Am J Cancer Res 2024;14(4):1784-1801
www.ajcr.us /ISSN:2156-6976/ajcr0156234

Review Article

Collagen type X expression and chondrocyte hypertrophic differentiation during OA and OS development

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Received February 29, 2024; Accepted March 15, 2024; Epub April 15, 2024; Published April 30, 2024

Abstract: Chondrocyte hypertrophy and the expression of its specific marker, the collagen type X gene (*COL10A1*), constitute key terminal differentiation stages during endochondral ossification in long bone development. Mutations in the *COL10A1* gene are known to cause schmid type metaphyseal chondrodysplasia (SMCD) and spondyloepiphyseal dyschondrodysplasia (SMD). Moreover, abnormal *COL10A1* expression and aberrant chondrocyte hypertrophy are strongly correlated with skeletal diseases, notably osteoarthritis (OA) and osteosarcoma (OS). Throughout the progression of OA, articular chondrocytes undergo substantial changes in gene expression and phenotype, including a transition to a hypertrophic-like state characterized by the expression of collagen type X, matrix metalloproteinase-13, and alkaline phosphatase. This state is similar to the process of endochondral ossification during cartilage development. OS, the most common pediatric bone cancer, exhibits characteristics of abnormal bone formation alongside the presence of tumor tissue containing cartilaginous components. This observation suggests a potential role for chondrogenesis in the development of OS. A deeper understanding of the shifts in collagen X expression and chondrocyte hypertrophy phenotypes in OA or OS may offer novel insights into their pathogenesis, thereby paving the way for potential therapeutic interventions. This review systematically summarizes the findings from multiple OA models (e.g., transgenic, surgically-induced, mechanically-loaded, and chemically-induced OA models), with a particular focus on their chondrogenic and/or hypertrophic phenotypes and possible signaling pathways. The OS phenotypes and pathogenesis in relation to chondrogenesis, collagen X expression, chondrocyte (hypertrophic) differentiation, and their regulatory mechanisms were also discussed. Together, this review provides novel insights into OA and OS therapeutics, possibly by intervening the process of abnormal endochondral-like pathway with altered collagen type X expression.

Keywords: Osteoarthritis, osteosarcoma, chondrocyte hypertrophy, *COL10A1*

Introduction

Osteoarthritis (OA), the most prevalent degenerative joint disease among the elderly, is responsible for mobility impairment and pain [1]. The incidence of OA keeps increasing year by year simply because of the aging population and the sharply increased number of obese people. OA progresses slowly and is often characterized by degenerative changes in the articular cartilage, including cartilage loss, osteophyte formation, subchondral osteosclerosis, and synovial inflammation [2]. Due to the limited self-healing ability of articular cartilage, untreated articular cartilage defects are further deformed, leading to joint pain and dysfunction, and eventually typical OA [3]. The pathogenesis of OA is complex, involving aging, mechanical, inflammation and metabolic factors, which eventually lead to the destruction and failure of

https://doi.org/10.62347/JWGW7377

the entire joint tissue structure. Its incidence continues to rise annually due to the increasing prevalence of obesity and an aging population. Obesity induces joint overloading, altering joint biomechanics, often leading to pronounced wear and tear, particularly in the medial cartilage [4]. Additionally, postmenopausal women experience a substantial decrease in estrogen levels, disrupting the balance between subchondral bone formation and resorption, thereby increasing susceptibility to joint trauma and elevating the risk of OA [5]. Normally, chondrocytes remain quiescent but actively synthesize an array of extracellular matrix (ECM) proteins, including collagen, hyaluronic acid, glycoproteins, and proteoglycans, to support and cushion stresses generated by movement. Conversely, metabolic abnormalities in chondrocytes and the ECM in articular cartilage injury predominantly manifest increased catabolism and decreased synthesis [6]. The ECM synthesized and secreted by chondrocytes is to support and cushion the stress generated during exercise. Progression of OA involves abnormal secretion of matrix degradation factors by chondrocytes, such as MMP13, MMP1, and ADAMTS5, resulting in ECM degradation and a marked reduction in type II collagen and proteoglycans, ultimately leading to defects in cartilage tissue [7].

In addition to the enhanced production of matrix-degrading enzymes, a prominent feature of OA is the presence of a hypertrophic chondrocyte phenotype. Chondrocyte hypertrophy constitutes a transient physiological process essential for bone formation within cartilage. In the context of OA, chondrocytes react to the accumulation of injurious biochemical and biomechanical stimuli by transitioning into a hypertrophic state [8]. Articular cartilage is a layer of hyaline cartilage, mainly composed of ECM and resting chondrocytes, including collagen (Collagen II, VI, IX), hyaluronic acid and proteoglycan [9]. With the progression of OA, articular chondrocytes are activated, and the expression of articular cartilage related markers such as proteoglycan, type II collagen, are significantly down-regulated [10], while hypertrophic markers, including type X collagen, MMP13, and ADAMTS5, may become highly expressed in chondrocytes undergoing hypertrophic changes [11]. The abnormal hypertrophy of articular chondrocytes results in cartilage matrix degradation, vascular invasion, and osteoid formation, mirroring the terminal differentiation of chondrocytes observed during endochondral bone formation [12]. This process is not limited to human patients, but is also observed in most animal models of OA [13, 14]. Indeed, numerous studies concerning OA have focused on hypertrophic chondrocyte-specific marker genes and signaling factors, such as *Col10a1*, *Mmp13*, and *Runx2* [15, 16]. However, a direct causal relationship between the development of a hypertrophic chondrocyte phenotype and the progression of OA remains to be firmly established. Whether the occurrence of OA is triggered by chondrocytes and the underlying mechanism of abnormal hypertrophic differentiation of articular chondrocytes in the development of OA need further study.

