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Di Chen

*Chinese Academy of Sciences*

Dongyeon J. Kim

*Washington University School of Medicine in St. Louis*

Jie Shen

*Washington University School of Medicine in St. Louis*

Zhen Zou

*Washington University School of Medicine in St. Louis*

Regis J. O'Keefe

*Washington University School of Medicine in St. Louis*

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## Review Article

## Runx2 plays a central role in Osteoarthritis development

Di Chen<sup>a,\*</sup>, Dongyeon J. Kim<sup>b</sup>, Jie Shen<sup>b</sup>, Zhen Zou<sup>b</sup>, Regis J. O'Keefe<sup>b</sup><sup>a</sup> Research Center for Human Tissues and Organs Degeneration, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen 518055, China<sup>b</sup> Department of Orthopedic Surgery, Washington University at St. Louis, MO, USA

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## ABSTRACT

Osteoarthritis (OA) is the most common form of arthritis, is the leading cause of impaired mobility in the elderly, and accounts for more than a third of chronic moderate to severe pain. As a degenerative joint disorder, OA affects the whole joint and results in synovial hyperplasia, degradation of articular cartilage, subchondral sclerosis, osteophyte formation, and chronic pain. Currently, there is no effective drug to decelerate OA progression and molecular targets for drug development have been insufficiently investigated. Anti-OA drug development can benefit from more and precise knowledge of molecular targets for drug development. Runt-related transcription factor 2 (Runx2) is a key transcription factor controlling osteoblast and chondrocyte differentiation and is among the most promising potential therapeutic targets. Notably, Runx2 expression is upregulated in several murine OA models, suggesting a role in disease pathogenesis. In this review article, we summarized recent findings on Runx2 related to OA development and evaluated its potential as a therapeutic target.

*The translational potential of this article:* A better understanding of the role of Runx2 in osteoarthritis pathogenesis will contribute to the development of novel intervention of osteoarthritis disease.

Osteoarthritis (OA) is the most widespread joint disease worldwide, and its prevalence has reached epidemic proportions [1]. By the year 2040, OA prevalence is projected to increase 49% to affect 78.4 million US adults [2,3] and will be the dominant cause of physical disability and morbidity among adults over 40 years of age [4]. OA is considered to be a whole joint disease that involves cellular and molecular abnormalities, such as articular cartilage degradation, hyperplasia of synovium, and osteophyte formation [5].

Although there are no current disease-modifying therapies or interventions to slow disease progression, significant progress has been made on OA research in recent years. Studies using murine models of OA indicate several growth factors, including transforming growth factor- $\beta$  (TGF- $\beta$ ), Wnt3a, and Indian hedgehog (*Ihh*), are involved in OA development [6–12]. Other signaling factors, such as Smad3,  $\beta$ -catenin, and hypoxia-inducible factors -2 $\alpha$  (HIF-2 $\alpha$ ), may also have a key role in OA disease progression [6–11]. Interestingly, one common hallmark of OA animal models and human OA cartilage samples is the upregulation of runt-related transcription factor 2 (Runx2). Therefore, it has been hypothesized that Runx2 is a major transcriptional mediator that directly regulates the expression of matrix degradation enzymes in articular

chondrocytes [12]. Since OA results from homeostatic failure of extracellular matrix degradation, Runx2 may serve as a key marker and regulator of OA development and progression.

Here, we have aimed to summarize the role of Runx2 in normal and diseased joint tissue, its regulation, and its regulatory targets. These findings point to a molecular pathway linking Runx2 expression to OA pathogenesis, which may be targeted for therapeutic intervention. The aim of this review article is to (1) provide a comprehensive overview of recent findings of Runx2 as a novel target for OA treatment, (2) describe Runx2 as a central mediator of OA development in joint tissue, and (3) understand OA molecular pathways to accelerate the development of novel therapeutic strategies.

## Runx2 upregulation in murine models of OA

Recent studies utilizing different murine OA models have illuminated the critical role of Runx2 in the development of OA. The chondroprotective effects of *Runx2* deletion from these studies, summarized below, suggest that Runx2 may be an attractive molecular target for future OA treatment research.

\* Corresponding author.

