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Chondrocyte senescence and osteoarthritis : role of oxidized LDL-induced oxidative stress

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Abstract

Although epidemiologic studies have shown that age is the chief risk factor for osteoarthritis (OA), the relationship between aging and the development of OA has not been completely understood yet. However, accumulating evidences in vivo and vitro have shown that the development of OA is, at least in part, attributable to the age-related chondrocyte senescence. This review focuses on how chondrocyte senescence affects the articular cartilage degeneration and how oxidative stress affects the chondrocyte senescence. Further, I would like to introduce our hypothesis that oxidized low-density lipoprotein, which is the most important molecule causing atherosclerosis, is involved in the pathogenesis of OA by playing a role as an oxidative stressor. It is interesting that even though mitotically inactive, senescent cells are far from being biologically inert. Many genes in senescent cells display higher expression levels that do not merely correlate with cell cycle arrest. Chondrocyte senescence is associated with an increased production of inflammatory mediators and matrix degrading enzymes characteristic of the senescent secretory phenotype. Age-related oxidative stress and damage may play a central role in cartilage aging through modulation of cell signaling pathways that regulate anabolic and catabolic activity.

Key words : osteoarthritis, chondrocyte senescence, oxidative stress, oxidized LDL, LOX-1

Abbreviations

OA : osteoarthritis
ox-LDL : oxidized low-density lipoprotein
hTERT : human telomerase reverse transcriptase
SIPS : stress-induced premature cell senescence
TNF-α : tumor necrosis factor-α
ROS : reactive oxygen species
EGF : epidermal growth factor
Rb : retinoblastoma protein
IL : interleukin
MMP : matrix metalloproteinase
IGFBP-7 : insulin-like growth factor binding protein-7
SA β-gal : senescent associated β-galactosidase
siRNA : small interfering RNA
IGF-I : insulin-like growth factor-1
FGF : fibroblast growth factor
TGF-β : transforming growth factor-β
LOX-1 : lectin-like oxidized low-density lipoprotein receptor-1
MAPK : mitogen activated protein kinase
ERK : extracellular signal-regulated kinase
MEK : mitogen-activated protein kinase kinase
JNK : c-jun N-terminal kinase
BAC : bovine articular chondrocyte
NAC : N-acetyl cystein
Akt : protein kinase B
PI3k : Phosphoinositide 3-kinase
COL10 : type X collagen
Runx 2 : runt-related transcription factor 2
VEGF : vascular endothelial growth factor
ALP : alkaline phosphatase

Introduction

Epidemiologic studies have shown that age is the chief risk factor for osteoarthritis (OA)[1,2] and that OA is the most common cause of chronic locomotive disability in the elderly people.[3,4]

Degenerative changes and thinning in articular cartilage is one of the most important pathologic findings in OA. It is not a simple "mechanically wearing out with time" and changes in the joint attributable to aging can be distinguished from those due to the disease. The relationship between aging and the development of OA has not been completely understood yet. However, accumulating evidences in vivo and vitro have shown that the development of OA is, at least in part, attributable to the age-related changes in the joints, which plays a role in conjunction with other factors such as excessive mechanical load, joint injury (injury of articular cartilage, menisci, subchondral bone and ligaments), life-style related diseases and genetics. The surgical destabilization of the knee in young animals, which can induce OA-like changes in the joint without any contribution of aging,[5,6] indicates that the excessive mechanical stress is one of the most important factors to develop cartilage degeneration. These evidences suggest that the aging and the development of OA are inter-related but not inter-dependent. However, there is a possibility that the excessive mechanical load may enhance chondrocyte aging in a cellular level, where chondrocytes undergo terminal differentiation to become hypertrophic chondrocytes. If it is the case, the chondrocyte senescence and the development of OA may be inter-related and inter-dependent.