Osteosarcoma (OS) is the most common malignant bone tumor and often affects children and adolescents [17]. The exact pathophysiology of OS is unknown, but the osteoid and immature bone in OS might be produced by malignant mesenchymal cells [18]. It is worth noting that cells of OS are often derived from osteoblasts, including pre-osteoblasts and osteoblasts. Chondrocytes and fibroblasts are also present in the tumor stroma of many osteosarcomas. In addition, in the induced mouse OS model, cartilage matrix was found present in the tumor tissue and ossification occurred through similar process of endochondral ossification [19, 20]. As a transcription factor, RUNX2 is known to play a crucial role in osteoblast differentiation and chondrocyte hypertrophy or maturation. Studies have shown that activation of Wnt/β-catenin in OS upregulates RUNX2 expression, and thus promoting the expression of metastasis-related genes [21]. *p53* gene is involved in the normal development and physiological mechanism of bone, and is considered an important factor in tumorigenesis. Deletion of *p53* and *Rb-1* leads to early OS [22]. Moreover, an interaction relationship between P53/RB-1 and RUNX2 has been proven [23]. We also notice that there are transcription factors, or regulators specifically secreted by chondrocytes are known to play a significant role in OS apoptosis and tumor drug resistance or sensitivity [24]. These findings together with the chondroid tissue found in osteochondrosarcoma may indicate a chondrogenic element during OS development.

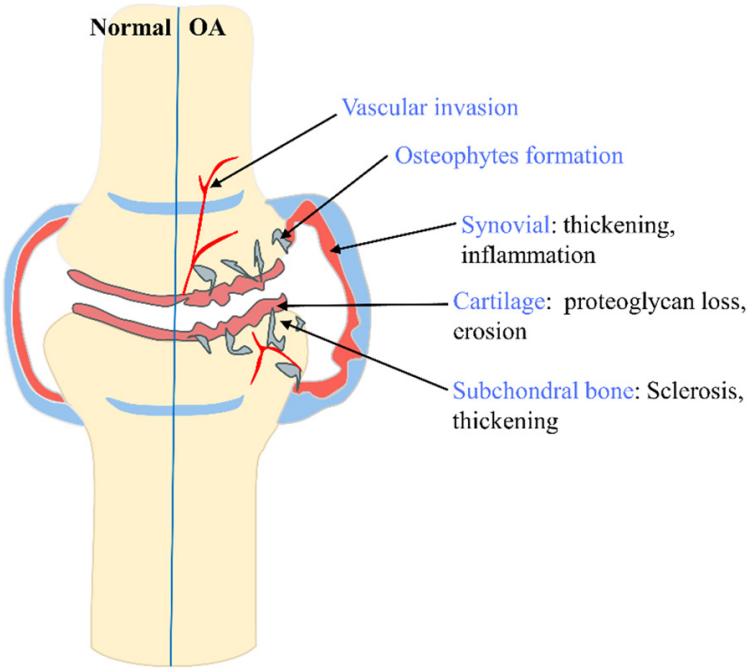

Figure 1. Healthy joint was compared with OA joint. Clinical data show that majority of OA patients have a variety of pathologic phenotypes, including vascular invasion, osteophytes formation, synovial inflammation, articular cartilage erosion, and subchondral bone sclerosis. Left side shows the normal joint structure. Right side shows the possible joint structural symptoms and changes of osteoarthritis.

Here we systematically reviewed the altered collagen X expression and chondrocyte (hypertrophic) differentiation in multiple OA models, as well as the potential chondrogenic element in OS pathogenesis, which may help with future OA and OS therapeutics.

Animal model of osteoarthritis

OA is the most common chronic joint disease and a leading cause of mobility impairment and pain in the elderly [25, 26]. When OA occurs, it always affects the joints under stress, usually the knees, hands, and spine [27]. OA is characterized by joint destruction, cartilage loss, and osteosclerosis and osteophyte formation (**Figure 1**) [28, 29]. In order to further understand the pathogenesis of osteoarthritis as well as newly developed treatment options, multiple OA animal models have been established, as OA is a heterogeneous disease, a single animal model won't reflect all aspects of disease [30]. Studies have also found that the underlying mechanism may be different in animal models of OA induced by different approaches [31]. Here, we reviewed following transgenic, surgical induction, chemically and mechanical load induced OA models, aiming to identify the different etiologies on the occurrence and development of OA.

Transgenic or genetically modified OA models

The application of transgenic mice has greatly improved our understanding of the physiological and molecular mechanism of many diseases [32]. In the past few decades, the number of transgenic or genetically modified OA models has been increasing and has gradually become the best candidate model to explore the molecular mechanism of OA (**Table 1**).

In heterozygous *Del1* mice, harboring a short deletion mutation in type II collagen gene lead to early-onset degenerative changes in the knee joint and progress to end-stage osteoarthritis at 12-15 months [33, 34]. Histological examination of *Del1* heterozygous mice showed earlier and more severe cartilage erosion. Subchondral osteosclerosis and osteophyte formation occurred in *Del1* heterozygous mice with age [34]. At the same time, studies have shown that lifelong running significantly increases the incidence and severity of OA in mice with *Col2a1* mutations [35]. Early-onset OA and up-regulation of related markers (*Ddr2, Mmp13, HtrA1*) in the temporomandibular joint were found in mice with *Col2a1* mutations [36, 37]. A recent study also showed that markers of chondrocyte hypertrophy were significantly upregulated in mice with *Col2a1* mutations, such as *Col10a1*, *Runx2*, and accelerated the progression of OA [8]. Mice that knocked out *Runx1* with *Col2-Cre* showed impaired cartilage formation and reduced bone density, and overexpression of *Runx1* prevented cartilage destruction in OA [38]. *Runx2* and *Col10a1* are molecular markers of chondrocyte hypertrophy [39, 40]. In view of the important role of abnormal hypertrophy of chondrocytes in OA, trans-

