

# Recent Advances in Molecular Mechanisms of Cartilage Degeneration during Osteoarthritis

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Osteoarthritis (OA) is the most common musculoskeletal disease, affecting around 50% of the population over 65 years of age [1]. OA is also a major cause of disability, which affects all joint tissues, but mainly the joints of knees, hands and hips. The high prevalence and disabled rate make OA a major social issue. However, to date, there is no efficacious therapy to slow and reverse joint damage during OA progression, mainly because the exact mechanisms driving OA development and progression remain largely unclear. Because the irreversible loss of articular cartilage is the main characteristic of OA, this seminar summarizes recent findings regarding OA articular cartilage to shed light on the molecular pathogenesis of cartilage degeneration.

## CHONDROCYTE CHANGES

Chondrocytes are the only cells in articular cartilage, and are responsible for cartilage integrity by synthesis of extracellular matrix. Thus chondrocytes changes may be a core component of cartilage degeneration. Osteoarthritis is characterized by destruction of extracellular matrix and loss of chondrocyte function, chondrocyte mainly experience following phenotype change.

## Chondrocyte Death

Chondrocyte depletion accompanied with large numbers of empty lacunae was found to be a persistent and important event in OA, and chondrocyte death was believed to be a major cause [2,3]. Cell dies usually by two processes, necrosis or apoptosis. Apoptosis is a programmed and energy-dependent cell death with distinctive morphological manifestations, including membrane blebbing, cell shrinkage, chromatin condensation, nuclear fragmentation, and formation of apoptotic bodies. Necrosis, however, is a non-programmed and energy-independent form of cell death. Many studies have demonstrated that there are both necrosis and apoptosis in OA cartilage, and the numbers of chondrocyte apoptosis is significantly correlated with severity of OA [2-4]. Although the increased cell death in OA is confirmed, the precise mechanism of chondrocyte death is still not established. Wei et al [5] discovered that the concentration of chemokine Stromal Cell-Derived Factor-1 (**SDF-1**) was significantly higher in Synovial Fluid (**SF**) of OA patients. While the pathological concentrations of SDF-1 (> or = 200 ng/mL) in SF induced death of human chondrocytes in a necrosis-dependent manner. In addition, Wei et al [3] discovered that the CD95 (Fas) pathway can mediate cell death in primary OA chondrocytes. Anti-CD95 induced both chondrocyte apoptosis and necrosis during development of OA, which depended on the activity of p38 mitogen-activated protein kinase (MAPK) within chondrocytes. The p38 MAPK inhibitor, SB203580, abolished anti-CD95 induced cell death by inhibiting the activities of ATF-2 and caspase-3.

## Chondrocyte Hypertrophy

Recent studies indicate that during OA development, chondrocytes in articular cartilage undergo hypertrophy-like changes, which has a remarkably resemblance to the phenotype of terminally differentiating chondrocytes during the growth plates development [6]. Normal chondrocytes is characterized by proliferation and expression of chondrogenic genes, including Sex Determining Region Y Box 9 (**SOX9**), Aggrecan, type II collagen (Col2A1). When the healthy chondrocyte phenotype switches toward a hypertrophic chondrocyte phenotype, chondrocyte become large about tenfold and expresses Runt-Related Transcription Factor 2 (**Runx2**), Indian Hedgehog (**Ihh**), Alkaline Phosphatase (**ALPs**), type X Collagen and Matrix Metalloproteinase 13 (**MMP-13**), which leads to cartilage breakdown and bone deposition [6,7]. Based on recent literature, multiple signaling pathways have been involved in regulation of hypertrophic differentiation in chondrocytes. The most important signaling pathways included WNT, Bone Morphogenetic Protein (**BMP**)/ Transforming Growth Factor- $\beta$  (**TGF- $\beta$** ), Parathyroid Hormone-Related Peptide (**PTHrP**), *Ihh*, Fibroblast Growth Factor (**FGF**), Insulin like Growth Factor (**IGF**) and Hypoxia-Inducible Factor (**HIF**) signaling pathways [8].

## Chondrocyte Senescence

A study showed that the density of chondrocytes had been reduced 30% in human hip joint cartilage between the ages of 30 and 70, the reduction of the cellularity with age is more

accentuated in the superficial than in the deeper zone and is even limited to the superficial zone during the latter part of aging; The loss of chondrocytes is more evident in aged cartilage with OA than without OA [9]. The loss of the chondrocytes can be attributed to chondrocyte death, or decrement of proliferation. The loss of resident cells in the articular cartilage results in disadvantageous impacts such as impaired matrix remodelling and development of OA [3]. Meanwhile, with age, chondrocytes exhibit senescent features, such as shortening of telomere and increase of  $\beta$ -galactosidase activity. Accordingly, aged chondrocytes decreased synthetic activity, and secrete smaller and more irregular proteoglycans [10]. The senescent chondrocytes also increased production of cytokines, including Interleukin 6 (**IL-6**) and Interleukin 1  $\beta$  (**IL-1  $\beta$** ), Matrix Metalloproteinases (**MMPs**), and growth factors such as FGF with some features in common with the OA chondrocyte phenotype. Studies have shown that expression of MMP-3 and MMP-13 were increased in aged cartilage, accompanied with accumulation of collagen neopeptides representing denatured or cleaved collagen [11]. These senescent changes impair the ability to maintain the surrounding extracellular matrix.