E-mail address: [di.chen@sait.ac.cn](mailto:di.chen@sait.ac.cn) (D. Chen).<https://doi.org/10.1016/j.jot.2019.11.008>

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### Destabilization of the medial meniscus (DMM) murine model

Destabilization of the medial meniscus (DMM) surgery induces changes in joint stability, similar to those seen in sport injury-induced and aging-related OA [14]. Therefore, the DMM mouse model is widely used to investigate OA mechanisms and treatment [14–17]. Using this model, Liao et al. deleted *Runx2* in chondrocytes in adult mice at 8 weeks of age and assessed the effect on OA progression [17]. Histological analysis on *Runx2* conditional knockout (cKO) mice following DMM surgery showed a significant decrease in the OA-like phenotype [17], suggesting that *Runx2* contributes to OA pathology. Consistent with this model, cartilage fissures, fibrillation, and degradation at 12 weeks postsurgery were reduced compared to the Cre-negative control [17]. Expression of matrix metalloproteinase-13 (MMP13), a potent enzyme that targets the cartilage for degradation and is upregulated in late-stage OA, was reduced at 8 to 12 weeks after DMM surgery in *Runx2* cKO mice [17]. Chondrocyte marker genes, such as *Mmp9*, *13* and a disintegrin and metalloproteinase with thrombospondin motifs (*Adams*) 4,5,7,12, were also markedly decreased [17]. Together, these findings indicate that the deletion of *Runx2* has substantial protective effects in murine chondrocytes following DMM surgery through the inhibition of multiple matrix degradation enzymes.

### TGF- $\beta$ receptor II (*Tgfr2*) conditional KO mice

The TGF- $\beta$ /Smad signaling pathway contributes to OA development and progression by mediating articular chondrocyte hypertrophy [7, 18–20]. TGF- $\beta$  binds to TGF- $\beta$  receptor II (*Tgfr2*), leading to phosphorylation of heteromeric Smad2,3,4 complexes. The Smad complex then translocates into the nucleus to interact with other DNA binding proteins to regulate TGF- $\beta$ /Smad signaling and induces OA pathology in chondrocytes, as loss of TGF- $\beta$ 2 or TGF- $\beta$ 2 isoform leads to bone defects in mice [21]. Overexpression of the dominant-negative *Tgfr2* (*dnTgfr2*) in transgenic mice causes extensive joint alterations that are similar to human OA, including skeletal degeneration, proteoglycan reduction, and progressive cartilage tissue degradation [18,22]. *Smad3* KO mice and *Tgfr2* cKO mice display the hallmarks of severe OA: progressive degradation of articular cartilage and osteophyte formation [6,7]. In chondrocytes of *Tgfr2* cKO mice, *Runx2* mRNA and *Runx2* protein levels were found to be increased approximately 3- and 8-fold, respectively [13,26], indicating that the pathology observed in *Tgfr2* cKO mice may be mediated through *Runx2*.

Additional studies have also illustrated a relationship between TGF- $\beta$  and *Runx2* expression. Using *Tgfr2* cKO mice [7,23], Shen et al. demonstrated that (1) *Tgfr2* inhibition in articular cartilage tissue upregulated the principal regulators of the matrix components *Mmp13* and *Adams5*, (2) Deletion of *Mmp13* and *Adams5* ameliorated the OA disease progression prompted by the reduction of TGF- $\beta$ /Smad signaling in *Tgfr2* cKO mice [7,23], and (3) *Runx2* binding site mutations largely prevented the inhibitory effect of TGF- $\beta$ . *Runx2* also influenced the expression of *Mmp13* and *Adams5* *in vitro* in cell culture studies [7,23]. In addition, TGF- $\beta$  treatment increased the expression of cell cycle proteins while reducing *Runx2* protein levels [24,25]. Among the cell cycle proteins, cyclin D1/cyclin dependent kinase 4 (CDK4) was specifically found to mediate the phosphorylation of *Runx2*, which contributes to its eventual degradation by the proteasome [25,26]. Together, these findings suggest that impaired TGF- $\beta$  signaling increases *Mmp13* expression through a pathway that involves *Runx2*. Since *Runx2* induces hypertrophic chondrocyte marker genes and *Mmp13* is the downstream target of *Runx2*, these findings suggest that *Runx2* plays a critical role in mediating *Mmp13* and *Adams5* expression during OA development.

In addition to the targeting of articular cartilage, TGF- $\beta$  also acts on subchondral bone tissue and affects subchondral bone remodeling leading to alterations in OA progression [27].