Table 1. List of transgenic or genetically modified mouse models

Gene	Mice model	Changes of mice bone	Molecular mechanisms
Type II collagen	*Del1*(+/-) [34]	Cartilage erosion Osteosclerosis Meniscus degeneration Various joint structures are mineralized	↓Col II
	Disproportionate micromelia (Dmm/+) [36, 37]	Abnormal condylay cartilage in TMJs Proteoglycan deficiency TMJs tremor at 6 months Cartilage erosion	↑HTRA1, MMP13, DDR2 in tempormandibular joint (TMJs) ↓Col II
Runx1	*Runx1*$^{F/F}$*Col2a1-CreER*^{T} [38]	Accelerated cartilage destruction Joint deformity Proteoglycan deficiency	↑MMP13 ↓COL2A1, SOX9, COL10A1
Runx2	*Col10a1-Runx2* [41]	Chondrocyte maturation is delayed Shortened femur length Cortical bone thinning Reduced joint destruction in induced OA	↑SOX9, COL10A1, RUNX2
Sox9	*Col10a1-Sox9* [43]	Chondrocyte columns disorder Reduced hypertrophic cartilage mineralization Hypertrophic chondrocytes increased adipogenesis Subchondral osteosclerosis Osteophyte formation	↑SOX9, MMP13, ADAMTS4/5, ↓COL10A1

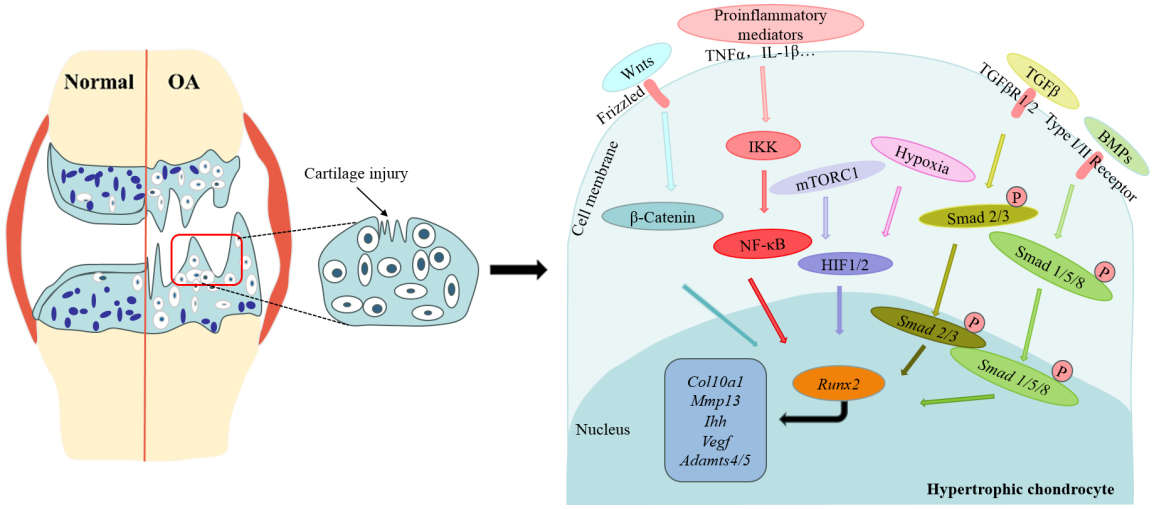

Figure 2. Regulatory mechanisms in hypertrophic chondrocytes of OA. Activation of Wnt/β-Catenin signaling pathway in OA induces *Runx2* gene expression [144, 145]. Activation of the inflammatory NF-κB pathway, upregulation of mTORC1 and hypoxia induced expression of HIFs protein in articular cartilage chondrocytes, and subsequently HIF-2α promoted cartilage destruction [146-148]. TGFβ/BMP regulates the function of *Runx2* through Smad pathway, affecting chondrocyte differentiation and possible development of OA [149].

genic animal models of hypertrophic chondrocyte related genes have also been extensively studied (**Figure 2**). Chondrocyte maturation was delayed in *Col10a1-Runx2* transgenic mice overexpressing *Runx2* and was able to protect the mouse joints against OA [41]. SOX9 is an essential regulator of cartilage development [42]. In a new *Col10a1-Sox9* transgenic mouse, OA was shown at six months of age. Histological findings showed that growth plate structure of the mouse was abnormal and the mineral content was significantly reduced [43]. A recent study induced OA lesions by using *Aggrecan-CreERT2* transgenic mice to delete the *Fermt2* gene encoding the key focal adhesion protein Kindlin-2 in chondrocytes of articular cartilage, highly mimicking human OA pathological changes [44]. In that study, the articular chondrocytes exhibited hypertrophic differentiation, with significant increases in the protein expression of the hypertrophy markers COL10A1, MMP13 and RUNX2. The *Col11a1*+/- mice

