011; 17 (15-16): 2017-24.

Philipot D, Guerit D, Platano D, Chuchana P, Olivotto E, Espinoza F, et al. p16INK4a and its regulator miR-24 link senescence and chondrocyte terminal differentiation-associated matrix remodeling in osteoarthritis. Arthritis Res Ther 2014; 16 (1): R58.

Pullig O, Weseloh G, Klatt A R, Wagener R, Swoboda B. Matrilin-3 in human articular cartilage: increased expression in osteoarthritis. Osteoarthritis Cartilage 2002; 10 (4): 253-63.

Roos H, Adalberth T, Dahlberg L, Lohmander L S. Osteoarthritis of the knee after injury to the anterior cruciate ligament or meniscus: the influence of time and age. Osteoarthritis Cartilage 1995; 3 (4): 261-7.

Rose J, Söder S, Skhirtladze C, Schmitz N, Gebhard P M, Sesselmann S, et al. DNA damage, discoordinated gene expression and cellular senescence in osteoarthritic chondrocytes. Osteoarthritis Cartilage 2012; 20 (9): 1020-8.

Sahin E, Colla S, Liesa M, Moslehi J, Müller F L, Guo M, et al. Telomere dysfunction induces metabolic and mitochondrial compromise. Nature 2011; 470 (7334): 359-65.

Sakata S, Hayashi S, Fujishiro T, Kawakita K, Kanzaki N, Hashimoto S, et al. Oxidative stress-induced apoptosis and matrix loss of chondrocytes is inhibited by eicosapentaenoic acid. J Orthop Res 2015; 33 (3): 359-65.

Salminen A, Kauppinen A, Kaarniranta K. Emerging role of NF-κB signaling in the induction of senescence-associated secretory phenotype (SASP). Cellular Signalling 2012; 24 (4): 835-45.

Schminke B, Frese J, Bode C, Goldring M B, Miosge N. Laminins and nidogens in the pericellular matrix of chondrocytes: their role in osteoarthritis and chondrogenic differentiation. Am J Pathol 2016; 186(2): 410-8.

Smeriglio P, Dhulipala L, Lai J H, Goodman S B, Dragoo J L, Smith R L, et al. Collagen VI enhances cartilage tissue generation by stimulating chondrocyte proliferation. Tissue Eng Part A 2015; 21 (3-4): 840-9.

Söder S, Hambach L, Lissner R, Kirchner T, Aigner T. Ultrastructural localization of type VI collagen in normal adult and osteoarthritic human articular cartilage. Osteoarthritis Cartilage 2002; 10 (6): 464-70.

Spector T D, Hart D J, Nandra D, Doyle D V, Mackillop N, Gallimore J R, et al. Low-level increases in serum C-reactive protein are present in early osteoarthritis of the knee and predict progressive disease. Arthritis Rheum 1997; 40 (4): 723-7.

Steinwachs M R, Guggi T, Kreuz P C. Marrow stimulation techniques. Injury 2008; 39 (1, Suppl): 26-31.

Stolzing A, Jones E, McGonagle D, Scutt A. Age-related changes in human bone marrow-derived mesenchymal stem cells: Consequences for cell therapies. Mech Ageing Dev 2008; 129 (3): 163-73.

Takahashi A, Ohtani N, Yamakoshi K, Iida S-i, Tahara H, Nakayama K, et al. Mitogenic signalling and the p16$^{INK4a\text{-}Rb}$ pathway cooperate to enforce irreversible cellular senescence. Nat Cell Biol 2006; 8 (11): 1291-7.

Toh W S, Lee E H, Guo X-M, Chan J K Y, Yeow C H, Choo A B, et al. Cartilage repair using hyaluronan hydrogel-encapsulated human embryonic stem cell-derived chondrogenic cells. Biomaterials 2010; 31 (27): 6968-80.

Toh W S, Foldager C B, Olsen B R, Spector M. Basement membrane molecule expression attendant to chondrogenesis by nucleus pulposus cells and mesenchymal stem cells. J Orthop Res 2013; 31 (7): 1136-43.

Toh W S, Foldager C B, Pei M, Hui J H. Advances in Mesenchymal Stem Cell-based Strategies for Cartilage Repair and Regeneration. Stem Cell Rev 2014a; 10 (5): 686-96.

Toh W S, Loh X J. Advances in hydrogel delivery systems for tissue regeneration. Mater Sci Eng C Mater Biol Appl 2014b; 45: 690-7.

