

# Research progress on epigenetic modification of genes in degenerative osteoarthritis

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**Abstract:** Degenerative osteoarthritis is one of the most common joint diseases, and its main pathological manifestations are joint synovitis, subchondral osteosclerosis, progressive damage to cartilage leading to osteophyte formation, and narrowing of joint spaces. A large number of studies have found a certain association between the pathogenesis of different pathological manifestations of OA, but the detailed pathogenesis is still not completely clear. Although the basic cell types of bone and cartilage tissue may all come from aquatic vertebral organisms, it has been found that osteoarthritis is mediated by epigenetic modifications of conserved developmental genes in response to excessive mechanical stress (among other factors), such as epigenetic modifications regulated by microRNAs that maintain the order of morphogenesis and temporal changes in response to stress rather than pathological phenotypes resulting from genetic mutations or new signaling pathways.

**Keywords:** Epigenetics, Genes, MicroRNAs, Osteoarthritis

## 1. Introduction

Mobile-functioning joints first appeared in teleost fish[1], followed by transcription factors and signaling pathways (BMP, Wnt, SOX9, FGF-2, ROS, etc.) involved in basic cell types involved in joint composition. Osteoarthritis is a multifactorial disease. In addition to lifestyle habits, self-constitution, it is also affected by many genes and environmental factors. The pathophysiology of osteoarthritis mainly includes degeneration of articular cartilage, subchondral osteosclerosis and synovitis. Although the pathological changes of chondrocytes are the key to the occurrence of osteoarthritis, the role of epigenetics in the occurrence and development of osteoarthritis has received more and more attention in recent years[2]. Some scholars describe the joint model of vertebrates as a complex system based on osteochondrium units, while others summarize the individual morphogenesis of this unit as the process of controlled differentiation and phenotypic expression of mesenchymal stem cells and their differentiated articular chondrocytes and growth plate cells[3]. Virtually all joint structures can be affected by mechanical stress during development, and their response to the direction and magnitude of stress vectors (gross and microscopic) produces an orderly process of adaptive remodeling to maintain bone and joint function. The adaptation and reconstruction of osteochondrocytes is evidence of their phenotypic diversity, and this process has been shown to be mediated by epigenetic modifications of gene networks rather than novel signaling pathways or genetic mutations[4].

## 2. Degenerative osteoarthritis

Degenerative osteoarthritis is a multifactorial degenerative disease involving single or multiple joints. Risk factors can be divided into two categories, one is the unmodifiable genetic factors associated with the degenerative OA phenotype, and the other is modifiable factors such as obesity, repetitive mechanical stress injury, muscle weakness, changes in bone lines, and nutritional metabolites such as advanced glycation endproducts[5]. Statistics have found that the incidence of OA is now more than twice that of 1950, and it is expected that the incidence of OA may increase by 3 percentage points in the next 10 years[6]. The incidence of OA in all parts increases with age, peaking at about 60 years of age and gradually decreasing with age after being greater than 60 years of age[7]. Among them, the incidence of OA in women, hands and knees, while the incidence of shoulder and cervical osteoarthritis is higher in

men. According to epidemiological surveys, the incidence of knee osteoarthritis in people over 60 years of age is 23%[8]. With the increasing aging of the population, coupled with the synergy of mechanical and biological mechanisms, osteoarthritis of the knee seriously endangers the health of patients. Its clinical characteristics are knee joint swelling and pain, aggravation of symptoms after activity, severe cases of varus deformity and even disability, which seriously affects the healthy life of patients and also increases the social and economic burden. Studies have found that the number of joint replacement surgeries worldwide increases by more than 10% every year, and more than 90% of them are knee replacements performed by OA patients. However, arthroplasty is not only expensive, but also has a variety of intraoperative and postoperative complications. OA is expected to become the fourth leading cause of disability worldwide in the near future. The total cost of care for OA patients is reported to be about \$14,521 per person per year, compared to \$3,629 for non-OA patients, an average of one-quarter of OA patients[9]. Although the pathogenesis of OA has received great attention in the past few decades, and some understanding of the structural changes in joints such as cartilage and synovium in OA has been obtained, the complex pathological mechanisms and physiological changes during the onset and development of OA remain elusive. However, we can still advance the understanding of the pathogenesis of OA through cartilage damage, subchondral bone sclerosis, synovial imbalance, epigenetic modifications, and changes in related signaling pathways.