Table 2. Surgical induction models of OA

Surgical induction models	Authors	Changes of the model	Molecular mechanisms
Destabilization of the medial meniscus (DMM)	Li et al. [47]	Medial meniscus displacement Osteophyte formation and synovial hyperplasia occurred at week 6	↑COL10A1, MMP13, ADAMTS 5, AMPKα1
	Fang et al. [150]	Gait change Proteoglycan loss, chondrocyte aggregation in medial articular ventricle and osteophytes formation at week 2 Mild to moderate cartilage damage at week 5 Intrachondral ossification in osteophytes at week 10	
Anterior cruciate ligament transection (ACLT)	Barbosa et al. [56]	Paw print area reduced Synovial inflammation Joint swelling	↑IL-1β, IL-17, TNF-α
	Aizah et al. [57]	Proteoglycan loss Subchondral trabecular bone thickness decreased	
	Go et al. [58]	Femoral cartilage surface erosion Osteophyte formation	↓Col II
	Jia et al. [59]	Cartilage lesions and loss Chondrocyte distribution irregular	↑SDF-1, MMP-13 ↓Col II, ACAN
Ovariectomized (OVX)	Andersen et al. [63]	Gain weight Cartilage erosion Cartilage turnover increased	↑CTX-I, CTX-II ↓Col II

developed several features of the typical OA knee at 15 months, including loss of articular cartilage and meniscal malformation, and a significant increase in MMP13 was also observed [45]. Although the transgenic mouse model cannot simulate the corresponding function of human joints, it is the main choice to study the molecular mechanism of OA. The genes that are increased or decreased in the transgenic model can be considered as potential targets for the treatment of OA and may provide an important pathway for the development of new therapeutic options.

Surgical induction OA models

Surgical induction model is the most common OA model in experimental animals (**Table 2**). The surgical instability of medial meniscus (DMM) is the most widely used OA model. After DMM, the medial meniscus was displaced, but the cruciate ligament remained intact, there was no ectopic chondrogenesis, and OA progression gradually progressed from mild to moderate [46]. Significant synovial hyperplasia and osteophyte formation were both observed at 6 and 12 weeks after DMM surgery [47]. At the same time, the results of immunohistochemical staining showed that the protein expression of COL10A1 and MMP13 were significantly up-regulated in the mice undergoing DMM surgery [47]. The results of a recent study also showed significantly increased COL10A1 expression in articular cartilage and subchondral bone in DMM-induced animal models [48]. Platelet-rich plasma (PRP) protects chondrocytes against destabilization of the DMM-induced OA and suppresses the dysregulation of chondrocyte matrix-related factors (*Sox9*, *Col10a1*, *Col2a1*) [49]. In one study, the protective effect of the EHFR pathway on OA was investigated by performing DMM surgery in *Col2-Cre HBEGF* overexpressed mice [50]. The DMM model has been used in many studies of OA disease progression and treatment options [51-53]. Anterior cruciate ligament rupture can lead to biomechanical changes and joint instability, eventually leading to OA [54, 55]. Anterior cruciate ligament transection (ACLT) is a surgical method alike to DMM. Severe OA symptoms were observed within a short time in a mouse model after ACLT surgery, with destruction of subchondral bone and growth plate, and ectopic bone formation at 8 weeks [46]. 30 days after ACLT was performed in rat and was also enough to induce the typical symptoms of knee OA, such as pain, dysfunction, synovial inflammation [56]. The model could also be used in follow-up studies within a short time after induction. Interestingly, one study used an ACLT rat model to investigate the correlation between subchondral bone and early cartilage changes

Table 3. Mechanical load models of OA

Mechanical load model	Authors	Load application frequency	Changes of the model
Stimulated jump	Radin et al. [79]	Be subjected to 1/2 the animal's body weight 40 times a minute for 20-40 minutes per day	Subchondral osteosclerosis Cartilage injury
Applied knee load	Poulet et al. [80]	Three times a week for two weeks or five weeks	Osteophyte formation Meniscus ossification Synovial hyperplasia and fibrosis
Right knee weight	Poulet et al. [81]	Once a week; Three times a week for two weeks; Three times a week for five weeks	Subchondral bone thickness of the femur and tibia increased Increased trabecula of epiphysis
	Heegde et al. [84]	9N/11N load, three times a week for two weeks	Mechanical sensitivity of paws decreased Articular cartilage injury Load a week synovial lining thickens
Repeated joint bucking load	King et al. [83]	Repeat bending of the front paw toe at 1 Hz for 2 hours per day for 60 hours	Thickness of calcified cartilage reduced Production of osteopontin increased
Lifelong moderate running training	Lapveteläinen et al. [89]	Speed 13.3 meters/min, distance 1000 meters/day	The incidence of medial and lateral tibial condyle OA increased

in OA, and the early progression of OA could be clearly appreciated [57]. And histological evaluation of the ACLT model found that the surgery group showed more hypertrophic differentiation of chondrocytes [57]. In addition to mice and rats, rabbits and pigs are also widely used in vivo preclinical studies [58, 59]. ACLT surgery in rabbit is more suitable for macroscopic evaluation because of its large joint compared to mice [58]. Histopathological analysis of rabbit ACLT at 12 weeks postoperatively revealed decreased staining of Alican blue and type II collagen on the surface of femur and tibia cartilage, suggesting loss of cartilage matrix [58]. In addition, meniscus injury after ACLT surgical induction will further accelerate the development of OA [60]. In a study of Micro-RNA, the effect of miR-26b-5p on chondrocyte hypertrophy was validated using an ACLT-induced animal model. The results show that miR-26b-5p can protect the articular cartilage of OA mice and reduce its synovial inflammation by targeting *Col10a1* [61]. The loss of estrogen will reduce the bone mass of subchondral bone and promote the progression of OA [62]. Estrogen physiologically decreased in postmenopausal women, and ovariectomy (OVX) rats have been shown to be useful as an in vivo study model for OA in postmenopausal women [63]. Researchers have induced experimental OA in rats by performing a meniscectomy (MSX). The role of chondromodulin-1 (ChM-1) in chondrocyte maturation and OA progression through the HIF-2α pathway was explored using this procedure-induced OA model [64]. It further showed that chondrocytes are abnormally hypertrophic and highly expressed COL10A1, MMP13 and VEGFA in surgically induced OA, while this study showed that ChM-1 can inhibit hypertrophic differentiation of chondrocytes and slow OA progression [64]. Surgical induction of OA can be used to study the molecular mechanism of OA and provide a new therapeutic mechanism for OA [65-68]. The surgically induced OA model is also preferred in many preclinical studies of new treatment option [51, 69, 70].