Toh W S, Foldager C B, Hui J H, Olsen B R, Spector M. Exploiting stem cell-extracellular matrix interactions for cartilage regeneration: a focus on basement membrane molecules. Curr Stem Cell Res Ther 2015; DOI: 10.2174/1574888X10666150723150525

Tsai C-C, Chen Y-J, Yew T-L, Chen L-L, Wang J-Y, Chiu C-H, et al. Hypoxia inhibits senescence and maintains mesenchymal stem cell properties through down-regulation of E2A-p21 by HIF-TWIST. Blood 2011; 117 (2): 459-69.

van der Kraan P M, Blaney Davidson E N, Blom A, van den Berg W B. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: Modulation and integration of signaling pathways through receptor-Smads. Osteoarthritis Cartilage 2009; 17 (12): 1539-45.

Volpin G, Dowd G, Stein H, Bentley G. Degenerative arthritis after intra-articular fractures of the knee. Long-term results. J Bone Joint Surg (Br) 1990; 72-B (4): 634-8.

Wang W, Sun L, Zhang P, Song J, Liu W. An anti-inflammatory cell-free collagen/resveratrol scaffold for repairing osteochondral defects in rabbits. Acta Biomater 2014; 10 (12): 4983-95.

Wu L, Leijten J C H, Georgi N, Post J N, van Blitterswijk C A, Karperien M. Trophic effects of mesenchymal stem cells increase chondrocyte proliferation and matrix formation. Tissue Eng Part A 2011; 17 (9-10): 1425-36.

Xu Y, Malladi P, Chiou M, Bekerman E, Giaccia A J, Longaker M T. In vitro expansion of adipose-derived adult stromal cells in hypoxia enhances early chondrogenesis. Tissue Eng 2007; 13 (12): 2981-93.

Yang Z, Zou Y, Guo X M, Tan H S, Denslin V, Yeow C H, et al. Temporal activation of β-catenin signaling in the chondrogenic process of mesenchymal stem cells affects the phenotype of the cartilage generated. Stem Cells Dev 2011; 21 (11): 1966-76.

Yu S-M, Kim S-J. Thymoquinone-induced reactive oxygen species causes apoptosis of chondrocytes via PI3K/Akt and p38kinase pathway. Exp Biol Med 2013; 238 (7): 811-20.

Yudoh K, van Trieu N, Nakamura H, Hongo-Masuko K, Kato T, Nishioka K. Potential involvement of oxidative stress in cartilage senescence and development of osteoarthritis: oxidative stress induces chondrocyte telomere instability and downregulation of chondrocyte function. Arthritis Res Ther 2005; 7 (2): R380 - R91.

Zhang X, Hirai M, Cantero S, Ciubotariu R, Dobrila L, Hirsh A, et al. Isolation and characterization of mesenchymal stem cells from human umbilical cord blood: Reevaluation of critical factors for successful isolation and high ability to proliferate and differentiate to chondrocytes as compared to mesenchymal stem cells from bone marrow and adipose tissue. J Cell Biochem 2011; 112 (4): 1206-18.

Zhang S, Peng J, Guo Y, Javidiparsijani S, Wang G, Wang Y, et al. Matrilin-2 is a widely distributed extracellular matrix protein and a potential biomarker in the early stage of osteoarthritis in articular cartilage. BioMed Res Int 2014; 2014: 10.

Zhang S, Chu W, Lai R, Hui J, Lee E, Lim S, et al. Human mesenchymal stem cell-derived exosomes promote orderly cartilage regeneration in an immunocompetent rat osteochondral defect model. Cytotherapy 2016a; 18 (6, Supplement): S13.

Zhang S, Chu W C, Lai R C, Lim S K, Hui J H, Toh W S. Exosomes derived from human embryonic mesenchymal stem cells promote osteochondral regeneration. Osteoarthritis Cartilage 2016b; DOI: 10.1016/j.joca.2016.06.022.

Zhou H W, Lou S Q, Zhang K. Recovery of function in osteoarthritic chondrocytes induced by p16INK4a-specific siRNA in vitro. Rheumatology 2004; 43 (5): 555-68.

Zhou S, Greenberger J S, Epperly M W, Goff J P, Adler C, LeBoff M S, et al. Age-related intrinsic changes in human bone-marrow-derived mesenchymal stem cells and their differentiation to osteoblasts. Aging Cell 2008; 7 (3): 335-43.