### 3. Cartilage damage mechanisms

Joint chondrocytes and their extracellular matrix (ECM) are important components of joint cartilage. In the early stage of osteoarthritis, proteoglycans in the cartilage matrix of the joint are reduced, collagen fibers on the cartilage surface are degenerated, and with repeated friction of the joint surface, the articular cartilage is destroyed, resulting in the exposure of subchondral bone and narrowing of the joint space[10]. Studies have shown that excessive loading will cause transmembrane mechanical receptor proteins (integrins) to initiate intracellular signaling, promoting catabolism and inflammatory cytokine production. Among them, a variety of pro-inflammatory genes, mainly mediated by the nuclear factor kappaB (NF- $\kappa$ B), will lead to ECM degradation. The presence of ECM degradation products and accumulated AGEs and other damage-associated molecular pattern DAMPs further involved in the early occurrence of chondrodegeneration and OA through inflammatory cytokines and NF- $\kappa$ B signaling pathways[11]. At the same time, with the increase of intracellular filaments and lysosom-like structures in degenerative chondrocytes, the destruction of cartilage is further aggravated. Although joints can be altered morphologically to redistribute weight-bearing surfaces and re-establish stability, joint surfaces tend to be destroyed faster than repaired. In contrast, moderate intermittent loading is necessary for homeostatic maintenance of normal cartilage[12].

### 4. Subchondral bone sclerosis

Subchondral bone not only maintains the shape of the joint surface, but also maintains the stability of the joint by absorbing 30% of the external force. Therefore, the subchondral bone adapts to changes in stress by remodeling when the joint moves, and when the external load is too large, the adaptability between the cartilage and the subchondral bone is broken, resulting in cartilage degeneration and hardening of the subchondral bone[13]. The subchondral bone, consisting of subchondral plates and trabeculae, represents the osteogenic niche. Among them, the bone plate should have sufficient channels, especially in areas of high stress, so that blood vessels can nourish the articular cartilage. With the degradation of the ECM collagen network, excessive loading upregulates alkaline phosphatase, collagen, growth factors, and inflammatory factors by NF- $\kappa$ B[14]. Thus, the homeostatic equilibrium between resorption and deposition is disrupted, leading to increased bone turnover, subchondral osteosclerosis, increased cartilage calcification, and decreased trabecular-bone diameter[15].

### 5. Synovial imbalance

Synovial membranes and synovial fluid make up the synovial niche. Necrotic and exfoliated cartilage irritate the synovium and joint capsule, leading to synovitis[16]. The synovial membrane regulates the balance of synovial fluid at the molecular level, including high molecular weight molecules (hyaluronic acid) and low molecular weight molecules (growth factors, cytokines, electrolytes, etc.), thereby maintaining the physiological homeostasis of joint cartilage. The imbalance between inflammatory synovial cells and synovial fluid molecules is an important cause of OA. For example, activation of the

Wnt signaling pathway in inflammatory synovial cells promotes the synthesis of matrix metalloproteinases (MMPs), which in turn leads to cartilage destruction[17]. In addition, synovial fibroblasts respond to mechanical stress by increasing the production of AGEs and ROS over time and increasing the level of oxidative stress in chondrocytes[18]. Given that synovial fluid is a source of nutrition for chondrocytes, inflammatory changes in the synovium can induce and exacerbate cartilage degeneration.