### $\beta$ -catenin activation mice

Human genetic association studies have correlated abnormal  $\beta$ -catenin signaling with OA development [28]. However,  $\beta$ -catenin deletion or activation causes embryonic or immediate postnatal lethality [29]; thus, our understanding of abnormal regulation of  $\beta$ -catenin remains incomplete in animal models. Conditional gene activation of  $\beta$ -catenin in cartilage has offered some insight into the role of this protein in early cartilage development. In  $\beta$ -catenin activation mice, Zhu et al. observed markedly diminished cartilage formation, severe cartilage damage, and accelerated articular chondrocyte maturation [8]. Consistent with the OA development,  $\beta$ -catenin expression was elevated in knee joint samples from OA patients [8]. Altogether, the data are consistent with a key role for  $\beta$ -catenin in chondrocyte differentiation and OA development.

*Runx2* appears to have an important role downstream of  $\beta$ -catenin in chondrocytes and endochondral bone development. Studies have established that  $\beta$ -catenin binds to the *Runx2* promoter and activates *Runx2* expression [30–33]. Analysis of gene expression and morphological changes *in vivo* further demonstrated that *Runx2* is upregulated in the bone collar, perichondrium, and primary spongiosa when  $\beta$ -catenin signaling is active [35–37]. Alterations in  $\beta$ -catenin mediated *Runx2* activation also led to dysregulation of osteoblast and chondrocyte function, degenerative joints, and bone mass alterations [32]. Furthermore, *Runx2* upregulation through  $\beta$ -catenin is essential for the full differentiation of osteoblasts and for repression of the chondrogenic potential of osteochondral progenitors [31,32]. These studies support the conclusion that  $\beta$ -catenin mediated *Runx2* action is essential in multiple steps of chondrocyte differentiation and endochondral bone development.

### *Ihh* transgenic mice

Hedgehog (Hh) signaling is a major modulator of skeletal development and chondrocyte differentiation during embryonic development and infancy [30,33–38]. The major Hh ligand in chondrocytes is the Indian hedgehog (*Ihh*), a protein that is produced and secreted by pre-hypertrophic chondrocytes. Mainly produced in cartilage, *Ihh* mediates chondrocyte hypertrophy and endochondral bone formation by regulating the conserved targets [39]. Of particular interest to the *Ihh* pathway is the activation of glioma-associated oncogene homolog (Gli). Gli is a major activator of *Runx2* expression that promotes osteoblast formation, and leads to chondrocyte hypertrophy [40].

In healthy adult cartilage, *Ihh* expression normally decreases with age to undetectable levels [41]. However, *Ihh* also mediates chondrocyte differentiation and hypertrophy in early OA and is associated with cartilage degeneration. Analysis of human OA cartilage and synovial fluid samples revealed that *Ihh* production was increased 2.6-fold in OA cartilage and by 37% in OA synovial fluid [42]. The *dnTgfr2* transgenic mice exhibited OA-like symptoms and increased catabolic marker gene expression, including elevated *Ihh* expression [18]. *Ihh* was also increased in response to early cartilage damage [41] and was associated with increased *Runx2*, *Mmp13*, and collagen type II alpha1 chain (*Col2a1*) expression. However, the complex network of *Ihh* has been difficult to study in murine models, as conventional *Ihh* knockout mice are embryonic lethal [9,39,40].

To explore the specific role of *Ihh* in OA, Zhou et al. utilized cartilage-specific, inducible loss-of-function (LOF) *Ihh* transgenic mice. The mice underwent DMM surgery at 3 months of age to induce posttraumatic OA. Histological analysis of mice after DMM surgery demonstrated that *Ihh* inactivation alleviates OA cartilage damage [43]. There was also a significant decrease in downstream targets of *Ihh*, including genes *Runx2*, *Gli1*, *Gli2*, *Col10a1*, and *Mmp13* in cartilage tissues of *Ihh* transgenic mice [43]. These findings demonstrate that the deletion of *Ihh* downregulates *Runx2* expression and suggests chondroprotection in patients in the early stage of OA [43].

Lin et al. confirmed these findings and established the role of *Ihh* in the regulation of Runx2 expression. In this study, articular chondrocytes were first transfected with *Runx2* small interfering RNA (siRNA) and were then treated with Hh-ligand or Hh-blocking agent. Hh positively regulated *Adamts5* in the control cells but did not regulate *Adamts5* in chondrocytes with *Runx2* deletion [9]. Interestingly, this relationship between Hh and Runx2 seems to be specific to OA [9]; Runx2 and Hh had similar functions yet discrete roles during normal chondrocyte growth and development [44,45]. Therefore, Hh was concluded to indirectly regulate *Adamts5* in OA through Hh-mediated expression of *Runx2* [9].