Mechanical load models

Appropriate stimulation may cause responsive stress of the body, leading to chondrocyte proliferation and synthesis of extracellular matrix. OA can develop when the intensity of stimuli increases beyond the body's ability to self-regulate and damage occurs [71, 72]. Therefore, such animal models with mechanic force change play an important role in studying the interaction between mechanical load and the occurrence and progression of OA (**Table 3**). In humans, overweight may promote the progression of OA people with OA are at increased risk of incident disability or disability progression [73, 74]. Many studies have shown that proper exercise is beneficial for cartilage development and improves joint symptoms, but excessive stress can destroy cartilage [74, 75]. At the same time, studies have observed that changes in the medial load position of the knee joint in early OA patients promote the development of OA [76]. Mechanical load is one of the fac-

tors in the occurrence of OA, so various animal load models are essential to study the association of machinery with various joint health and diseases [77, 78]. The first load test was carried out in rabbits. The effect of the load test on the knee joint of rabbits was investigated by simulating the jumping force and repeating the impulse load [79]. Blandine Poulet et al. characterized a new noninvasive mouse joint load model, showing that increased load can lead to osteophyte formation, meniscus ossification, and cruciate ligament injury, the severity of which depends on the load regimen [80]. When an appropriate degree of load was applied, subchondral bone thickened, epiphyseal trabecular thickness and mass increased, and gait changed [81], cells in the meniscus show loss of fibrocyte-like shape, like hypertrophic chondrocyte changes. In the study of temporomandibular cartilage in response to mechanical loading, the results found that increased loading caused increased mineralized cartilage areas and increased expression of COL10A1 [82]. Cartilage thinning and osteopontin production in a periodic joint load rabbit model [83]. The model can be used to study the effect of cyclic load on joint. In the study of OA growth plate dynamics, increased expression of COL10A1 and MMP13, markers of chondrocyte hypertrophy, were observed in articular cartilage and tibial growth plate in a mouse load model [11]. One study has shown that mechanical joint loading can be used as a suitable model to study mechanical OA pain, effectively avoiding the risk of postoperative pain and infection that may result from surgical intervention [84]. Interestingly, obesity can increase mechanical load-induced cartilage damage, and animal load models are critical to study the association between obesity and metabolic syndrome with OA [85, 86]. The combination of diet and exercise will significantly reduce the load on the hips and knees, and improve the clinical and biomechanical outcomes of obese OA patients [87, 88]. The mice received running training from 2 to 18 months of age, and the results showed that moderate and sustained running training accelerated the development of OA in the mice [89]. In previous studies, repeated intra-articular injection of TGFβ-1 and treadmill overrunning into *Col10a1-Runx2* transgenic mice to induce knee OA caused joint damage [41]. Mechanical loading models are also widely used in the study of mechanical-related molecular mechanisms. One study suggests that interleukin-6 (IL-6), a mechanosensitive cytokine, may play a key role in the biomechanical regulation of OA bone remodeling in mechanical load models [90]. Mechanosensitive PIEZO1 channels can drive mechanical damage in chondrocytes and may provide pathways for the prevention and treatment of OA [91]. In short, mechanical loading models are crucial for biomechanical study of OA, the correlation study of obesity and metabolism, and the study of molecular mechanisms.

Chemical induction models

Monosodium iodoacetate (MIA) is a metabolic inhibitor that disrupt glycolysis by inhibiting the activity of glycolysis-3-phosphate dehydrogenase in chondrocytes and ultimately leads to chondrocyte death [92, 93]. Intra articular injection of MIA is often used to induce OA models. A recent study showed that MIA injection in the joint of rats caused immediate inflammation, resulting in histological destruction of functional knee units and ultimately OA pain [94]. The MIA-induced OA model has also been applied to the treatment of OA [95, 96]. Curcumin nanoparticles can improve the progression of MIA-induced OA in rats, and the content of MMP13 in articular cartilage is significantly increased in OA models [97]. *p53* is a key factor in OA pain. Sirtuin 1 (*SIRT1*) mediates MIA-induces OA pain by upregulating *p53* expression, which makes targeting SIRT1/P53 pathway a possible new therapeutic option [98]. In a study of early biomarkers of OA induced OA in donkeys using MIA, significant up-regulation of miR-146b, miR-27b and *Col10a1* expression in serum and synovial fluid was observed in early OA in this animal model [99]. It can be seen from the above studies that the MIA-induced OA model is often used in the pain research of OA, and it can be selected in the pain treatment. Papain is a proteolytic enzyme that can degrade proteoglycans and cause cartilage damage [100]. Different concentrations of papain induced knee OA in rabbits shown different severity of symptoms [101]. To elucidate the effect of many developed tissue engineering methods and novel biomaterials on osteochondral defects in OA in an inflammatory environment, papain was used to construct a model of persistent cartilage defects in rabbits with OA [102]. In this model, compared with non-OA