OA is clinically defined as joint dysfunction due to progressive changes in several structures of the joint, including the articular cartilage, the menisci in the knee, bone, muscle, synovium, and other soft tissues (ligaments and tendons). Among these joint structures, the articular cartilage has been extensively studied especially in regards to aging. This review focuses on how chondrocyte aging (senescence in a cellular level) affects the articular cartilage degeneration (senescence in a tissue level) from the point of view of oxidative stress. Further, I would like to introduce our hypothesis that oxidized low-density lipoprotein (ox-LDL), which is the most important molecule causing atherosclerosis, is involved in the pathogenesis of OA through one of oxidative stressors inducing cell senescence.

Cell Senescence and Oxidative Stress

Definition of cell senescence generally accepted is a significant decrease in proliferative ability, where cells cannot further divide in culture after a period of 30-40 population doublings, often referred to as the "Hayflick limit".[7] This form of cell senescence is called "replicative senescence", which is supposed to result from a cell cycle arrest. There are some evidences to indicate that replicative senescence play an important role in aging in a tissue level and an individual level. For example, fibroblasts isolated from older human or animal skin reach replicative senescence sooner than cells isolated from younger individuals.[8] In addition, the cells with replicative senescence in older adults have shortened telomeres and the formation of senescence-associated (SA) heterochromatin.[8] It is reasonable to consider that cell senescence is a essential mechanism to prevent proliferation of cells with damaged DNA and thus to avoid tumorigenesis.

Replicative senescence is associated with alterations in DNA structure and function including telomere shortening and dysfunction.[9,10] Telomeres are found at the ends of chromosomes and it's structures, the terminal guanine-rich sequences of chromosomes (TTAGGG repeats in humans and other vertebrates), work to stabilize the chromosome during replication by protecting the chromosome end against exonucleases. Telomere length decreases with incomplete replication during mitosis and, when decreased to a critical length, it signals a cell to stop dividing and to enter replicative senescence.[11,12] Meanwhile, 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.[13,14] Although hTERT is generally repressed in normal somatic cells, telomerase activation in human vascular smooth muscle cells protects telomere shortening with replication.[15] Because vascular cell senescence occurs in human atherosclerotic lesions and is associated with telomere shortening, telomerase activity seems to be important in guarding against cell senescence.[16] Telomere shortening has been shown in OA chondrocytes,[17–19] and the lifespan of senescent chondrocytes retrieved from OA cartilage can be increased by exogenous expression of telomerase,[20] indicating an important relationship between chondrocyte senescence and telomerase

activity.

In addition to replicative senescence, stress-induced premature cell senescence (SIPS) also occurs by which cells without discernible attrition of telomeres show a growth arrest.[21,22] In quiescent cells such as chondrocytes, this mode of cell senescence (SIPS) may be more important than the replicative senescence, because progressive telomere shortening due to repeated cycles of cell division does not completely explain senescence in those cells. Some stressors identified include DNA damage, oxidative stress, suboptimal culture conditions, and PI3k inhibitors. Proatherogenic and proinflammatory factors such as ox-LDL, tumor necrosis factor-α (TNF-α), and hydrogen peroxide have also been implicated in SIPS,[10,21-23] and these can suppress telomerase activity by inactivating the PI3k/Akt pathway.[24] Oxidative damage to DNA can directly contribute to SIPS and because the ends of chromosomes are particularly sensitive to oxidative damage, it can result in telomere instability similar to that seen with replicative senescence.[9,10,25] SIPS due to oxidative stress fits quite well with one of the long-standing theories of aging first proposed by Harman in the 1950s that invoked free radicals, or reactive oxygen species (ROS), as mediators of aging.[26] Oxidative stress has been found to induce cell senescence in vitro and there is in vivo evidence for age-related oxidative stress in many tissues.[8] Both modes of senescence are associated with suppressed cell proliferation, impaired physiological cell function. It is likely that both the telomere shortening-initiated and stress-induced cell senescent modes may contribute jointly to the pathogenic process of many chronic diseases in vivo.[21]