#### Nuclear factor- $\kappa$ B (NF- $\kappa$ B) KO mice

Nuclear factor- $\kappa$ B (NF- $\kappa$ B) orchestrates a wide range of stress-related inflammatory responses and controls the growth, survival, and development of many cell types [46]. The pathology of OA chondrocytes in aging and inflammatory models is exacerbated by prolonged NF- $\kappa$ B activation [47,48]. Once activated, NF- $\kappa$ B homodimers translocate to the nucleus and regulate genes involved in extracellular matrix remodeling and chondrocyte terminal differentiation [49].

Many *in vivo* and *in vitro* studies demonstrate a role for NF- $\kappa$ B signaling in promoting the production of procatabolic mediators, increased production of proinflammatory cytokines, and the modification of inflammatory transcription factors [10,11,47,50–52]. Two pivotal kinases, I- $\kappa$ B kinase (IKK)- $\alpha$  and - $\beta$ , activate NF- $\kappa$ B homodimers, but studies have shown that these two kinases have differential effects on chondrocyte differentiation [53,54]. Stable knockdown (KD) of *IKK- $\alpha$*  or *IKK- $\beta$*  compromised extracellular matrix (ECM) remodeling by different pathways and to different degrees [53,54]. Of note, *IKK- $\alpha$*  KD chondrocytes resulted in pronounced hypertrophic differentiation of articular chondrocytes [55]. Interestingly, the effects of IKK-mediated NF- $\kappa$ B signaling were intrinsic to OA chondrocytes since only ECM remodeling was affected, and OA-like differentiation is conserved only to chondrocytes [55].

Using *IKK- $\beta$*  cKO mice as an OA model, Chang et al. also investigated the role of IKK-NF- $\kappa$ B activation and its relation to Runx2 in OA development: specifically, they studied the role of NF- $\kappa$ B in inflammation-mediated inhibition of tissue regeneration [56]. Deletion of *IKK- $\beta$*  repressed expression of a well-established target of NF- $\kappa$ B, *IL-6*, in cells treated with proinflammatory cytokines, TNF, and *IL-17* [56]. This suggests that IKK-NF- $\kappa$ B may modulate hypertrophic-like conversion via the control of Runx2, and thus, may be a pathway of interest to regulate chondrocyte homeostasis in OA.

#### Hif-2 $\alpha$ KO mice

One of the downstream effectors of NF- $\kappa$ B signaling is HIF-2 $\alpha$  [47]. Studies have shown that HIF-2 $\alpha$  regulates genes involved in endochondral ossification by connecting inflammation-related chondrocyte hypertrophy and ECM degradation [10]. In *HIF-2 $\alpha$*  heterozygous KO mice, surgically induced OA development and progression was markedly suppressed [57], and *HIF-2 $\alpha$*  haploinsufficiency decreased catabolic factors, such as *Mmp13*, *Mmp9* and *Vegfa* (vascular endothelial growth factor A) [10,57]. HIF-2 $\alpha$  also upregulated the production of the proinflammatory cytokines *IL-1 $\beta$*  and *TNF- $\alpha$* , which have been reported to be reduced during the treatment with an IKK inhibitor [36,58]. These data indicate that the NF- $\kappa$ B-targeted transcription factor HIF-2 $\alpha$  increases the production of catabolic factors and proinflammatory cytokines.

Further studies suggest that the combined action of C/EBP- $\beta$  (CCAAT-enhancer-binding protein beta) and Runx2 is essential to HIF-2 $\alpha$  to trigger *Mmp13* expression [59]. Site-directed mutagenesis within the *Mmp13* promoter significantly represses *HIF-2 $\alpha$*  promoter activity induced by C/EBP- $\beta$  and Runx2 [59]. Similarly, retroviral overexpression of *HIF-2 $\alpha$*  enhanced *Cebpb* expression in primary chondrocytes, while dominant-negative *HIF-2 $\alpha$*  suppressed *Cebpb* expression in chondrocytes [59].

In addition, bioinformatics predictions identified that Runx2 and C/EBP- $\beta$  are potent transcriptional partners in chondrocytes. Mice with dual KO of *Cebpb* and *Runx2* were resistant to OA, exhibiting decreased cartilage degradation and decreased expression of *Mmp13* [59]. *Cebpb* and *Runx2* were also coexpressed and colocalized in highly differentiated chondrocytes during OA development in humans and in mice [59]. Hence, HIF-2 $\alpha$  is a functional inducer, and *Mmp13* is a target of Runx2 and C/EBP- $\beta$ . Recent studies also demonstrated that excessive mechanical loading promotes OA development through activation of the gremlin-1-NF- $\kappa$ B pathway [60].