There are two types of cell senescence: intrinsic senescence and extrinsic senescence. Intrinsic senescence, also terms the classic replicative senescence, is attributed to telomeres shortening and telomere dysfunction. Extrinsic senescence, also terms stress-induced senescence, results from telomere-damaging stimuli, including oxidative damage [12], activated oncogenes and inflammation [13]. It has been evidenced that telomeres become short in chondrocytes of older adults [14]. But considering those telomere-damaging stimuli frequently occurs in aging cartilage and OA, chondrocytes senescence in articular cartilage seems more attributed to the extrinsic senescence [10,14].

The mediators of chondrocytes senescence mainly include Telomeric Repeat Binding Factor (**TRF**), X-ray repair complementing defective repair in Chinese Hamster Cells 5 (**XRCC5**), and Sirtuin 1 (**Sirt1**). TRF1 and TRF2 are telomeric proteins to form and maintain telomere structure [15]. XRCC5 plays an important role in repairing DNA double-strand damage [16]. Sirt1 promotes growth, and prevents senescence and apoptosis as an inhibitor of p53 [17]. Oxidative stress induces senescence and accelerates telomere shortening in human chondrocytes [12]. Acute oxidative insult induced up regulation of TRF1, TRF2, XRCC5 and Sirt1 in the early passages of human chondrocytes, but induced to a less extent in aged chondrocytes [12]. This finding suggests that TRF1, XRCC5, and Sirt1 help young chondrocytes to resist damage from oxidative stress by repairing DNA damage accumulation and preventing telomere shortening. Consistently, aged chondrocytes with lower induction levels of these regulatory proteins lose this ability to withstand oxidative challenge, and may trigger chondrocyte senescence. Membrane protein caveolin-1 is also involved in regulation of chondrocytes senescence. Study revealed that expression of caveolin proteins is increased in old rats; over expression of caveolin-1 contributes to a senescent phenotype, while reduction of caveolin-1 can reversed senescent phenotype [18].

# CARTILAGE MATRIX DEGENERATION

The most representative characteristic of OA is irreversible and ingravescent degeneration of articular cartilage. The articular cartilage is composed of chondrocytes and extracellular matrix, essential components of which include collagen and aggrecan. The negatively charged glycosaminoglycan chains of aggrecan attract water molecules, endowing the cartilage with the high osmotic pressure; while the cartilaginous collagen network provides the tissue with its tensile resistance. Chondrocyte, embedded in it, has a responsibility for maintaining homeostasis of cartilage via expression of various matrix proteins. The fundamental function of articular cartilage is bearing loading, and its function relies on integrity of its structure, cartilage matrix degeneration will impair its function and contribute to OA.

## Age-Related Matrix Changes

Many evidences demonstrate that increase of age is the most remarkable risk factor for the primary OA [19]. With aging, cartilage matrix was experiencing age-related changes, which maybe contributed to OA development.

**Accumulation of AGEs:** With age, there is much accumulation of Advanced Glycation Endproducts (**AGEs**) in articular cartilage. AGEs are produced via a nonenzymatic reaction between reducing sugars and free amino groups of proteins, lipids, or nucleic acids. Accumulation of AGEs has a pathogenetic effect on the body, such as enhanced oxidative stress and inflammation [20]. In chondrocytes, AGEs stimulates the production of inflammatory cytokine, such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$  and IL-6, and inflammatory mediators, such as nitric oxide and prostaglandin E<sub>2</sub>; Meanwhile, AGEs inhibits the expression of aggrecan and type II collagen, and promotes the expression of Metalloproteinases (**MMPs**) and A Disintegrin And Metalloproteinase With Thrombospondin-Like Motifs (**ADAMTS**) [21]. AGEs accumulation also has adverse effects on the cartilaginous matrix. AGEs promotes collagen cross-linking, which increases tissue stiffness and makes cartilage more brittle and susceptible to fatigue failure [10].

**Aggrecan degradation:** Aggrecan is a large “aggregating” proteoglycan, and consists of a core protein which is covalently attached by numerous highly sulfated glycosaminoglycan chains. Owing to the hydrophilic nature of negatively charged sulfated glycosaminoglycan chains, articular cartilage contains around 70%–80% water and is very resilient. With aging, aggrecan, the second most abundant cartilage matrix protein, has some age-related changes in size, structure, and sulfation [10]. At same time the degraded fragments from aggrecan will be left behind and accumulate in cartilage with age. All this will impair cartilage resiliency and hydration [10]. MRI studies showed that knee cartilage become thin with aging, particularly on the femoral side of the joint and in patellae [22]. This could be due to loss of chondrocytes and cartilage matrix, but also to reduction of water content.

**Increase of ROS:** With age, levels of Reactive Oxygen Species (**ROS**) are increased in cartilage, and chondrocytes are more susceptible to impairment from ROS. Excessive ROS can cause oxidative

damages to proteins, lipids, and DNA; Excessive ROS also results in telomere shortening, reduced production of matrix, chondrocyte senescence, and chondrocyte apoptosis. Increase of ROS can up regulate pro-inflammatory cytokines and MMPs, which mediate degradation of cartilage [23]. The ROS imbalance may be caused by an age-related change of mitochondria which is a major regulator of ROS and can protect cells from ROS damage [24]. Many inflammatory cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ , can lead to mitochondrial DNA damage, which contributes to chondrocyte death [24].