ROS are generated by intracellular enzymes such as nicotine amide adenine dinucleotide phosphate (NADPH) oxidase and 5-lipoxygenase in response to activation of specific cell signaling pathways. These ROS serve as secondary messengers that regulate signal transduction by activating redox-sensitive kinase and inhibiting redox-sensitive phosphatase.[27,28] Insufficient levels of ROS can be detrimental to certain signaling pathways, such as the epidermal growth factor (EGF) pathway that regulates cell proliferation, while excessive levels of ROS may inhibit pathways, such as the insulin-signaling pathway, through activation of the stress-induced kinase JNK.[27,29] A direct role for ROS in cell senescence has been shown where mitogenic signals increases the ROS level and elicits a positive feedback activation of ROS-protein kinase C delta (PKCδ) signaling pathway, which cooperates with the $p16^{INK4A}$-retinoblastoma protein (Rb) pathway, to promote cell senescence.[30] Senescent cells exhibit altered activity and expression of regulatory proteins that control growth and proliferation, including p53 and the cyclin-dependent kinase inhibitors $p21^{CIP1}$, and $p16^{INK4A}$.[8,23] Activation of p53 occurs from DNA damage or from telomere shortening and serves to inhibit cell-cycle progression. Activated p53 increases the expression of p21, which contributes to senescence. As p21 declines in senescent cells, p16 is increased which appears to serve a more long-term role in the inhibition of cell-cycle progression through inhibition of Rb.[8]

It is interesting that even though mitotically inactive, senescent cells are far from being biologically inert. Many genes in senescent cells display higher expression levels that do not merely correlate with cell cycle arrest.[31] Senescent cells can secrete proteins, including degradative enzymes, inflammatory cytokines, and growth factors that may stimulate tissue aging and tumorigenesis and hence possess a more complex role in promoting chronic diseases.[8,32,33] Sometimes referred to as "the senescent secretory phenotype",[23,34] these activities of senescent cell may be particularly relevant to the development of OA. This phenotype is characterized by the increased production of cytokines, including interleukin-6 (IL-6) and interleukin-1 (IL-1), MMPs, and growth factors such as EGF. Recent studies have also provided evidence for a role of the IL-8 receptor chemokine XC receptor 2 (CXCR2)[35] and insulin-like growth factor binding protein-7 (IGFBP-7)[36] in senescence, suggesting autocrine loops of secreted proteins contribute to cell senescence. The accumulation of cells expressing the senescent secretory phenotype can also contribute to tissue aging through damage to the extracellular matrix, such as seen with the degradation of dermal collagen due to an age-related increase in collagenase.[23,34]

Chondrocyte Senescence and Oxidative Stress

Many studies have shown that chondrocytes isolated from the elderly exhibit distinct features of typical senescent cell (Figure). Chondrocytes after multiple passages in cell culture undergo replicative senescence with telomere shortening.[37] Evidence of telomere shortening in chon-