In summary, (1) overexpression of *Runx2* in mice increased the number of cartilage proteases expressed in chondrocytes [12,17], (2) *Runx2* expression level was elevated in human cartilage obtained from OA patients [12,17], (3) Upregulation of *Runx2* activated MMP13 and *Adamts5*, which are matrix degradation enzymes, (4) Runx2 regulates *Mmp13* gene by directly mediating *Mmp13* gene transcription [61] or through mitogen-activated protein kinase (MAPK) pathways [12,17], and (5) After induction of knee joint instability, heterozygous global *Runx2* KO mice or chondrocyte-specific *Runx2* deletion had decreased cartilage destruction and osteophyte formation [11–13].

#### Runx2 functions in different joint tissues

Cartilage degradation is the hallmark of OA progression and indicates the irreversibility of the disease. However, OA affects the whole joint, and pathological changes seen in OA patients include thickening of subchondral bone, osteophyte formation, synovial inflammation [63,64], degeneration of ligaments, and hypertrophy of the joint capsule [65,66]. Changes in periarticular muscles, nerves, fat pads, and bursa can also contribute to OA development [65]. All these pathological changes could impact the joint and OA as a whole joint failure [65].

#### Articular chondrocytes

Articular chondrocytes are suspected to be major players in the initiation and progression of OA. Chondrocytes are mainly affected by inflammatory cytokines and cartilage-degrading enzymes, which work together to create the characteristic phagocyte infiltration in joint tissue associated with inflammation and OA [48].

Previous studies have shown that *Runx2* was significantly upregulated during hypertrophic differentiation and was associated with catabolic phenotypes observed in OA. Runx2 expression was tightly correlated with increased expression of hypertrophic indicators, such as *Col10a1*, *Ihh*, *Mmp13*, and *Alp* [67,68]. In articular chondrocytes, upregulation of Runx2 is induced by a variety of factors, including (1) the  $\beta$ -catenin/lymphoid enhancer-binding factor (LEF)/T-cell factor (TCF) complex through Wnt signal pathway [69], (2) canonical Wnt signaling, which mediates the switch from Sox9 to Runx2 pathway [29,70], (3) initiation of rapidly accelerated fibrosarcoma (Raf)-mitogen-activated protein (MEK1)/2-extracellular receptor kinase (ERK1)/2 cascade through fibroblast growth factors (FGF2) [71,72], and (4) HIF-2 $\alpha$  communication with both the  $\beta$ -catenin and NF- $\kappa$ B pathways [30].

In addition, it has been reported that during the progression of OA, type X collagen, alkaline phosphatase, Runx2, and MMP13 are expressed in articular chondrocytes with decreased proteoglycans and expanded calcified cartilage zones in articular cartilage [73,74].

In contrast, it has also been shown that the knocking down of Runx2 could not inhibit *Col10a1* and *Adamts5* expression and increased *Mmp13* expression in human OA mesenchymal stem cells (MSCs). These findings are opposite to the effects of Runx2 in chondrocytes [75].

In addition, DNA methylation studies by Bui et al. showed significant differential expression of *Runx2* and changes in methylation patterns in primary articular cartilages derived from patients with OA compared to patients with no history of OA [76]. Consistent with this data, genetic analysis by Roach et al. and Fernández-Tajes et al. showed changes in *Runx2* methylation in human OA articular chondrocytes [77]. These



studies indicate a role for Runx2 as a master transcription factor in articular chondrocyte differentiation in OA.

### Synovial cells

Studies by Scanzello et al. have characterized synovial pathology in the development or progression of OA, especially in the context of inflammation and pain [63]. Synovia with increased inflammation were associated with unique chemokines and cytokines that may represent a signature for OA development [75,78]. Specifically, cytokines known to have catabolic effects on chondrocytes, including IL-8, CCL-5, IL-1, IL-6, and TNF- $\alpha$ , were increased in the synovia [75,79–81]. Even in the absence of phagocyte infiltration and inflammation of joint tissue inflammation, OA synovial fluid had elevated levels of inflammatory cytokines [75]. Synovial inflammation, whether dependent or independent of joint inflammation, is a critical factor in OA pathogenesis and warrants further investigation.