ROS plays an important role in cartilage degeneration via several signaling pathways. Excessive ROS inhibits matrix synthesis by activation of the IRS-1-PI-3 kinase-Akt signaling pathway; Meanwhile, ROS suppresses the expression of aggrecan, type II collagen, and SOX9 by activation of ERK and MAP kinase in chondrocytes [25,26]. Continuing activation of ERK can induce cell senescence. Moreover, extracellular ROS can inhibit the Akt pathway through oxidization of Low-Density Lipoprotein (**LDL**). The binding of oxidized LDL to cell surface receptor LOX-1 can induce chondrocyte senescence. Oxidized LDL can also promote chondrocyte hypertrophic phenotype [27].

## Imbalance of Matrix Metabolism

In healthy articular cartilage, chondrocytes maintain the balance between anabolism and catabolism. In OA, however, damaging stimulus disrupts this balance. At early stage of OA, chondrocytes increased proliferation, which results in formation of chondrocyte clusters; meanwhile chondrocytes increased matrix synthesis, but synthesized lots of irregular matrix ingredient, such as smaller and more irregular collagens and proteoglycans. With OA progression, excessive catabolic activity from chondrocyte broke the balance of cartilage homeostasis and lead to decomposition of cartilage matrix [28]. During OA development, chondrocytes in articular cartilage also underwent hypertrophy-like changes to express type X collagen and MMP-13, which lead to cartilage breakdown and bone deposition [6,7]; Meanwhile, chondrocytes death resulted in reduction of number of functional chondrocytes.

The balance of anabolism and catabolism is regulated by several mediators. Catabolic factors include TNF- $\alpha$ , IL-1 $\beta$ , IL-6 and matrix-degrading enzymes, such as the collagenases (MMP-1, -3 and -13) and Aggrecan-Degrading Enzymes (**ADAMTS-4 and -5**). Anabolic factors include IGF-1, Transforming Growth Factor- $\beta$  (**TGF- $\beta$** ), Osteogenic Protein-1 (**OP-1**) and bone morphogenic protein-7. In OA cartilage, anabolic factors remarkably reduced, while catabolic factors abundantly increased [1].

## SIGNAL PATHWAY INVOLVING IN CARTILAGE DEGENERATION

### TGF- $\beta$ Signal Pathway

TGF- $\beta$  plays a vital role in development and homeostasis of cartilage. It can regulate cell proliferation and differentiation, control ECM synthesis and degradation. TGF- $\beta$  is secreted in an inactive form and requires activity via binding to two of its receptors in cytomembrane,

the type I receptors and type II receptors, also termed receptor-like kinases 1 (ALK1) and receptor-like kinases 5 (ALK5). TGF- $\beta$  mainly signals via formation of heteromeric complexes of TGF- $\beta$ , transmembrane type I and type II receptors to activate ALK5, which results in phosphorylation of Smad2 or Smad3. Phosphorylated Smad2 or Smad3 will form complex with the co-Smad (Smad4) and move to the nucleus to promote anabolism and proliferation. However, TGF- $\beta$  is also able to signal via only binding to TGF- $\beta$  type I receptor, and activating ALK1, which will lead to phosphorylation of Smad1, Smad5, or Smad8. Phosphorylated Smad1, Smad5, or Smad8 can also form complex with the Smad4 and move to the nucleus to modify gene expression in opposite directions [29,30]. During OA, the ratio of ALK1 to ALK5 is increased to promote OA development [30]. In OA, ALK5 expression decreased much more than ALK1, resulting in increase of the ALK1/ALK5 ratio, which was associated with an increased of Id-1 (inhibitor of DNA binding-1)/PAI-1 (plasminogen activator inhibitor-1) ratio [30,31]. In chondrocytes, overexpression of active ALK1 increased MMP-13 expression, while siRNA against ALK1 resulted in decrease of MMP-13 expression to nondetectable levels; Overexpression of constitutive active ALK5 increased aggrecan expression, whereas siRNA against ALK5 increased MMP-13 expression. Similarly, in human OA cartilage ALK1 was highly correlated with expression of MMP-13, and ALK5 was correlated with expression of aggrecan and type II collagen [30,31]. Moreover, in the DMM (meniscus destabilization OA model) and the STR/ORT mice (spontaneous OA model), development of the disease was correlated with decreased ALK5 expression. In STR/ORT mice model, the ALK1/ALK5 ratio was increased from 5 to 18 at the medial tibia, increased from 1 to 5 at the lateral tibia during 1-year OA. Thus, increase of ALK1/ALK5 ratios in chondrocytes is associated with OA development [31].

The Smad pathway is the most important for TGF- $\beta$  signaling, but is not the solely only pathway. Erk, Jun N-Terminal Kinase (**JNK**) and p38MAPK pathways are all involved in TGF- $\beta$  signaling [32,33]. The Smad-pathway can intersect with other TGF- $\beta$  signaling pathways via phosphorylated by ERK or JNK, or via interaction between Smad complexes and MAPK-activated transcription factors. But MAPK activation can also be triggered by various extracellular cytokines, such as TNF- $\alpha$  and IL-1. Therefore, the Smad/MAPK interactions are a result lead by interaction of various cytokines [32,33].

## Ihh Signaling Pathways

Indian Hedgehog (**Ihh**) is mainly expressed by prehypertrophic chondrocytes. Vertebrate Ihh signaling is transduced through two multipass transmembrane proteins, Patched1 (**Ptch1**) and Smoothed (**Smo**). In the absence of Ihh signal, the Ihh binding receptor Ptch1 inhibits the signaling component Smo and represses the downstream gene expression by suppressing the Gli/Ci zinc finger transcription factors (Gli1, 2, and 3). When Ihh is present, Ihh binds to Ptch1 and releases Smo, allowing Smo to transduce the Ihh signal to intracellular components and allowing the active Gli/Ci transcription factors to enter the nucleus and enhance the transcription level of downstream targets [6,7].

