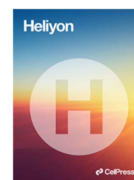
Heliyon 8 (2023) e12656

Contents lists available at ScienceDirect

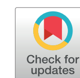
Heliyon

journal homepage: www.cell.com/heliyon



Review article

The roles of Runx1 in skeletal development and osteoarthritis: A concise review



Yi Liu [a], Chengdong Huang [b], Mingru Bai [a], Caixia Pi [a], Demao Zhang [c], Jing Xie [a,*]

[a] State Key Laboratory of Oral Diseases, West China Hospital of Stomatology, Sichuan University, Chengdu, China
[b] School of Nursing, Chengdu University of Traditional Chinese Medicine, China
[c] Institute of Biomedical Engineering, School of Preclinical and Forensic Medicine, Sichuan University, Chengdu, China

ARTICLE INFO

Keywords:
Runx1
Skeletal development
Osteogenesis
Chondrogenesis
Osteoarthritis
Bone
Gene therapy

ABSTRACT

Runt-related transcription factor-1 (Runx1) is well known for its functions in hematopoiesis and leukemia but recent research has focused on its role in skeletal development and osteoarthritis (OA). Deficiency of the Runx1 gene is fatal in early embryonic development, and specific knockout of Runx1 in cell lineages of cartilage and bone leads to delayed cartilage formation and impaired bone calcification. Runx1 can regulate genes including collagen type II (Col2a1) and X (Col10a1), SRY-box transcription factor 9 (Sox9), aggrecan (Acan) and matrix metalloproteinase 13 (MMP-13), and the up-regulation of Runx1 improves the homeostasis of the whole joint, even in the pathological state. Moreover, Runx1 is activated as a response to mechanical compression, but impaired in the joint with the pathological progress associated with osteoarthritis. Therefore, interpretation about the role of Runx1 could enlarge our understanding of key marker genes in the skeletal development and an increased understanding of Runx1 could be helpful to identify treatments for osteoarthritis. This review provides the most up-to-date advances in the roles and bio-mechanisms of Runx1 in healthy joints and osteoarthritis from all currently published articles and gives novel insights in therapeutic approaches to OA based on Runx1.

1. Introduction

The Runx1 protein, one of the members of the Runx family, is a transcription factor that can directly bind to DNA. The function of Runx1 is best understood in hematopoiesis [1], but its effects on regulating skeletal development were found recently, although the mechanisms have not yet been fully understood. Runx1 can be expressed in the early stage of cartilage formation [2, 3], and is essential for the condensation and differentiation of bone marrow mesenchymal stem cells (BMSCs), which are pluripotent stem cells that produce osteoblasts, chondrocytes and adipocytes [4]. Moreover, Runx1 can upregulate chondrogenic genes and suppress the expression of hypertrophic genes, indicating that Runx1 can be a promising treatment for osteoarthritis (OA), because patients with OA often have cartilage and bone damage and abnormal skeletal development [5]. Therefore, Runx1 is vital for skeletal development, involving chondrogenesis and osteogenesis, and is a promising molecule to help treat OA, and the mechanisms of Runx1 in OA can be better understood if skeletal development is further investigated.

Osteoarthritis (OA) is one of the most common joint diseases around the world [6, 7] and can cause synovitis, lesions of the meniscus, degeneration of the anterior cruciate ligament, bone turnover, such as thickened subchondral plates, osteophyte formation and bone marrow lesions [8–10]. Besides, research demonstrates that adverse factors including bone marrow lesions, meniscal abnormalities and cartilage trauma presented in the modern environment would further induce the occurrence and development of OA [11]. OA is caused by the imbalance of joint tissue destruction and repair, and can result from ectopic endochondral ossification under chronic overloading [5, 12]. In OA, articular cartilage is characterized by dysregulated hypertrophy and proliferation, and a loss of extracellular matrix components such as collagen and proteoglycans [8, 13]. Type X collagen (Col10a1), a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS), and matrix metalloproteinase-13 (MMP-13) are catabolic factors and are signals of hypertrophic chondrocytes, which can trigger cartilage degradation and mineralization [14]. Anabolic factors such as aggrecan (ACAN), type II collagen (Col2a1), and sex-determining region Y-type high mobility group box protein 9 (Sox9) are helpful for chondrogenesis. In healthy joints, articular homeostasis is maintained via a balance between chondrocyte synthesis of catabolic and anabolic factors [15, 16], while in cartilage with OA, hypertrophic genes and chondrogenic genes are observed to decline due to tissue loss [14, 17]. Research has found that