Table 4. Chemical induction models of OA

Drugs	Authors	Changes of the model	Molecular mechanisms
Monosodium iodoacetate (MIA)	Kwon et al. [94]	Knee swelling Contralateral hind leg load increased Articular cartilage and subchondral bone disorganized Osteophyte formation Cartilage erosion	
	Hamdalla et al. [97]	Knee joint space stenosis Osteophyte formation Cartilage erosion Chondrocytes reduced	↑TNF-α, IL-6, IL-1β, TGF-β, NF-κB, iNOS, Caspase-3, MMP13 ↓IL-10, Col II
Papain	Meng et al. [102]	Cartilage injury Knee swelling Subchondral cyst Subchondral sclerosis Synovial inflammation	↑MMP13 ↓COL I, COL II
	Jia et al. [59]	Cartilage injury Synovial inflammation Fracture of cartilage	↑SDF-1 in synovium, MMP-3 ↓Col II, ACAN
Collagenase	van der Kraan et al. [109]	Cartilage erosion Subchondral osteosclerosis Osteophyte formation Knee deformity	

group, COL I and COL II in the regenerated cartilage of OA group were significantly reduced, while MMP13 protein expression was increased [102]. At the same time, some studies compared the pig OA model induced by surgery and papain, and found that joint injection of papain can produce more and faster cartilage erosion, synovial inflammation, similarly to human OA [59, 103]. However, both OA models showed cartilage lesions and irregular distribution of chondrocytes at 8 weeks of induction [59]. Papain induced animal models have also been used in the study of aerobic exercise combined with glucosamine in the treatment of OA [104]. After the intervention of aerobic exercise in the OA model, the cartilage lesions were improved, the chondrocyte arrangement was more regulate, and the aggregation was significantly reduced [104]. Cyclooxygenase-2 (*Cox2*) inhibitors are used to treat pain associated with OA [105]. To investigate the mechanism of action of COX2 on chondrocytes, papain induced OA model was used. The results showed that COX2 prevented the terminal differentiation of articular chondrocytes by up-regulating the expression of PTHRP in the early stage of OA and promoted the mRNA expression of *Col10a1* and *Mmp13* [106]. Collagenase destroys the joint structure containing type I collagen, leading to cartilage degradation and eventually OA [107, 108]. After a single injection of collagenase in the knee joint, the knee joint of mice developed degeneration, subchondral osteosclerosis, osteophyte formation, and then knee deformity [109]. The collagenase-induced OA mouse model can also be selected in the study of OA pain treatment [110]. The animal model of chemically induced OA is less traumatic, simple to operate, and more economical, and can also applied to the validation of some treatment schemes of OA and the mechanism research (**Table 4**).

There is no single optimal experimental animal model for OA research. The selection of animal models should be considered from multiple perspectives, such as research objectives, economic budget, modeling feasibility and so on. The molecular mechanism of OA disease is more suitable to use the transgenic model, the pathological mechanism is more suitable to choose the surgical model, and the MIA induction model is preferred to study the pain mechanism and analgesic treatment. In the summary of the various models mentioned above, most of the OA models showed abnormal chondrocyte hypertrophy and changes in the expression of hypertrophy markers (*Col10a1*, *Mmp13*, *Runx2*) (**Figure 3**). Thus, chondrocyte hypertrophy may be a necessary process for the development of OA. Therefore, an understanding of each animal model is essential.