Interestingly, fibroblast growth factor 2 (FGF-2) was found to be present in synovial fluid and highly correlated with the severity of cartilage degeneration in OA [72]. FGF2 has been implicated in cartilage homeostasis by controlling chondrocyte differentiation [82,83]. In human OA cartilage, FGF-2 activated Runx2 through MEK/ERK signaling and upregulated the expression of *Mmp13*. Correspondingly, inhibition of the MEK/ERK pathway impeded Runx2 activation by FGF-2, and treatment with FGF-2 increased Runx2 phosphorylation [84,85]. Together, FGF-2, which accrues in synovial fluids of OA joints [72], is suggested to contribute to Runx2 activation leading to *Mmp13* upregulation.

### Subchondral bone cells

It has been well established that Runx2 is critical for skeletal, cartilage, and condylar development [86–88]. Shibata et al. have demonstrated that the absence of condylar cartilage development in *Runx2*<sup>−/−</sup> mice and *Runx2* global KO mice display stunted growth and die shortly after birth due to an absolute absence of bone tissue [86–88]. This suggests that Runx2 is essential for condylar cartilage formation [87].

Since Runx2 plays a central role in chondrocyte hypertrophy, the characteristic of OA pathogenesis, Liao et al. studied its specific role in subchondral bone cells using *Runx2* cKO mice. Interestingly, *Runx2* deletion blocks chondrocyte translocation into the subchondral bone region and inhibits chondrocyte transdifferentiation into progenitor cells [89]. Comparatively, histological analysis demonstrated extensive chondrocyte growth in condylar cartilage and subchondral bone in control mice, indicating a key role for Runx2 in subchondral bone remodeling [89]. Runx2 was also highly expressed in proliferative and hypertrophic chondrocytes, suggesting Runx2 regulates subchondral bone remodeling. Combined with studies demonstrating that *Runx2* deletion causes loss of hypertrophic chondrocytes [17], the findings by Shibata et al. indicate that Runx2 is essential in orchestrating the proliferation and hypertrophic progression at the postnatal stage.

### Meniscus

Changes in the meniscus are well documented in OA, and knee joint degeneration often starts with meniscal lesions [67,90–92]. In fact, this high interdependency of OA and the meniscus is why meniscectomy is now obsolete, as the procedure inevitably leads to the development of OA [90–92]. While the outer, vascularized meniscus has been shown to return to normal function after surgical repair [93,94], it is traditionally thought that degeneration of the inner meniscus is difficult to address because it is avascular, and thus, unable to self-regenerate to full functionality [95–97]. However, recent studies have revealed that the inner meniscus responds to growth factors and fibrin clots [92,94,98,99], and thus, it has been proposed that the inner meniscus harbors cells capable of regeneration [91]. Of note, Muhammad et al. described the regenerative potential of avascular meniscal tissue obtained from late-stage OA

patients prior to knee replacement. Surprisingly, an explant culture of the avascular part of the inner meniscus obtained from an OA patient led to the discovery of a group of migratory, multilineage, and multipotent cells [92]. These new cells, which have been termed as human meniscus progenitor cells (MPCs), were found only in the diseased tissue but not in healthy controls. These MPCs seem to be modulated by Runx2, consistent with previous studies. There was a greater level of Runx2 and a simultaneous reduction of TGF- $\beta$  in diseased meniscal specimens. There were almost undetectable levels of Runx2 and a reduction in *Runx2* mRNA in healthier specimens. When 3D explant cultures of MPCs from human inner meniscal tissue were differentiated into cells of chondrogenic lineage, cells from damaged meniscus showed no Runx2 levels and increased Sox9 levels. Knocking down of *Runx2* via siRNA in MPCs demonstrated the upregulation of Sox9 and *Smad2*. The data correlate with previous findings that the regulation of Runx2 is the key to OA development. These findings also suggest that Runx2 is a major regulatory factor of OA progression in the meniscus and is a potential drug target for regeneration in the diseased meniscus.

These data were also recapitulated in the aforementioned studies using the DMM model in *Runx2* KO mice [17,100]. Surgical destabilization of the medial meniscus is a technique widely used as an OA model, as DMM surgery leads to degeneration of articular cartilage and shows OA-like pathology in the knee joint [17]. With the inhibition of Runx2 in the DMM-induced OA mouse model, Liao et al. have demonstrated that Runx2 is a significant contributor to OA disease in the meniscus and can serve as a target for potential therapeutics.

### Up-regulation of Runx2 in joint tissue

Since OA is a whole joint disease, its initiation and progression remain poorly understood, especially at the molecular level. Recent evidence suggests epigenetic and microRNA (miRNA) alterations may play a role in OA disease pathology. Several miRNAs and DNA methylation patterns have been reported to regulate *Runx2* expression in OA cartilage. These findings suggest that epigenetic modifications or miRNA regulation may serve as important mediators of OA.