* Corresponding author.
E-mail address: xiejing2012@scu.edu.cn (J. Xie).

https://doi.org/10.1016/j.heliyon.2022.e12656
Received 25 January 2022; Received in revised form 12 July 2022; Accepted 19 December 2022
2405-8440/© 2022 The Author(s). Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

Runx1 can regulate these factors and that the upregulation of Runx1 maintains joint homeostasis. Moreover, Runx1 is produced as a response to mechanical compression, but decreases in the joint with OA due to a loss of tissue [12].

To sum up, some reports have elucidated on the role of Runx1 during chondrogenesis, matrix metabolism and osteoarthritis. However, there lacks relevant summary to reorganize the achievements, therefore, in this review, we describe the structural components and the major functions of Runx1, and summarize the impacts of Runx1 on joints and OA that are recently discovered. We then focus on the biomechanism of Runx1, including the interaction with other molecular factors, the current progression of research, the potential gaps in knowledge and the potential treatment to OA related to Runx1.

2. The overview of Runx1

2.1. The Runx family of transcription factors

The Runx transcription factor family has three members in mammals, Runx1 (AML1/CBFα2/PEBP2αB), Runx2 (AML3/CBFα1/PEBP2αA), and Runx3 (AML2/CBFα3/PEBP2αC) [17, 18], and is vital for hematopoiesis, osteogenesis, neurogenesis and gastric epithelial cell growth. In humans, Runx1, Runx2, and Runx3 are located on chromosomes 21q22.12, 6p21, and 1p36.1 respectively [2].

The Runx gene is also called AML, CBFα and PEBP2α. The Runx gene was first named AML, whose mutation is one of the causes of acute myeloid leukemia (AML) [1, 19]. Then, a conserved sequence of 128 amino acids was found in three proteins in mammals, which can also be seen in the Drosophila melanogaster segmentation gene Runt [17, 20–22]. Therefore, the proteins were identified as runt-related (Runx) proteins and the domain was named the runt homology domain (RHD). Moreover, Runx is the DNA-binding subunit of core-binding factor (CBF) or polyomavirus enhancer-binding protein 2 (PEBP2), whose DNA-binding function can be enhanced by combining CBFβ (also known as PEBP2β) [23, 24].

2.2. The structure of Runx1

Runx proteins have various functions in different tissues and during different stages of development due to their structures. Runx genes have two promoters (P1, P2), and they are triggered at distinct stages, generating various isoforms [2, 25]. The proximal P2 promoter drives the expression of Runx1a and Runx1b, whereas the distal P1 promoter generates Runx1c [26]. At the N-terminus, RHD is the region that binds to the DNA motif 5'-PuACCPuCA-3′ (where Pu indicates purines) [27]. All Runx family members have a conserved C-terminal region containing a nuclear matrix-targeting signal (NMTS), which is responsible for localization to distinct subnuclear sites for specific gene regulation [22, 28, 29]. NMTS is the binding site of multiple proteins co-regulating with Runx proteins, including Smad2 and Smad3 [22, 30]. At the carboxy-terminus of Runx, there also has a less conserved five amino motif (VWRPY in most cases), known as the recruitment signal for Groucho/TLE corepressors [22, 26, 31].