## 6. Epigenetic modification of OA

The three main epigenetic modifications of OA (DNA methylation, histone modifications, and noncoding RNAs) play important roles in joint health and disease. Studies have found that DNA methylation can directly or indirectly regulate metalloproteases to affect the occurrence of osteoarthritis, such as MMP-9 and MMP-13 activators single point in osteoarthritis can appear methylation decrease, resulting in osteoarthritis[19]. Similarly, leptin in chondrocytes is regulated by DNA and expression is reduced, thereby preventing the development of osteoarthritis[20]. DNA methylation of the DNMT3b gene can cause inflammation and osteophyte formation by regulating the catabolism and eventual differentiation of chondrocytes.

The histone-modified EZH2 gene can reduce the number of mesenchymal stem cells, thereby accelerating osteoblast differentiation and affecting bone patterns[21]. Histone deacetylases (HDACs) can promote the development of osteoarthritis by inducing the expression of MMPs. At the same time, HDACs can also directly target different special chondrocytes genes, such as HDAC4, which interacts with Runx2 to prevent excessive hypertrophy of immature chondrocytes, thereby promoting the occurrence of osteoarthritis[22].

MicroRNAs are an evolutionarily conserved class of double-stranded RNA molecules, mainly transcribed by RNA polymerase II, and then transcribed into primary microRNAs containing hairpin structures by a 5' methylguanine nucleoside cap and 3' poly(A) tail. Primary microRNAs are recognized and cleaved into precursor microRNAs by the nucleus type II molecule Drosha and its cofactor DGCR8, which are recognized by output protein 5 and transported to the cytoplasm. A small number of microRNAs are spliced and debranched by mirtrons to produce precursor microRNAs. Finally, the nuclease type III molecule Dicer cleaves it in the cytoplasm into double-stranded short RNAs(microRNAs)[23]. A large number of studies have shown that microRNAs, as negative regulators of gene expression, play a crucial role in maintaining cartilage homeostasis at different ages, and can be stably associated with microRNAs-induced silencing complexes, negatively regulating gene expression by binding to specific sequences in target mRNA, thereby inhibiting post-transcriptional products. Therefore, microRNAs have significant construction, enzymatic and regulatory effects on osteochondrocytes[24]. Moreover, a microRNA can bind to multiple mRNAs to regulate the transcriptional translation of multiple proteins, and a protein synthesis gene receives targeted regulation by multiple microRNAs at the same time. Current studies have shown that the expression of approximately 30 microRNAs is strongly associated with OA of varying severity[25].

Taking microRNA-140 as an example, it was found that miR-140 can not only directly target Smad3 of TGF- $\beta$  signaling pathway to promote chondrocytes differentiation, but also promote chondrocytes proliferation by targeting SP1. MicroRNA-140 plays an important role in regulating cartilage homeostasis and inhibiting inflammatory pathways at the same time, so it is considered a cartilage-specific microRNA and is highly conserved [26]. There is abundant evidence that microRNAs also play an important role in regulating chondrage autophagy. For example, microRNA-155 induces autophagy through the mTOR signaling pathway[27], and the inhibition of microRNA-30b can also lead to upregulation of autophagy, preventing apoptosis and cartilage degradation[28]. MicroRNA-27a can promote the proliferation of chondrocytes by targeting the nuclear factor  $\kappa$ B signaling pathway, activating chondrocytes autophagy, and regulating the activity of chondrocytes. Some microRNAs have a negative effect on joint cartilage. For example, microRNA-4262 can inhibit SIRT1 by activating the AKT/mTOR pathway, thereby inhibiting chondrocytes autophagy, which ultimately leads to the occurrence of osteoarthritis. MicroRNA-214 can inhibit osteoblast activity and matrix mineralization by targeting the expression of transcription activator (ATF4), leading to the occurrence of osteoporosis[29].