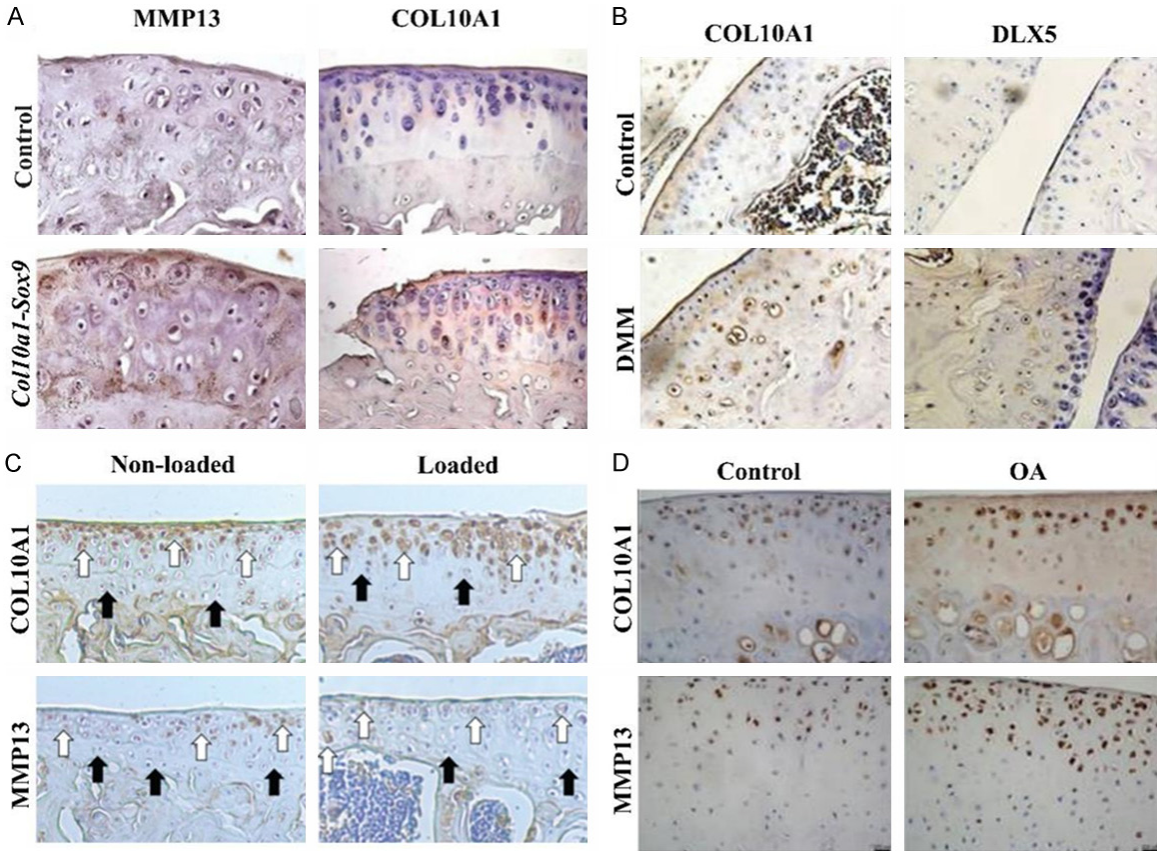

Figure 3. Expression of COL10A1 in various OA-induced models. A. Immunohistochemical results of COL10A1and MMP13 in articular cartilage of transgenic OA model and control group [43]. B. Expression of COL10A1 and DLX5 in cartilage of DMM induced mouse OA model and control group [48]. C. Immunohistochemical results of COL10A1 and MMP13 in tibial articular cartilage of knee joint in mechanical load model and control group [11]. D. Immunohistochemical results of COL10A1 and MMP13 in articular cartilage in papain induced OA model and control group [106].

Osteosarcoma and its chondrogenic elements

OS is a rare primary bone malignancy, and most OS occurs in children and adolescents [111]. To date, the exact causes of OS are unknown. OS is largely considered a malignant tumor that originates in connective tissue, and mainly with tumor cells that produce bone or osteoid [112]. Although osteoblasts are the main cells of OS, there is no evidence that osteoblasts can be restored to primitive cells or malignant cells after differentiation from bone progenitor cells [113]. In fact, in most OS, there are also chondrocytes and fibroblasts that present in the matrix. It has been reported that there is cartilage matrix in the tumor tissues of mice transplanted with OS cell lines, and it has ossification similar to the process of endochondral ossification, suggesting that chondrogenesis or endochondral ossification plays a role in the development of OS [19, 20]. RUNX2 is a transcription factor belonging to the Runt domain gene family, which is to play a crucial role in osteoblast differentiation and chondrocyte maturation [39, 114, 115]. Chondrocyte maturation of *Runx2*$^{-/-}$ mice was seriously disturbed [116]. RUNX2 is also the main transcription factor that regulates the expression of *Col10a1* gene, a specific molecular marker of hypertrophic chondrocytes [117]. RUNX2 is highly expressed in developing human bone and in various tumors, including OS [118, 119]. RUNX2 plays a role in the evolution of the OS in several ways (**Figure 4**). Previous studies have demonstrated that interfering with *Runx2* expression in OS cells can further inhibit the invasion of OS cells by inhibiting VEGF, MMP-2, MMP-9 expression [120]. Within chondrocytes, SOX9 is involved in cell cycle and differentiation [121]. In OS, *Sox9* interacts with *Runx2* and is part of

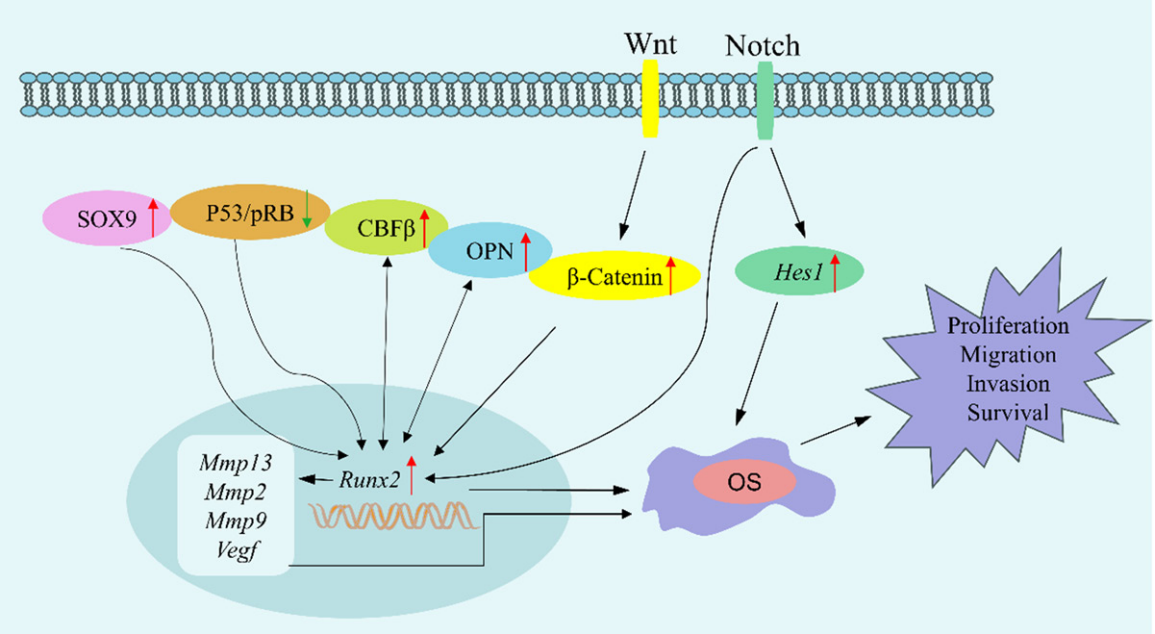

Figure 4. Regulatory mechanisms of *Runx2* in OS. The interaction between SOX9 and RUNX2 promotes the cell survival of OS [122]. The deletion of P53 and pRB increases the expression of *Runx2* and promotes the proliferation of OS cells [124, 126]. The interaction of CBFβ and OPN with RUNX2 promotes cell proliferation, metastasis, and survival of OS [133, 138]. Abnormal activation of Notch and Wnt/β-Catenin signaling pathways can play a role in cell proliferation and invasion of OS by acting to *Runx2* [129, 135, 136]. *Runx2* can promote the expression of *Mmp13*, *Mmp2*, *Mmp9* and *Vegf* to further promote OS invasion [120, 135].