Recent studies have demonstrated that *Ihh* expression is very low in healthy human articular cartilage but increases during OA development [7,34]. During OA development, articular cartilage chondrocytes recapitulate a process of chondrocyte hypertrophy, terminal differentiation, ossification and finally apoptosis. The expression of *Ihh*, MMPs, and genes associated with chondrocyte hypertrophy and cartilage degradation is upregulated in early human articular cartilage lesions, suggesting that *Ihh*-mediated chondrocyte hypertrophy differentiation may be associated with early cartilage degeneration in OA. Meanwhile, type X collagen expression, a specific marker for hypertrophic chondrocytes, significantly increases around chondrocyte clusters in damaged cartilage, but not in normal cartilage [6].

Genetic studies using knockout mice have demonstrated that activation of *Ihh* downstream signaling pathways increased chondrocyte hypertrophy and symptoms resembling human OA in the affected joints. Elevated *Ihh* induced cartilage damage in adult *Ptch1* C/C, *Col2a1*-CreER transgenic mice, including thinner articular cartilage lining and decreased Proteoglycan (PG) content. In contrast, reduced *Ihh* activity has a chondroprotective effect, causing thickening of articular cartilage lining and increased PG content in *Smo* C/C, *Col2a1*-CreER. These observations imply that *Ihh* may play a role in cartilage degradation. Consistent with these observations, increased *Hh* signaling is involved in mouse OA development and increased *Ihh* expression is associated with the severity of OA cartilage damage [35]. Previous study, in which human OA tissues were analyzed for *Ihh* and hypertrophic marker contents, as well as the effect of *Ihh* signaling on OA chondrocyte hypertrophy, provided strong evidence that *Ihh* signaling may promote OA development by driving chondrocyte hypertrophy [7]. Later, another study demonstrated that *Ihh* signaling is part of the pathobiology of OA development by utilizing *Ihh* conditional knockout mice (*Col2a1*-CreERT2; *Ihh*<sup>fl/fl</sup>), a Tamoxifen (TM)-inducible CreERT2 recombinase and partial medial meniscectomy [6]. Moreover, *Ihh* is also involved in cartilage endplate degeneration [36].

In addition to inducing chondrocyte hypertrophy and cartilage degradation in OA, *Ihh* is also a mechanoresponsive gene related to mechanical stress. It is likely that the redistribution of the stress in the knee following injury, such as Anterior Cruciate Ligament (ACL) injury, stimulates the synthesis and release of *Ihh* into the synovial fluid. Thus, evaluating the biosynthesis of *Ihh* will provide insight into mechanisms of cartilage degeneration mediated by *Ihh* pathway and provide important data regarding the potential efficacy of *Ihh*-targeted therapies for treating OA [7].

## SDF-1/CXCR4 Signal Pathway

Chemokines and their receptors play important role in cell immune, migration of stem cells, and invasion of cancer cell. Of which particular interest in cartilage biology is Stromal Cell-Derived Factor 1 (SDF-1) and Chemokine Receptor Four (CXCR4). SDF-1 is an 8-kDa chemokine originally isolated from bone marrow stromal cells; CXCR4 is a seven-transmembrane G-protein-coupled receptor, whose activation leads to intracellular signaling cascades. The SDF-1/CXCR4 axis is unique in that SDF-1 is the only known ligand of CXCR4. When binding to CXCR4, SDF-1 will

activate a wide variety of primary cells to stimulate proliferation, differentiation, and apoptosis [37]. In the joint, CXCR4 is expressed by chondrocytes, while SDF-1 is synthesized by the synovium. SDF-1/CXCR4 plays a role in the progression of OA. In chondrocytes, SDF-1 activates the calcium, Erk and p38 MAP kinase signaling pathways, thereby inducing the release of MMPs, such as MMP-3 and MMP-13. *In vivo*, SDF-1 markedly increased in the synovial fluid from the knee joints of rheumatoid arthritis and OA patients, and synovectomy significantly reduces the serum concentrations of SDF-1, MMP-9 and MMP-13; The binding of SDF-1 to CXCR4 induces OA cartilage degeneration [5,38]. The catabolic processes can be disrupted by pharmacologic blockade of SDF-1/CXCR4 signaling.

The drug AMD3100 is a specific inhibitor of SDF-1 pathway, also is the prototypical CXCR4 blocking drug with high specificity for CXCR4. AMD3100 has been approved for human use. *In vitro*, AMD3100 blocked CXCR4-induced expression of MMP-1 and invasion in chondrosarcoma cells. *In vivo*, AMD3100 inhibited collagen-induced joint inflammation. Recent studies have demonstrated that AMD3100 relieved OA via blockade of the SDF-1/CXCR4 signaling pathway in primary guinea pig OA model [39]. Therefore, Blocking SDF1/CXCR4 signaling pathway is a novel therapeutic target for the prevention and treatment of OA.

## EPIGENETICS

Accumulating evidence indicates that environmental OA risk factors can mediate cartilage homeostasis by epigenetic mechanisms, mainly including DNA methylation, histone modification and Micro-RNA (**miRNA**) [40].