Cell signaling pathways are complex epigenetic networks composed of chemical messengers, mainly regulated by microRNAs, which have been shown to mediate intracellular and cell-to-cell connections during morphogenesis and remodeling. In the pathophysiology of OA, five signal transduction pathways have been reported: (1) BMPs pathway: this pathway is highly conserved and participates in and regulates bone and cartilage formation. Studies have found that in joint tissues, BMPs can not only promote the synthesis of cartilage ECM, but also accelerate the differentiation of chondrocytes. (2) Wnt pathway: Wnt pathway not only strictly regulates bone formation and regeneration, but also participates in the joint

development process in the early embryonic stage. The expression of Wnt pathway proteins at different stages and locations can lead to significant differences[30]. (3) SOX-9 pathway: expressed by mesenchymal stem cells, SOX-9 maintains the survival of chondrocytes by promoting the synthesis of cartilage-specific matrix proteins during cartilage formation[31]. (4) FGF-2 pathway: Studies have found that it plays an important role in the formation of joint cavities in the early embryonic stage. (5) ROS pathway: free radicals produced by many normal and abnormal cellular processes that are involved in structural modification of microRNAs[32]. At the same time, microRNAs under oxidative stress can lead to misidentification of cell signals and abnormal expression of genes, which in turn leads to defects in protein synthesis and changes in cellular programs[33].

The study found that early intervention in the development of OA has obvious advantages over late drug therapy and joint replacement[34], but the complex pathogenesis of OA and our limited understanding of the specific mechanism make early diagnosis and targeted therapy more difficult. Therefore, it is crucial to understand the role of epigenetic modifications in OA.

## 7. Conclusion

In summary, we believe that degenerative osteoarthritis is a biological response of highly conserved gene-regulated epigenetic modifications represented by microRNA-140 in response to excessive mechanical load. The regulation of the occurrence and development of OA through epigenetic modification is bound to be a future development situation, of course, the relevant conclusions have yet to be further confirmed by the relevant studies on the pathophysiology of OA.

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## References

- [1] Godivier, J., Lawrence, E. A., Wang, M., Hammond, C. L., & Nowlan, N. C. (2022). Growth orientations, rather than heterogeneous growth rates, dominate jaw joint morphogenesis in the larval zebrafish. *Journal of Anatomy*, 241(2), 358–371. <https://doi.org/10.1111/joa.13680>
- [2] Abramoff, B., & Caldera, F. E. (2020). Osteoarthritis: Pathology, Diagnosis, and Treatment Options. *The Medical Clinics of North America*, 104(2), 293–311. <https://doi.org/10.1016/j.mcna.2019.10.007>
- [3] Cheng, H. W., Chik, T. K., Weir, J., & Chan, B. P. (2022). Differentiation of equine mesenchymal stem cells into cells of osteochondral lineage: potential for osteochondral tissue engineering. *Biomedical Materials (Bristol, England)*, 17(6). <https://doi.org/10.1088/1748-605X/ac8c76>
- [4] van Meurs, J. B., Boer, C. G., Lopez-Delgado, L., & Riancho, J. A. (2019). Role of Epigenomics in Bone and Cartilage Disease. *Journal of Bone and Mineral Research: The Official Journal of the American Society for Bone and Mineral Research*, 34(2), 215–230. <https://doi.org/10.1002/jbmr.3662>
- [5] Martel-Pelletier, J., Barr, A. J., Cicuttini, F. M., Conaghan, P. G., Cooper, C., Goldring, M. B., ... Pelletier, J.-P. (2016). Osteoarthritis. *Nature Reviews. Disease Primers*, 2, 16072. <https://doi.org/10.1038/nrdp.2016.72>
- [6] Ij, W., S, W., Dt, F., Rd, J., Kt, W., H, M., ... De, L. (2017). Knee osteoarthritis has doubled in prevalence since the mid-20th century. *Proceedings of the National Academy of Sciences of the United States of America*, 114(35). <https://doi.org/10.1073/pnas.1703856114>
- [7] Dj, H., & S, B.-Z. (2019). Osteoarthritis. *Lancet (London, England)*, 393(10182). [https://doi.org/10.1016/S0140-6736\(19\)30417-9](https://doi.org/10.1016/S0140-6736(19)30417-9)
- [8] G, P., & Mj, T. (2021). Osteoarthritis year in review 2020: epidemiology & therapy. *Osteoarthritis and cartilage*, 29(2). <https://doi.org/10.1016/j.joca.2020.10.007>
- [9] Sx, W., Ax, G., A, B., Jk, M., Wm, R., & D, M. (2017). Healthcare resource utilization and costs by age and joint location among osteoarthritis patients in a privately insured population. *Journal of medical economics*, 20(12). <https://doi.org/10.1080/13696998.2017.1377717>
- [10] Mobasheri, A., & Batt, M. (2016). An update on the pathophysiology of osteoarthritis. *Annals of Physical and Rehabilitation Medicine*, 59(5–6), 333–339. <https://doi.org/10.1016/j.rehab.2016.07.004>
- [11] Millerand, M., Berenbaum, F., & Jacques, C. (2019). Danger signals and inflammaging in osteoarthritis. *Clinical and Experimental Rheumatology*, 37 Suppl 120(5), 48–56.
- [12] McKay, C. D., Cumming, S. P., & Blake, T. (2019). Youth sport: Friend or Foe? *Best Practice & Research. Clinical Rheumatology*, 33(1), 141–157. <https://doi.org/10.1016/j.berh.2019.01.017>
- [13] Hügle, T., & Geurts, J. (2017). What drives osteoarthritis?-synovial versus subchondral bone