the *Runx2*-regulated transcriptional circuit that promotes OS cells survival [122]. *p53*-deficient mice can lead to the development of OS and are enhanced by loss of pRB, suggesting an important role for P53 and pRB in the development of OS [123]. However, high expression of RUNX2 was shown in mouse osteosarcoma models lacking P53 and pRB [124]. Interestingly, RUNX2 has been shown to interact directly with P53 and pRB [23, 125]. In normal osteoblast differentiation, RUNX2 interacts specifically with pRB during cell cycle exit [125]. In OS, pRB is absent, and RUNX2-pRB mediated cell cycle cannot withdraw and infinite proliferation occurs [126]. At the same time, the proliferation of bone progenitor cells and the expression of RUNX2 were increased in *p53* deficient mice, thus promoting the maturation of osteoblasts and the occurrence of OS [23]. The specific interaction between RUNX2, P53 and pRB not only maintains the normal cycle process of osteoblasts, but also dysregulates it in OS [127]. Wnt/β-Catenin signaling is crucial in determining whether mesenchymal progenitors differentiate into osteoblasts or chondrocytes [128]. Studies have shown that abnormal activation of Wnt/β-Catenin pathway involved in autocrine Wnt signaling drives the proliferation of OS cells [129], so targeting Wnt/β-Catenin signal transduction may provide a new therapeutic approach. The Wnt/β-Catenin pathway has been shown to promote OS metastasis by activating RUNX2 expression in some OS [21]. The specific fusion protein EWS-FLI expressed in Ewing's sarcoma has been shown to bind the FLI sequence to *Runx2*, thereby blocking osteoblast differentiation. Disrupting the interaction between RUNX2 and EWS-FLI can promote the differentiation of tumor cells [130]. The interaction between CBFβ and RUNX2 plays a key role in bone development. Mice without CBFβ showed severely delayed bone formation, chondrocyte maturation, and no endochondral ossification [131, 132]. In OS, the expression of both CBFβ and RUNX2 is increased, resulting in impaired cell cycle. This control by heterodimer CBFβ-RUNX2 may contribute to the occurrence and development of OS [133]. Notch signal transduction is an old-fashioned cell interaction mechanism and an important regulator of the proliferation and differentiation of mouse chondrocytes [134, 135]. Normally, Notch sig-

naling pathway can regulate the expression of MMP13 through RUNX2 and promote hypertrophic differentiation of chondrocytes [135]. At the same time, high level expression of the Notch signal transduction target gene *Hes1* has been found in metastatic OS cell lines, and studies have shown that *Hes1* is required for the aggressiveness of OS [136]. Osteopontin (OPN) plays an important role in the process of bone formation and absorption, and is an crucial component of bone matrix [137]. Relevant studies have found that the combined expression of RUNX2/OPN promotes OS metastasis and may be a valuable independent predictor of OS metastasis and survival [138]. High mobility group box 1 protein (HMGB1) is a chromatin protein specifically secreted by differentiated chondrocytes to regulate endochondral ossification [139]. The expression of HMGB1 in OS cells can inhibit apoptosis and increase drug resistance [24]. A recent study showed that the chondrocyte derived exosome miR-195 can inhibit tumor cell proliferation and increase drug sensitivity of OS cells [140]. Therefore, chondrocytes play an important role in occurrence, development and treatment of OS, further investigation is needed.

Conclusion

OA is a multifactorial disease that not only causes degeneration of articular cartilage but also affects the subchondral bone, synovium, and surrounding muscles and ligaments. Although the exact pathogenic mechanism of OA is unknown, it is hypothesized that abnormal hypertrophy of articular chondrocytes in OA leads to matrix degradation and production of pro-inflammatory factors, ultimately resulting in inflammation in different animal models of OA. Notably, the suppression of inflammatory markers has been demonstrated by chondrocyte hypertrophy inhibitors [141]. At the same time, abnormal activation of chondrocyte hypertrophy and aging can accelerate the progression of OA, and regulating hypertrophic or senescent chondrocytes may be a potential therapeutic target to alleviate the progression of OA [142]. However, the exact link between cartilage degradation, inflammation, and hypertrophy of chondrocytes remains unclear [143]. Investigating the effects of chondrocyte hypertrophy on OA would thus provide a promising area of research for the development of potential therapeutic targets for OA.

OS is characterized by abnormal bone formation, but chondroid components were also found in OS tumor tissues [19]. Besides RUNX2, an essential transcription factor for collagen X expression and chondrocyte hypertrophy, which is highly expressed in OS and can cause infinite cell cycle proliferation of OS [126], another protein HMGB1, which is specifically secreted by differentiated chondrocytes in OS, can also inhibit apoptosis and promote cell proliferation in OS [24]. However, how chondrogenesis, hypertrophic differentiation or related signaling pathways may play a role in OS, and hold therapeutic potential for OS, remain to be explored.

Acknowledgements

This study was supported by the grant from the Jiangsu provincial key research and development program (#BE2020679 to Q.Z.) and the Science and Research Innovation Program for Graduate Students in Jiangsu Province (KYCX23_3761 to T.H.).

Disclosure of conflict of interest

None.

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