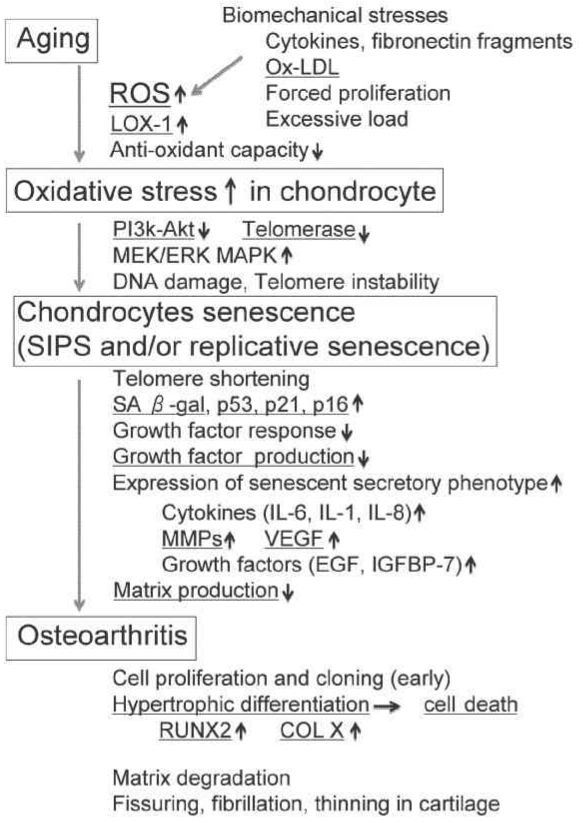

Fig. A tentative model of the development of OA related to aging, oxidative stress, chondrocyte senescence. Pathological biomechanical stresses, including cytokines, fibronectin fragments, ox-LDL, and so on, causes an increase in ROS levels in chondrocytes. The pathological increase in oxidative stress results in both modes of chondrocyte senescence, SIPS and replicative senescence. The role of ox-LDL and sequels attributable to the ox-LDL/LOX-1 system that has already been demonstrated in our studies are shown as issues marked with underlines. See the list of abbreviations.

drocytes has also been reported in cells isolated from older adults.[18] However, it is much more likely that the SIPS induced by chronic extrinsic stress cause chondrocyte senescence, because it has been reported that telomere shortening in chondrocytes could occur due to DNA damage caused by ROS.[25,38] ROS generated from excessive mechanical loading and/or stimulation by cytokines could also contribute to DNA damage and subsequent telomere shortening.[39–41]

Evidence of cell senescence in tissues from older adults can be obtained by examining for the presence of senescence markers. These markers currently include histological staining for senescent associated (SA)-βgal, SA heterochromatin, increased p53, p21, and p16 and reduced Wnt2.[35] Staining for SA-βgal has been shown to be present in articular chondrocytes from older adults[18] and in OA chondrocytes.[37] 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 SIPS.[42] Dai et al have provided evidence that SIPS in vitro is also accompanied by an increase in chondrocyte p53 and p21 expression as additional markers of the senescent phenotype.[42] The senescence marker $p16^{INK4A}$ has also been examined and found to be present at greater levels in OA chondrocytes relative to age-matched normal tissue, which in turn had higher levels than fetal tissue.[43] In the latter study, siRNA knockdown of $p16^{INK4A}$ was noted to enhance chondrocyte proliferation and matrix gene expression.

There is accumulating evidence that chondrocytes can exhibit features of "the senescent secretory phenotype", which has important implications for the role of chondrocyte senescence in the development and progression of OA. When compared to cells isolated from young tissue donors, human articular chondrocytes from older adults were found to secrete more MMP-13 into the media after stimulation with either IL-1β or fibronectin fragments.[44] Isolated human chondrocytes were also found to produce more IL-1[44] and more IL-7[45] with increasing donor age and, like IL-1 and fibronectin fragments, IL-7 can also induce MMP-13 production.[45] MMP-13 serves as a major mediator of type II collagen cleavage in the cartilage.[46,47] Studies have shown increased immunostaining for MMP-3 and MMP-13 in cartilage with aging[48] as well as an age-related accumulation of collagen neoepitopes representing cleaved collagen.[49,50]

Chondrocyte senescence can contribute to a decline in chondrocyte numbers due to increased cell death, although the extent of cell death with aging or in OA has varied among studies.[51–53] There are certainly reasons to expect an age-related increase in death of chondrocytes including the decline in growth factor activity, the loss of survival promoting matrix proteins, and the increase in oxidative damage. The response of chondrocytes to IGF-I declines with age and IGF-I is an important autocrine survival factors in cartilage.[54] Although matrix alterations occur with aging, it is not known if these affect the ability of either type II collagen[55] or fibronectin signaling through the $\alpha5\beta1$ integrin[56] to promote chondrocyte survival. Oxidative damage

from ROS could also contribute to chondrocyte death. Levels of ROS increase in cartilage with aging and chondrocytes from older adults are more susceptible to ROS-mediated cell death.[57] Because of a low ability of recruiting cells in cartilage, any loss of cells could cause negative changes in cartilage function.