- pathology. *Rheumatology (Oxford, England)*, 56(9), 1461–1471. <https://doi.org/10.1093/rheumatology/kew389>
- [14] Segarra-Queralt, M., Piella, G., & Noailly, J. (2023). Network-based modelling of mechano-inflammatory chondrocyte regulation in early osteoarthritis. *Frontiers in Bioengineering and Biotechnology*, 11, 1006066. <https://doi.org/10.3389/fbioe.2023.1006066>
- [15] Aho, O.-M., Finnilä, M., Thevenot, J., Saarakkala, S., & Lehenkari, P. (2017). Subchondral bone histology and grading in osteoarthritis. *PloS One*, 12(3), e0173726. <https://doi.org/10.1371/journal.pone.0173726>
- [16] Mathiessen, A., & Conaghan, P. G. (2017). Synovitis in osteoarthritis: current understanding with therapeutic implications. *Arthritis Research & Therapy*, 19(1), 18. <https://doi.org/10.1186/s13075-017-1229-9>
- [17] B, K., E, V., & Ee, N. (2019). Regulatory Effects and Interactions of the Wnt and OPG-RANKL-RANK Signaling at the Bone-Cartilage Interface in Osteoarthritis. *International journal of molecular sciences*, 20(18). <https://doi.org/10.3390/ijms20184653>
- [18] Aj, K., Ec, F., Om, E., L, L., Lm, J., Pm, R., ... T, M. (2023). Synovial fibroblasts assume distinct functional identities and secrete R-spondin 2 in osteoarthritis. *Annals of the rheumatic diseases*, 82(2). <https://doi.org/10.1136/ard-2022-222773>
- [19] Hi, R., N, Y., Ks, C., S, T., Nm, C., Ro, O., ... F, B. (2005). Association between the abnormal expression of matrix-degrading enzymes by human osteoarthritic chondrocytes and demethylation of specific CpG sites in the promoter regions. *Arthritis and rheumatism*, 52(10). <https://doi.org/10.1002/art.21300>
- [20] Iliopoulos, D., Malizos, K. N., & Tsezou, A. (2007). Epigenetic regulation of leptin affects MMP-13 expression in osteoarthritic chondrocytes: possible molecular target for osteoarthritis therapeutic intervention. *Annals of the Rheumatic Diseases*, 66(12), 1616–1621. <https://doi.org/10.1136/ard.2007.069377>
- [21] Shen, J., Wang, C., Li, D., Xu, T., Myers, J., Ashton, J. M., ... O'Keefe, R. J. (2017). DNA methyltransferase 3b regulates articular cartilage homeostasis by altering metabolism. *JCI insight*, 2(12), e93612, 93612. <https://doi.org/10.1172/jci.insight.93612>
- [22] Gu, X., Li, F., Gao, Y., Che, X., & Li, P. (2022). HDAC4 mutant represses chondrocyte hypertrophy by locating in the nucleus and attenuates disease progression of posttraumatic osteoarthritis. *BMC musculoskeletal disorders*, 23(1), 8. <https://doi.org/10.1186/s12891-021-04947-6>
- [23] Tx, L., & Me, R. (2018). MicroRNA. *The Journal of allergy and clinical immunology*, 141(4). <https://doi.org/10.1016/j.jaci.2017.08.034>