### DNA Methylation

DNA methylation plays an essential role in regulating gene expression by alteration of chromatin structure. DNA methylation is controlled by different DNA Methyltransferases (**DNMTs**), among which DNMT1 and DNMT3A are present at high levels in cartilage tissue [41]. DNA methylation patterns exhibit dynamic alterations at the promoter regions of individual genes in OA chondrocytes. First, methylation of DNA is likely to influence the expression levels of anabolic factors in OA. For example, the Collagen Type **IX** A1 (**COL9A1**) enhancer was hypermethylated, and the hypermethylation of COL9A1 enhancer attenuates the binding of SOX9 to the COL9A1 promoter, and leads to transcriptional repression of COL9A1 during OA development [42]. Similarly, DNA methylation also influences the expression of inflammatory cytokines and catabolic factors. The expression of IL-1 $\beta$  gene requires demethylation of the CpG sites of IL-1 $\beta$  promoter in OA [43]. The MMP-13 promoter region was also demethylated in OA chondrocytes. The demethylation of MMP-13 promoter can make the cAMP response element bind to the promoter region to upregulate MMP-13 expression [43, 44]. In addition, the promoters of other catabolic factors, such as MMP-3,-9 and ADAMTS4, are all demethylated when the expression of these genes increased under OA-related pathogenic conditions [41].



## Histone Deacetylase

Histone Deacetylase (**HDACs**) deacetylates histone to achieve transcriptional regulation of gene by altering the interaction between histone and DNA. Histone Deacetylase 4 (**HDAC4**), a member of the class IIa histone deacetylase family, is highly expressed in the cartilage [45]. Recent studies show HDAC4 prevents chondrocyte hypertrophy by repressing the activity of Runx2. The lacking HDAC4 mutant mice display chondrocyte hypertrophy, increased MMP-13, and premature ossification of developing bones owing to constitutive expression of Runx2 [46]. Our recent findings reveal that HDAC4 decreases dramatically in human OA cartilage, and decrease of HDAC4 was associated with increase of Runx2 and other OA-related genes in human OA cartilage, specifically: MMP-13, *Ihh* and type X collagen. While over expression of HDAC4 reduced the mRNA expression of Runx2, MMP-1, MMP-3, MMP-13, type X collagen, *Ihh*, ADAMTS-4 and -5 and promoted the mRNA expression of type II collagen and aggrecan. HDAC4 inhibited Runx2 and MMP-13 promoter activities in a dose dependent manner [47]. Thus, increase of HDAC4 may attenuate OA.

Sirtuins, a family of NADt-dependent deacetylases, is the class III histone deacetylase, also involved in regulation of chondrocyte energy and metabolism [48]. The Sirt1, a member of the Sirtuins, promotes chondrocyte survival and matrix gene expression. Moreover, TNF- $\alpha$  signaling triggers cathepsin B-mediated cleavage of Sirt1, which lead to reduced matrix gene expression [49]. Heterozygous Sirt1+/- or Sirt1 mutant mice, lacking Sirt1 enzymatic activity, developed premature OA-like changes at 9 months of age, which may be due to increased chondrocyte apoptosis [50]. Similarly, the expression of Sirt1 is decreased over the course of OA; chondrocyte-specific deletion of the Sirt1 gene significantly accelerated OA pathogenesis in surgically-induced mouse OA model, accompanied with down regulation of anabolism and up regulation of cartilage degrading enzymes [51].

## miRNAs

miRNAs are a class of non-coding RNA that has been implicated in important cellular processes. They are post-transcriptional regulators that bind to 3'-untranslated sequences on target messenger RNAs (**mRNAs**), usually resulting in translational repression or target degradation and gene silencing [52,53]. The regulative effects of miRNAs on OA are obvious through studies comparing miRNA expression between OA cartilage tissues and their normal cartilage counterparts [52]. Iliopoulos et al [52] measured the expression of 365 miRNAs and identified 9 significantly up regulated miRNAs and 7 down regulated miRNAs in OA cartilage, compared with normal controls, suggesting that miRNAs are involved in OA development. Jones et al [53] examined the expression of 157 human miRNAs and identified 17 miRNAs whose expression varied by 4-fold or more when comparing normal with late-stage OA cartilage.

It is well known that IL-1 $\beta$  contributes to the progression of OA. Recent studies demonstrated that miRNAs are implicated in the processes of OA cartilage breakdown triggered by IL-1, including

miR-140, miR-27b, miR-146, miR-9, miR-98 and miR-558. Several functional experiments indicated miR-9 in the regulation of MMP-13 expression, as well as miR-9, miR-98, and miR-146a in the control of TNF expression, suggesting that these miRNAs may play a protective role in OA [53-56].

The miR-146a gene is significantly upregulated in human knee OA joint cartilage. Functional experiments implicated miR-146a significantly suppressed extracellular matrix associated proteins in human knee joint chondrocytes and regulated inflammatory cytokines in synovial cells from human knee joints. MiR-146a controls knee joint homeostasis and OA-associated algia by balancing inflammatory responses in cartilage. MiR-146a functions in an anti-catabolic manner in articular cartilage by antagonizing the IL-1 $\beta$  induced expression of cartilage degrading enzymes MMP-13 and ADAMTS5 [57].