As same in other tissues, oxidative stress may play an important role in aging of the cartilage. Oxidative stress results when the amount of ROS exceeds the anti-oxidant capacity of the cell (Figure). This can be due to either increased production of ROS or decreased levels of anti-oxidants and in aging both are often responsible.[27] Glutathione is a major intracellular anti-oxidant that also participates in regulating redox-signaling events. An increase in levels of oxidized glutathione can be a sign of oxidative stress.[8] Evidence for an age-related increase in oxidative stress in human chondrocytes was obtained by finding an increase in the ratio of oxidized to reduced glutathione in isolated cells.[57] Increased levels of intracellular ROS were also detected in cartilage from old rats when compared to young adults.[58] It may be important that an age-related increase in intracellular oxidative stress can make human chondrocytes[57] and rat chondrocytes[58] more susceptible to cell death induced by oxidants.

As additional evidence for oxidative stress playing a role in chondrocyte senescence, chondrocyte senescence in vitro was associated with oxidative stress[59] and exogenous addition of ROS to cultured chondrocytes was found to induce markers of the senescent phenotype.[42] There is also evidence for reduced levels of anti-oxidant enzymes in cartilage with aging and in OA that would contribute to chondrocyte oxidative stress. In chondrocytes from aged rats, catalase, but not superoxide dismutase or glutathione peroxidase, was found at lower levels than in young adults.[58] Proteomic studies of human articular chondrocytes found a decrease in mitochondrial superoxide dismutase with aging[60] as well as a decrease in OA cells when compared to cells from normal tissue.[61] Although not studied in aging, cartilage from adults with OA also had less extracellular superoxide dismutase than normal cartilage[62] and gene array studies performed with RNA isolated from OA cells revealed a decreased expression of superoxide dismutase and glutathione peroxidase.[63]

One marker of protein oxidation is the presence of nitrotyrosine, which can be detected using anti-nitrotyrosine antibodies. Nitrotyrosine is created by the reaction of protein tyrosine residues with peroxynitrite (ONOO-) formed when the ROS superoxide (O_2*-) and nitric oxide ($NO*$) react.[64] Increased immunostaining for nitrotyrosine has been noted with aging in normal human and monkey cartilage.[65] Nitrotyrosine has also been detected in OA tissue.[63,65] In monkey cartilage, the presence of positive immunostaining for nitrotyrosine correlated with a reduced anabolic response to IGF-I in chondrocytes isolated from nearby tissue, suggesting that oxidative damage may be one mechanism for the reduced growth factor response.[65] In addition, excess levels of NO, a reactive nitrogen species, have also been found to reduce the chondrocyte response to IGF-I.[66] Likewise, earlier studies noted that treatment with H_2O_2 inhibits chondrocyte proteoglycan synthesis.[67]

The source of ROS contributing to oxidative stress and oxidative damage can include both free radicals generated as by-products of aerobic metabolism as well as ROS generated in response to specific stimuli such as growth factors and cytokines. Although chondrocytes live in an environment with a low oxygen tension, they do consume oxygen and therefore exhibit aerobic metabolism.[68] It has been demonstrated that stimulation by cytokines and growth factors including IL-1, TNF-α, FGF, TGF-β[69-72] and fibronectin fragments[73] increases ROS levels in chondrocytes. As discussed further below, we previously demonstrated that ox-LDL binding to lectin-like oxidized low-density lipoprotein receptor-1 (LOX-1) also increases ROS production in cultured bovine articular chondrocytes. ROS produced by those stimulation has been reported to result in DNA damage of chondrocyte.[40]

The underlying mechanisms by which oxidative stress contributes to chondrocyte senescence have not been well defined. Studies in other cell types have provided evidence that oxidative stress contributes to senescence through modulation of the activity of specific cell signaling pathways.[8,74] As noted above, this can be due to modulation of the activity of a number of redox-sensitive kinases and phosphatases. The activity of MAP kinase pathways, which include ERK, JNK, and p38, may be particularly important. Caveolin-1 is an integral

membrane protein that serves as a scaffold and can regulate cell-signaling pathways involved in senescence.[8] Caveolin-1 has been found to play a role in chondrocyte senescence induced by IL-1 and H_2O_2 through activation of the p38 MAP kinase.[42] More recently, Yin et al have demonstrated that ROS can contribute to chondrocyte IGF-I resistance and reduced proteoglycan synthesis by causing an imbalance in the activity of the phosphoinositide-3 (PI-3) kinase-Akt pathway.[75] Because IGF-I can also stimulate chondrocyte anti-oxidant capacity,[72] resistance to IGF-I could further contribute to a redox imbalance.