- [24] Swingler, T. E., Niu, L., Smith, P., Paddy, P., Le, L., Barter, M. J., ... Clark, I. M. (2019). The function of microRNAs in cartilage and osteoarthritis. *Clinical and Experimental Rheumatology*, 37 Suppl 120(5), 40–47.
- [25] Zhang, M., Lygrisse, K., & Wang, J. (2017). Role of MicroRNA in Osteoarthritis. *Journal of Arthritis*, 6(2), 239. <https://doi.org/10.4172/2167-7921.1000239>
- [26] R, Z., J, M., & J, Y. (2013). Molecular mechanisms of the cartilage-specific microRNA-140 in osteoarthritis. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]*, 62(10). <https://doi.org/10.1007/s00011-013-0654-8>
- [27] Z, L., Y, H., X, J., L, L., & H, G. (2022). PCB153 suppressed autophagy via PI3K/Akt/mTOR and RICTOR/Akt/mTOR signaling by the upregulation of microRNA-155 in rat primary chondrocytes. *Toxicology and applied pharmacology*, 449. <https://doi.org/10.1016/j.taap.2022.116135>
- [28] Chen, Z., Jin, T., & Lu, Y. (2016). AntimiR-30b Inhibits TNF- $\alpha$  Mediated Apoptosis and Attenuated Cartilage Degradation through Enhancing Autophagy. *Cellular Physiology and Biochemistry: International Journal of Experimental Cellular Physiology, Biochemistry, and Pharmacology*, 40(5), 883–894. <https://doi.org/10.1159/000453147>
- [29] S, Y., W, Z., Q, O., L, L., X, L., & Y, W. (2017). MicroRNA-214 Suppresses Osteogenic Differentiation of Human Periodontal Ligament Stem Cells by Targeting ATF4. *Stem cells international*, 2017. <https://doi.org/10.1155/2017/3028647>
- [30] Sassi, N., Laadhar, L., Allouche, M., Achek, A., Kallel-Sellami, M., Makni, S., & Sellami, S. (2014). WNT signaling and chondrocytes: from cell fate determination to osteoarthritis physiopathology. *Journal of Receptor and Signal Transduction Research*, 34(2), 73–80. <https://doi.org/10.3109/10799893.2013.863919>
- [31] Zhang, M., Theleman, J. L., Lygrisse, K. A., & Wang, J. (2019). Epigenetic Mechanisms Underlying the Aging of Articular Cartilage and Osteoarthritis. *Gerontology*, 65(4), 387–396. <https://doi.org/10.1159/000496688>
- [32] Portal-Núñez, S., Esbrit, P., Alcaraz, M. J., & Largo, R. (2016). Oxidative stress, autophagy, epigenetic changes and regulation by miRNAs as potential therapeutic targets in osteoarthritis. *Biochemical Pharmacology*, 108, 1–10. <https://doi.org/10.1016/j.bcp.2015.12.012>
- [33] Jx, W., J, G., Sl, D., K, W., Jq, J., Y, W., ... Pf, L. (2015). Oxidative Modification of miR-184 Enables It to Target Bcl-xL and Bcl-w. *Molecular cell*, 59(1). <https://doi.org/10.1016/j.molcel.2015.05.003>

[34] de Girolamo, L., Kon, E., Filardo, G., Marmotti, A. G., Soler, F., Peretti, G. M., ... Chubinskaya, S. (2016). Regenerative approaches for the treatment of early OA. *Knee surgery, sports traumatology, arthroscopy: official journal of the ESSKA*, 24(6), 1826–1835. <https://doi.org/10.1007/s00167-016-4125-y>