Reduced miR-140 expression was observed in human OA cartilage compared with normal cartilage [54,58] and decreased miR-140 expression was also reported in OA chondrocytes [58]. *In vitro* treatment of chondrocytes with IL-1 $\beta$  suppressed miR-140 expression. Transfection of chondrocytes with ds-miR-140 down-regulated IL-1 $\beta$  induced ADAMTS5 expression and rescued the IL-1 $\beta$  dependent repression of aggrecan gene expression [54]. Moreover expression of MMP-13 is inhibited by miRNA-140 in C28/I2 cells [59]. MiR-140-/- mice showed age-related OA-like changes, characterized by proteoglycan loss and fibrillation of articular cartilage. In contrast, over expression of miR-140 in chondrocytes protected cartilage from damage in an antigen-induced arthritis model [55].

The miR-558 was mainly expressed by normal human articular cartilage, and its expression level was significantly lower in OA cartilage. When stimulated by IL-1 $\beta$ , there was a significant reduction of miR-558 mRNA level in both normal and OA chondrocytes. IL-1 $\beta$  induced activation of MAPK and Nuclear Factor-kB (**NF-kB**) decreased miR-558 expression and induced COX-2 expression in chondrocytes. Interestingly, IL-1 induced activation of NF-kB and expression of MMP-1 and MMP-13 was significantly inhibited by miR-558 overexpression. MiR-558 directly targets COX-2 and regulates catabolic effects stimulated by IL-1 $\beta$  in human chondrocytes [56].

Increased levels of miR-203 led to elevated secretion of MMP-1 and IL-6, suggesting that miR-203 might be a pro-inflammatory and joint destructive factor [60]. Over-expression of miR-203 resulted in increased secretion of nitric oxide, which was one of the major pro-inflammatory factors in OA [61].

In summary, the function of joint depends on integrity of articular cartilage, and progressive cartilage degeneration is a pivotal aspect of OA. Over the past few decades, researches have obtained many important findings. It is sure that more extensive researches focusing on this topic will provide a much more clues for understanding or treatment of OA in future.

## References

1. Musumeci G, Aiello FC, Szychlińska MA, Di Rosa M, Castrogiovanni P. Osteoarthritis in the XXIst century: risk factors and behaviours that influence disease onset and progression. *Int J Mol Sci.* 2015; 16: 6093-6112.
2. Sharif M, Whitehouse A, Sharman P, Perry M, Adams M. Increased apoptosis in human osteoarthritic cartilage corresponds to reduced cell density and expression of caspase-3. *Arthritis Rheum.* 2004; 50: 507-515.
3. Wei L, Sun XJ, Wang Z, Chen Q. CD95-induced osteoarthritic chondrocyte apoptosis and necrosis: dependency on p38 mitogen-activated protein kinase. *Arthritis research & therapy.* 2006; 8: R37.
4. Zamli Z, Sharif M. Chondrocyte apoptosis: a cause or consequence of osteoarthritis? *Int J Rheum Dis.* 2011; 14: 159-166.
5. Wei L, Sun X, Kanbe K, Wang Z, Sun C. Chondrocyte death induced by pathological concentration of chemokine stromal cell-derived factor-1. *J Rheumatol.* 2006; 33: 1818-1826.
6. Zhou J, Wei X, Wei L. Indian Hedgehog, a critical modulator in osteoarthritis, could be a potential therapeutic target for attenuating cartilage degeneration disease. *Connective tissue research.* 2014; 55: 257-261.
7. Wei F, Zhou J, Wei X, Zhang J, Fleming BC. Activation of Indian hedgehog promotes chondrocyte hypertrophy and upregulation of MMP-13 in human osteoarthritic cartilage. *Osteoarthritis Cartilage.* 2012; 20: 755-763.
8. Zhong L, Huang X, Karperien M, Post JN. The Regulatory Role of Signaling Crosstalk in Hypertrophy of MSCs and Human Articular Chondrocytes. *Int J Mol Sci.* 2015; 16: 19225-19247.
9. Ritter MA, French ML, Eitzen H. Evaluation of microbial contamination of surgical gloves during actual use. *Clin Orthop Relat Res.* 1976; : 303-306.
10. Li Y, Wei X, Zhou J, Wei L. The age-related changes in cartilage and osteoarthritis. *Biomed Res Int.* 2013; 2013: 916530.
11. Dejica VM, Mort JS, Laverty S, Antoniou J, Zukor DJ, et al. Increased type II collagen cleavage by cathepsin K and collagenase activities with aging and osteoarthritis in human articular cartilage. *Arthritis research & therapy.* 2012; 14: R113.
12. Brandl A, Hartmann A, Bechmann V, Graf B, Nerlich M, et al. Oxidative stress induces senescence in chondrocytes. *J Orthop Res.* 2011; 29: 1114-1120.
13. Campisi J, d'Adda di Fagagna F. Cellular senescence: when bad things happen to good cells. *Nat Rev Mol Cell Biol.* 2007; 8: 729-740.
14. Campisi J. Senescent cells, tumor suppression, and organismal aging: good citizens, bad neighbors. *Cell.* 2005; 120: 513-522.
15. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet.* 2008; 42: 301-334.
16. Thacker J, Zdzienicka MZ. The XRCC genes: expanding roles in DNA double-strand break repair. *DNA Repair (Amst).* 2004; 3: 1081-1090.
17. Dang W. The controversial world of sirtuins. *Drug Discov Today Technol.* 2014; 12: e9-9e17.
18. Dai SM, Shan ZZ, Nakamura H, Masuko-Hongo K, Kato T, 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 and rheumatism.* 2006; 54: 818-831.
19. Meulenbelt I. Osteoarthritis year 2011 in review: genetics. *Osteoarthritis Cartilage.* 2012; 20: 218-222.
20. Uribarri J, Woodruff S, Goodman S, Cai W, Chen X. Advanced glycation end products in foods and a practical guide to their reduction in the diet. *J Am Diet Assoc.* 2010; 110: 911-916.
21. Nah SS, Choi IY, Yoo B, Kim YG, Moon HB. Advanced glycation end products increases matrix metalloproteinase-, -3, and -13, and TNF- $\alpha$  in human osteoarthritic chondrocytes. *FEBS Lett.* 2007; 581: 1928-1932.
22. Eckstein F, Cicuttini F, Raynaud JP, Waterton JC, Peterfy C. Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. *Osteoarthritis Cartilage.* 2006; 14: 46-75.
23. Scott JL, Gabrielides C, Davidson RK, Swingler TE, Clark IM, et al. Superoxide dismutase downregulation in osteoarthritis progression and end-stage disease. *Annals of the rheumatic diseases.* 2010; 69: 1502-1510.
24. Wu L, Li L, Liu H, Cheng Q. Mitochondrial pathology in osteoarthritic chondrocytes. *Curr Drug Targets.* 2014; 15: 710-719.
25. Yin W, Park JI, Loeser RF. Oxidative stress inhibits insulin-like growth factor-I induction of chondrocyte proteoglycan synthesis through differential regulation of phosphatidylinositol 3-Kinase-Akt and MEK-ERK MAPK signaling pathways. *The Journal of biological chemistry.* 2009; 284: 31972-31981.
26. Beier F, Loeser RF. Biology and pathology of Rho GTPase, PI-3 kinase-Akt, and MAP kinase signaling pathways in chondrocytes. *J Cell Biochem.* 2010; 110: 573-580.