Ox-LDL-induced oxidative stress and chondrocyte senescence

Ox-LDL has been recognized as one of the most important molecules causing atherosclerosis.[76] A novel receptor for ox-LDL, designated lectin-like ox-LDL receptor 1 (LOX-1), was cloned recently from cultured bovine aortic endothelial cells.[77] Ox-LDL uptake through this receptor, which is expressed on the vascular endothelium, is critically involved in endothelial activation and dysfunction in atherogenesis.[78] While, the involvement of lipid peroxidation in cartilage degeneration associated with aging and the pathogenesis of OA has been suggested by in vivo[79,80] and in vitro[81,82] studies. Some epidemiologic studies have suggested that OA and atherosclerosis share a common epidemiologic background in terms of the involvement of lipid peroxidation.[83,84] Other epidemiologic studies have shown that age is the chief risk factor for atherosclerotic diseases.[85] Further, endothelial cells in atherosclerotic lesions also show attributes of cell senescence.[86]

Interestingly, Nakagawa et al showed that LOX-1 is expressed and ox-LDL is found in chondrocytes in rat zymosan-induced arthritis (ZIA) and that treatment with anti-LOX-1 blocking antibody suppresses articular cartilage degeneration in ZIA, suggesting that ox-LDL binding to LOX-1 is involved in cartilage degeneration.[87] Their in vitro study using cultured rat articular chondrocytes showed that LOX-1 expression is detectable in basal culture conditions and that ox-LDL reduces rat chondrocyte viability through LOX-1, which induces nonapoptotic cell death.[88] As mentioned above, we previously demonstrated that ox-LDL binding to LOX-1 in cultured bovine articular chondrocytes (BACs) increases the production of intracellular ROS, suggesting that ox-LDL increases intracellular oxidative stress similar to IL-1β or fibronectin fragments[89,90] and that the ox-LDL/LOX-1 system plays a role in both endothelial and chondrocytic dysfunction.

More recently, Kakinuma et al[91] and Akagi et al[92] reported the presence of ox-LDL and LOX-1 expression in articular cartilage from patients with rheumatoid arthritis and OA, respectively. They also showed that ox-LDL can penetrate the cartilage matrix and associate with LOX-1, increasing MMP-3 production from cultured explants of human articular cartilage.[91] They further demonstrated that the presence of ox-LDL and expression of LOX-1 in chondrocytes correlates with degenerative grades of OA cartilage.[92] Simopoulou et al also showed that ox-LDL is detectable in the synovial fluid of OA joints and that LOX-1 mRNA and protein are expressed in chondrocytes from OA cartilage.[93] Further, we demonstrated that mechanical tensile load and ox-LDL synergistically induce LOX-1 in cultured BACs, resulting in decreased cell viability and proteoglycan synthesis.[94] These accumulating evidences possibly suggest that binding of ox-LDL to LOX-1 may cause cartilage degeneration in the context of chondrocyte senescence.

Clusters or clones of proliferating chondrocytes surrounded by newly synthesized matrix molecules constitute one of the histologic hallmarks of the chondrocytic response in the early phase of OA.[94–97] Anabolic growth factors trapped previously in the matrix may be released in a process of matrix degradation, which activates chondrocytes to proliferate and synthesis matrix macromolecules.[98] These factors in the synovial fluid may have better access to chondrocytes because of fissuring or loosening of the collagen network or damage to the collagen matrix itself.[95] These phenomena are thought to represent repairing responses of damaged cartilage. The progressive degeneration of cartilage in the later phase of OA may be attributed to limited repairing responses caused by cell senescence associated with reduced cell function and proliferative ability.[98,99]