### MicroRNA regulation of Runx2

MicroRNAs are short non-coding RNA molecules that regulate gene expression, especially in the context of embryonic and hematopoietic stem cells [101]. Although our understanding of miRNAs and their role in mesenchymal stem cells (MSCs) is scant, several transcription factors are known to modulate MSC differentiation into chondrocytes and osteoblasts. Of the transcriptional factors identified, Runx2 plays a unique multifunctional role in chondrogenesis and osteogenesis [102–104]. The deletion of the miRNA processing enzyme Dicer significantly reduced the expression of Runx2-related miRNAs and interrupted bone formation. Specifically, miR-105 binds to Runx2 in chondrocytes [105], and the downregulation of miR-105 in OA cartilage was associated with decreased Runx2, Adamts7, and Adamts12 expression [105]. Additionally, miRNA-145 indirectly regulates Runx2 by relieving Sox9-mediated repression of *Runx2*, and thus, the overexpression of miR-145-induced mRNA levels of Runx2 [106]. Similarly, miR-140 targeted and promoted chondrocyte hypertrophy and was specifically expressed in cartilage tissue [107]. Its chondroprotective effect against OA progression has been linked to the downregulation of several proteins involved in cartilage destruction, such as *Mmp13* and *Admats5* [108,109]. Lastly, miR-204 decreased chondrocyte proliferation and ameliorated the OA-like phenotype in rodent models in a Runx2-dependent mechanism [102,104]. Since Runx2 protein expression was significantly increased by miR-204 Antagomir, it suggests that miR-204 is an endogenous attenuator of Runx2 in MSCs [102,104,110]. Consistent with the *in vitro* findings, miR-204/miR-211 double KO mice developed severe, time-dependent OA development and progression, indicating the key role of regulation of Runx2 in OA development [111]. Together, it can be

concluded that Runx2-miRNA interaction is an area of interest to OA development and progression.

### Epigenetic regulation of Runx2

Epigenetics alter the gene expression without changes in the DNA sequence and may significantly contribute to gene regulation and protein expression. Although the genetic code is identical for every cell in the body, epigenetic regulation and changes are often location- and cell-type-specific [112]. Therefore, disruption of an established epigenetic network can cause several major pathologies, including OA [114,115].

Several genome-wide profile studies revealed that the DNA methylation-dependent alteration might contribute to biological processes in human OA articular chondrocytes [113–115]. Fernández-Tajes et al. identified 91 differentially methylated probes that were tightly associated with a specific cluster of OA patients. Among these genes, Runx2 methylation was markedly decreased [113]. Similarly, studies by Bui et al. examined the effect of DNA methyltransferase inhibitor Aza on the expression of genes associated with cartilage, epigenetics, and cell senescence. Gene expression analysis revealed that *Runx2* was differently expressed between control and OA chondrocytes treated with Aza [76].

Most significantly, genome-wide methylation analysis using cartilage DNA revealed seven CpG sites located 82 kb upstream of *Runx2* and *Supt3h*, a chromatin remodeling protein [115]. Additionally, the UK GWAS arcOGEN study [116] identified a common single nucleotide polymorphism (SNP) associated with OA susceptibility, and Rice et al. showed that this SNP was located within and flanking differentially methylated regions. OA disease progression was strongly affected by genetic and epigenetic activity within this region. It was concluded that *Runx2* was strongly associated with OA susceptibility and was a principal target in modulating the methylation patterns of OA disease [117]. Because *Runx2* expression is modulated by DNA methylation and is associated with OA genetic risk, *Runx2* epigenetic regulation is a realm for further investigation and potential intervention.

### Runx2 downstream target genes

OA disease progression is highly complex and involves numerous interrelated events that eventually lead to decreased chondrocyte protein synthesis and catabolic protease activation. Specifically, matrix metalloproteinases (MMPs) and aggrecanases (Adamts) contribute widely to aggrecan loss. A subfamily of collagenases, MMP1, 8, and 13, are involved in the cartilage destruction. MMP1 is expressed in fibroblast and macrophages [118], MMP8 cleaves aggrecan at specific sites [119], and MMP13 interacts primarily with type II collagen [119]. Adamts4 (aggrecanase 1) and Adamts5 (aggrecanase 2) have been associated with fundamental structural degradation underlying human OA. Although there is no universal agreement of which primary aggrecanase is responsible for aggrecan degradation in human OA [120–122], Adamts have significant potential for novel OA drug design.