27. Kishimoto H, Akagi M, Zushi S, Teramura T, Onodera Y, et al. Induction of hypertrophic chondrocyte-like phenotypes by oxidized LDL in cultured bovine articular chondrocytes through increase in oxidative stress. *Osteoarthritis and Cartilage*. 2010; 18: 1284-1290.
28. Schroepfel JP, Crist JD, Anderson HC, Wang J. Molecular regulation of articular chondrocyte function and its significance in osteoarthritis. *Histol Histopathol*. 2011; 26: 377-394.
29. Blaney Davidson EN, van der Kraan PM, van den Berg WB. TGF-beta and osteoarthritis. *Osteoarthritis Cartilage*. 2007; 15: 597-604.
30. van der Kraan PM, Blaney Davidson EN, Blom A, van den Berg WB. TGF-beta signaling in chondrocyte terminal differentiation and osteoarthritis: modulation and integration of signaling pathways through receptor-Smads. *Osteoarthritis and Cartilage*. 2009; 17: 1539-1545.
31. Blaney Davidson EN, Remst DF, Vitters EL, van Beuningen HM, Blom AB. Increase in ALK1/ALK5 ratio as a cause for elevated MMP-13 expression in osteoarthritis in humans and mice. *J Immunol*. 2009; 182: 7937-7945.
32. van der Kraan PM, Goumans MJ, Blaney Davidson E, ten Dijke P. Age-dependent alteration of TGF-beta signalling in osteoarthritis. *Cell and tissue research*. 2012; 347: 257-265.
33. Kamato D, Burch ML, Piva TJ, Rezaei HB, Rostam MA, et al. Transforming growth factor-beta signalling: role and consequences of Smad linker region phosphorylation. *Cellular signaling*. 2013; 25: 2017-2024.
34. Wei L, Fleming BC, Sun X, Teeple E, Wu W, et al. Comparison of differential biomarkers of osteoarthritis with and without posttraumatic injury in the Hartley guinea pig model. *J Orthop Res*. 2010; 28: 900-906.
35. Zhang C, Wei X, Chen C, Cao K, Li Y. Indian hedgehog in synovial fluid is a novel marker for early cartilage lesions in human knee joint. *Int J Mol Sci*. 2014; 15: 7250-7265.
36. Wang S, Yang K, Chen S, Wang J, Du G, et al. Indian hedgehog contributes to human cartilage endplate degeneration. *European spine journal*. 2015; 24: 1720-1728.
37. Sun X, Wei L, Chen Q, Terek RM. CXCR4/SDF1 mediate hypoxia induced chondrosarcoma cell invasion through ERK signaling and increased MMP1 expression. *Mol Cancer*. 2010; 9: 17.
38. Wei L, Kanbe K, Lee M, Wei X, Pei M. Stimulation of chondrocyte hypertrophy by chemokine stromal cell-derived factor 1 in the chondro-osseous junction during endochondral bone formation. *Dev Biol*. 2010; 341: 236-245.
39. Wei F, Moore DC, Wei L, Li Y, Zhang G. Attenuation of osteoarthritis via blockade of the SDF-1/CXCR4 signaling pathway. *Arthritis Res Ther*. 2012; 14: R177.
40. Barter MJ, Bui C, Young DA. Epigenetic mechanisms in cartilage and osteoarthritis: DNA methylation, histone modifications and microRNAs. *Osteoarthritis Cartilage*. 2012; 20: 339-349.
41. Kim H, Kang D, Cho Y, Kim JH1. Epigenetic Regulation of Chondrocyte Catabolism and Anabolism in Osteoarthritis. *Mol Cells*. 2015; 38: 677-684.
42. Imagawa K, de Andres MC, Hashimoto K, Itoi E, Otero M, et al. Association of reduced type IX collagen gene expression in human osteoarthritic chondrocytes with epigenetic silencing by DNA hypermethylation. *Arthritis & rheumatology*. 2014; 66: 3040-3051.
43. Hashimoto K, Otero M, Imagawa K, de Andrés MC, Coico JM. Regulated transcription of human matrix metalloproteinase 13 (MMP13) and interleukin-1 $\beta$  (IL1B) genes in chondrocytes depends on methylation of specific proximal promoter CpG sites. *J Biol Chem*. 2013; 288: 10061-10072.
44. Bui C, Barter MJ, Scott JL, Xu Y, Galler M, et al. cAMP response element-binding (CREB) recruitment following a specific CpG demethylation leads to the elevated expression of the matrix metalloproteinase 13 in human articular chondrocytes and osteoarthritis. *FASEB journal*. 2012; 26: 3000-3011.
45. Guan Y, Chen Q, Yang X, Haines P, Pei M. Subcellular relocation of histone deacetylase 4 regulates growth plate chondrocyte differentiation through Ca $^{2+}$ /calmodulin-dependent kinase IV. *Am J Physiol Cell Physiol*. 2012; 303: C33-40.
46. Vega RB, Matsuda K, Oh J, Barbosa AC, Yang X. Histone deacetylase 4 controls chondrocyte hypertrophy during skeletogenesis. *Cell*. 2004; 119: 555-566.
47. Cao K, Wei L, Zhang Z, Guo L, Zhang C, et al. Decreased histone deacetylase 4 is associated with human osteoarthritis cartilage degeneration by releasing histone deacetylase 4 inhibition of runt-related transcription factor-2 and increasing osteoarthritis-related genes: a novel mechanism of human osteoarthritis cartilage degeneration. *Arthritis research & therapy*. 2014; 16: 491.
48. Gabay O, Sanchez C. Epigenetics, sirtuins and osteoarthritis. *Joint Bone Spine*. 2012; 79: 570-573.
49. Dvir-Ginzberg M, Gagarina V, Lee EJ, Booth R, Gabay O. Tumor necrosis factor  $\beta$ -mediated cleavage and inactivation of SirT1 in human osteoarthritic chondrocytes. *Arthritis Rheum*. 2011; 63: 2363-2373.