SA β-gal activity is recognized as an important biological marker of cell senescence[100] and is higher in cloned chondrocytes in the OA cartilage.[17,19] In culture, ox-LDL increased the number of SA β-gal-positive BACs in a dose-dependent manner, which can be reversed by pretreat-

ment anti-oxidant N-acetyl cystein (NAC). Further, ox-LDL reduced the cell proliferative ability, as evaluated by BrdU incorporation, in a dose-dependent manner. Pretreatment with anti-LOX-1 blocking antibody cancelled these effects of ox-LDL on BACs as well as reported in the endothelial progenitor cells.[101] The induction of cell senescence caused by ox-LDL occurred within 24 hours and did not need subculturing, indicating that ox-LDL can induce the SIPS through the oxidative stress in chondrocytes.

As mentioned above, the "telomere hypothesis" is generally accepted to explain the replicative cell senescence. Telomerase is activated in the proliferating cells of tissues under repair, which prolongs the cellular replicative capacity and postpones cell senescence.[102-104] The regulation of telomerase activity is thought to play an important role in tissue repair and regeneration. We investigated whether the telomerase activity of BACs changes with the culture conditions that induced distinct proliferating activity in chondrocytes. Cultured chondrocytes with a higher proliferating activity have a higher telomerase activity, although the activity in all chondrocytes is lower than in HeLa cells. This suggests that the telomerase activity in BACs is upregulated during cell expansion, agreeing with previous reports on chondrocytes[37,105] and somatic cells.[106,107] Taken together, these data imply that the telomerase activity in proliferating and cloning chondrocytes in the early phase of OA is upregulated and plays an important role in tissue repair by postponing cell senescence and maintaining cell function. Thus, we investigated the effects of ox-LDL on the telomerase activity of the 70% confluent BACs. The telomerase activity was suppressed significantly in a time- and dose-dependent manner by adding ox-LDL. This suppressive effect on the telomerase activity was reversed by pretreatment with the LOX-1 blocking antibody, indicating that ox-LDL suppresses telomerase activity through its receptor LOX-1. Ox-LDL probably impairs the tissue repair of degenerative cartilage in the early phase of OA because suppression of telomerase activity in proliferating cells results in telomere shorting and instability, leading to cell senescence.[17,19] We also investigated the intracellular signaling pathway by which ox-LDL alters telomerase activity. Telomerase activity is regulated by phosphorylation of the reverse transcriptase (hTERT), and protein kinase C or protein kinase B (Akt) plays a critical role in the phosphorylation of hTERT.[108] In general, the PI3k/Akt pathway plays important roles in the progress of the cell cycle, cell proliferation, regulation of nuclear transcription factors, cell survival,[109,110] and chondrocyte differentiation and apoptosis.[111] Activation of this pathway increases the production of aggrecan,[112] and inactivation of this pathway suppresses cell viability in articular chondrocytes.[88] We found that ox-LDL and LY294002 (a specific inhibitor of PI3k) suppressed the telomerase activity in a dose-dependent manner and that IGF-1 (an activator of PI3k) recovered the ox-LDL-induced suppression of telomerase activity in BACs as well as in endothelial cells.[24] In addition, ox-LDL reduced the amount of the pAkt without changing the amount of Akt. Taken together, these results suggest that ox-LDL-induced suppression of telomerase activity can be attributed to inactivation of the PI3k/Akt pathway through binding to LOX-1.

An interesting question is whether ox-LDL activates the pathways that are linked mechanically to replicative senescence and SIPS, including the ATM-p53-p21-Rb pathway and the p38-MAPK-p16-Rb pathway, respectively.[113-114] We are especially interested in whether adding ox-LDL stabilizes p53 because a recent report shows that p53 destabilizes and permeabilizes lysosomes to shift β-galactosidase from the lysosomes to the cytosol, which is recognized as cytosolic staining of SA β-gal.[114] We have already the ox-LDL/LOX-1 system increases expression of p53 in mRNA and protein levels (unpublished data) (Figure).