### MMPs

In recent studies, MMP13 has been shown as a downstream target of Runx2. In *Runx2* KO mice, OA pathology and progression were ameliorated due to a mechanism that involved a significant decrease in *Mmp13* expression [123]. Runx2 directly regulates *Mmp13* promoter activity and MMP13 expression *in vivo*. Real-time RT-PCR analysis demonstrated upregulated Runx2 mRNA expression was correlated with decreased *Mmp13* expression *in vivo* [124]. Hypertrophic chondrocyte markers, such as *Mmp13* and *Col10a1*, were upregulated *in vitro* under cyclic tensile strain (CTS) [125]. This complex mechanoresponsive mechanism was found to be under the control of the Runx2/Cbfa1 pathway that leads to the regulation of *Mmp13* expression in primary chondrocytes [13,84,124–126]. Tetsunaga et al. also demonstrated the upregulation of *Mmp13* with overexpression of Runx2 and the downregulation of *Mmp13* with

Runx2 siRNA [127]. Chromatin immunoprecipitation (ChIP) assay demonstrated that Runx2 directly binds to the *Mmp13* promoter in rat chondrosarcoma cells [7], which was confirmed by clustered regularly interspaced short palindromic repeats (CRISPR) gene deletion studies. It has been determined that *Mmp13* expression was mediated by Runx2 via a complex arrangement of enhancers [124,128,129]. Combined with the observation that *Mmp13* is a marker of chondrocyte hypertrophy [38], Runx2 is a key mediator of stress-induced *Mmp13* expression [125,127] and play a key role in OA pathogenesis [62,84,126,130].

### Adamts

Over the past decade, many *ex vivo* and *in vivo* studies have demonstrated that significant upregulation of Runx2 may lead to catabolic responses in chondrocytes [19,20,131–134]. The observed changes in TGF- $\beta$ ,  $\beta$ -catenin, *Ihh*, and FGF pathways have shown to converge on Runx2 regulation, primarily throughout the expression of MMPs and Adamts activity [59,120,134]. Thirunavukkarasu et al. demonstrated that *Runx2* overexpression could increase *Adamts5* expression by 5- and 7-fold over the control in human chondrosarcoma cells and in primary bovine chondrocytes, respectively [135]. In another study, cotransfection with *Runx2* siRNA together with *Tgfr2* siRNA repressed stimulation of *Adamts5* and *Mmp13* expression [7]. CTS studies also confirmed that *Adamts5* expression is controlled by Runx2, as CTS induction of *Runx2* expression was also associated with increased *Adamts5* expression [127]. Similarly, the transfection of Runx2 siRNA resulted in significant downregulation of *Adamts5* [127]. In addition, *Adamts5* KO mice noticeably reduced the severity of cartilage destruction with surgically induced joint instability. This study demonstrated that *Adamts5* single gene deletion abrogated OA-like cartilage destruction and concluded that Adamts5 is a primary factor responsible for OA cartilage degradation [13]. With noteworthy data showcasing Runx2 as a key transcription factor regulating these genes, targeting Runx2 may be a key therapeutic strategy in OA cartilage to ameliorate disease onset and progression.

### Conclusion

In summary, Runx2 was identified as a major marker of OA disease that is highly expressed in OA murine models and human patients. Runx2 was upregulated in multiple OA mouse models, including DMM, *Tgfr2* conditional KO,  $\beta$ -catenin activation, *Ihh* transgenic, and NF- $\kappa$ B KO mice models. In most studies, multiple matrix degradation enzymes, *Mmp13* and *Adamts5*, and their corresponding regulatory genes were highly involved, providing compelling evidence for Runx2 as a transcriptional factor that controls the expression of these genes. Together, these studies suggest that expression of Runx2 and development of OA is through a fine balance of multiple factors, especially through the activation of  $\beta$ -catenin, Wnt, *Ihh*, IKK- $\alpha$ , TGF- $\beta$ , and Hif-2 $\alpha$  signaling pathways. Many studies also implicated the role of Runx2 in different joint tissues, such as the meniscus, synovial cells, and subchondral bone cells. Therefore, Runx2 could serve as a novel molecular target for not only OA progression but also a target to reduce pain and inflammation associated with OA disease. In addition to Runx2, Runx1 has also been shown to be involved in OA development which may serve as a drug target for OA treatment [136].

### Conflict of Interest

The authors have no conflicts of interest to disclose in relation to this article.

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