50. Gabay O, Oppenheimer H, Meir H, Zaal K, Sanchez C. Increased apoptotic chondrocytes in articular cartilage from adult heterozygous SirT1 mice. *Ann Rheum Dis.* 2012; 71: 613-616.
51. Matsuzaki T, Matsushita T, Takayama K, Matsumoto T, Nishida K1. Disruption of Sirt1 in chondrocytes causes accelerated progression of osteoarthritis under mechanical stress and during ageing in mice. *Ann Rheum Dis.* 2014; 73: 1397-1404.
52. Iliopoulos D, Malizos KN, Oikonomou P, Tsezou A. Integrative microRNA and proteomic approaches identify novel osteoarthritis genes and their collaborative metabolic and inflammatory networks. *PLoS One.* 2008; 3: e3740.
53. Jones SW, Watkins G, Le Good N, Roberts S, Murphy CL. The identification of differentially expressed microRNA in osteoarthritic tissue that modulate the production of TNF-alpha and MMP13. *Osteoarthritis Cartilage.* 2009; 17: 464-472.
54. Miyaki S, Nakasa T, Otsuki S, Grogan SP, Higashiyama R. MicroRNA-140 is expressed in differentiated human articular chondrocytes and modulates interleukin-1 responses. *Arthritis Rheum.* 2009; 60: 2723-2730.
55. Miyaki S, Sato T, Inoue A, Otsuki S, Ito Y. MicroRNA-140 plays dual roles in both cartilage development and homeostasis. *Genes Dev.* 2010; 24: 1173-1185.
56. Park SJ, Cheon EJ, Kim HA. MicroRNA-558 regulates the expression of cyclooxygenase-2 and IL-1 $\beta$ -induced catabolic effects in human articular chondrocytes. *Osteoarthritis Cartilage.* 2013; 21: 981-989.
57. Li X, Gibson G, Kim JS, Kroin J, Xu S. MicroRNA-146a is linked to pain-related pathophysiology of osteoarthritis. *Gene.* 2011; 480: 34-41.
58. Tardif G, Hum D, Pelletier JP, Duval N, Martel-Pelletier J. Regulation of the IGFBP-5 and MMP-13 genes by the microRNAs miR-140 and miR-27a in human osteoarthritic chondrocytes. *BMC Musculoskelet Disord.* 2009; 10: 148.
59. Liang ZJ, Zhuang H, Wang GX, Li Z, Zhang HT, et al. MiRNA-140 is a negative feedback regulator of MMP-13 in IL-1beta-stimulated human articular chondrocyte C28/I2 cells. *Inflammation research.* 2012; 61: 503-509.
60. Stanczyk J, Ospelt C, Karouzakis E, Filer A, Raza K. Altered expression of microRNA-203 in rheumatoid arthritis synovial fibroblasts and its role in fibroblast activation. *Arthritis Rheum.* 2011; 63: 373-381.
61. Hu F, Zhu W, Wang L. MicroRNA-203 up-regulates nitric oxide expression in temporomandibular joint chondrocytes via targeting TRPV4. *Arch Oral Biol.* 2013; 58: 192-199.