Ox-LDL-induced oxidative stress and chondrocyte hypertrophy

Under physiologic conditions, articular chondrocytes maintain a stable phenotype to retain their function as a permanent cartilage. Differentiation of chondrocytes is strictly regulated so that the cartilage does not undergo ossification through hypertrophic differentiation, as is the case with endochondral bone formation.[115] However, the characteristic pathological changes in the early phase of osteoarthritis (OA) cartilage show formation of clusters of activated and proliferating chondrocytes.[116] These activated chondrocytes are hypertrophic in size and exhibit sustained functional and phenotypic changes, including an increase in alkaline phosphatase activity and expression of type X col-

lagen (COL10), runt-related transcription factor 2 (Runx2), vascular endothelial growth factor (VEGF), and MMP-13, which indicates that chondrocytes in OA cartilage assume the characteristics of hypertrophic chondrocytes in growth plate cartilage.[97,117,118] Hypertrophic chondrocytes, terminally differentiated chondrocytes in the growth plate that induce endochondral ossification expressing COL 10,[119] destroy the cartilage matrix via MMP expression, introduce vascular channels and bone cells from the bone marrow to the cartilage, and eventually cause apoptosis.[119] Interestingly, Morita et al recently showed that ROS regulates chondrocyte proliferation and the initiation of hypertrophic differentiation in the growth plate,[121] indicating that production of ROS by chondrocytes may also have an important physiologic role in vivo in the endochondral bone formation, suggesting a potential connection between ROS production in articular cartilage and chondrocyte hypertrophy observed in the early phase of OA. Oxidative stress, which may be induced by many mechanisms in OA, causes chondrocytes to differentiate into hypertrophic chondrocyte-like cells, resulting in degeneration of cartilage because of cartilage matrix degradation and cell death.

Then, we recently investigated and reported that ox-LDL binding to LOX-1 increased the expression of COL10 and the activity of ALP in a dose-dependent manner, suggesting that ox-LDL promotes hypertrophic differentiation in OA cartilage. The results of our study showed that both ox-LDL and H_2O_2 upregulate COL10 expression through upregulation of Runx2 in cultured BACs, and that NAC, an antioxidant, canceled the effect of ox-LDL and H_2O_2 on COL10 expression. These results make it possible to propose a mechanism for the involvement of ox-LDL in the development of OA in which ox-LDL-induced oxidative stress causes pathologic hypertrophic differentiation of articular chondrocytes through Runx2 upregulation, following which the activated chondrocytes upregulate the hypertrophic cell markers, COL10 and ALP. We previously demonstrated in vitro that ox-LDL upregulates another hypertrophic chondrocyte marker, VEGF, by binding to LOX-1.[122] Furthermore, we have recently observed that ox-LDL upregulates MMP-13 expression in BACs (unpublished data) (Figure).

Conclusions

Although the relationship between chondrocyte senescence and development of OA has not been completely understood yet, accumulating evidences in vivo and vitro have shown that the development of OA is, at least in part, attributable to the age-related changes in the joints. Senescent chondrocytes show both a decline in the local availability of growth factors, as well as a decline in the chondrocyte's response to stimulation with growth factors. Chondrocyte senescence is associated with an increased production of inflammatory mediators and matrix degrading enzymes characteristic of the senescent secretory phenotype. Age-related oxidative stress and damage may play a central role in cartilage aging through modulation of cell signaling pathways that regulate anabolic and catabolic activity (Figure).

We propose a mechanism for the involvement of ox-LDL in the development of OA where ox-LDL-induced oxidative stress causes SIPS of chondrocytes and results in suppression of telomerase activity through inactivation of the PI3k/Akt pathway. Furthermore, ox-LDL could induce pathologic chondrocyte hypertrophy through Runx2 upregulation, following which the activated chondrocytes upregulate the hypertrophic cell markers, COL10 and ALP. We have previously demonstrated in vitro that ox-LDL upregulates other hypertrophic chondrocyte markers, MMPs[91] and VEGF[122]. The ox-LDL-induced oxidative stress may play a significant role in the pathogenesis of OA through chondrocyte senescence.

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