The transcriptional function of Runx1 is considerably weak, but with the combination of other coactivator molecules, such as GATA-1, ETS-1, C/EBPα, ALY, p300, YAP, MOZ, NERF-1/2, c-Fos, and AP1, its activating properties are enhanced, and the interaction with the SIN3A complex and the Groucho/TLE complex has a repressive function [24–26, 32–37] (Figure 1). Additionally, diverse posttranscriptional modifications, such as phosphorylation [38], acetylation [39], methylation [24] and sumoylation [40], all contribute to the complexity of Runx proteins [35, 36].

2.3. The functions of Runx1

Runx1 plays a critical role in many systems (Table 1). The most well-known function of Runx1 is in hematopoiesis [22, 25], without which no definitive erythroid, myeloid, or megakaryocytic cells can be seen [19], the quantity of T cells and B cells would decrease [25] and abnormal vasculature development can be seen in many organs [41]. The expression of Runx1 is upregulated in cardiac injury, inducing changes in cardiac cell function and injury response through various signaling pathways, which indicates that Runx1 can be a promising target for adverse cardiac remodeling and heart failure [42]. Moreover, Runx1 plays an important role in bone formation by regulating BMSC differentiation [43, 44], promoting chondrogenesis and osteogenesis. Furthermore, Runx1 is involved in the development of other tissues, such as hair follicles, the nervous system, mammary glands, oral epithelium, and muscles [18, 22, 23, 45–48]. Runx1 contributes to nervous system development, such as the establishment of cutaneous sensory circuits, motor circuits and the olfactory system [49], cell type specification and axonal targeting projections of nociceptive dorsal root ganglion neurons [50]. Runx1 is involved in mammary gland development, and Runx1 could be involved in breast tumor progression depending on Foxp3, which normally inhibits the transcriptional activity of Runx1 [51]. Runx1 is expressed in several epithelial tissues in the craniofacial area such as the salivary gland and olfactory epithelium, and is detected in the dental epithelium of the bud and cap stage tooth germs at embryonic day 13 (E13) and E14 [18]. Runx1 deletion in the hair follicle impairs the hair homeostasis cycle and blocks hair follicle stem cells in quiescence [45].

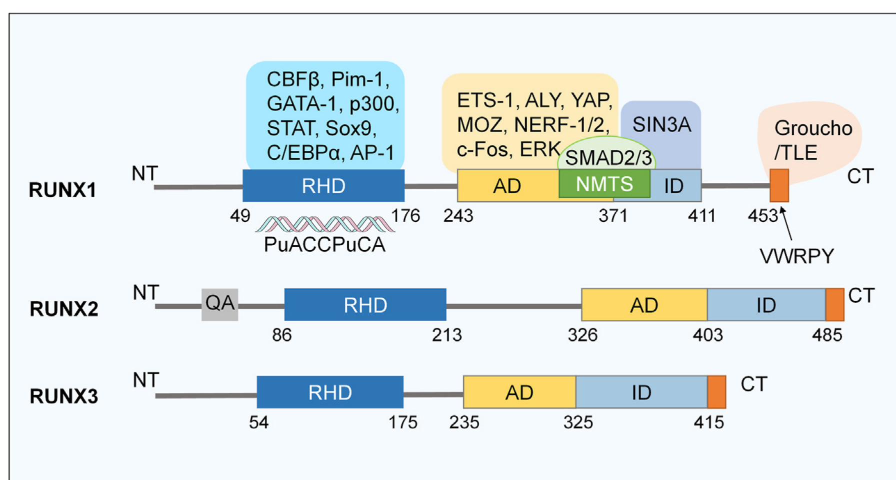


Figure 1. The structure of Runx1. The abbreviations are as follows: NT, N-terminus; CT, C-terminus; AD: activation domain; ID: inhibitory domain; QA, glutamine/alanine-rich sequence. The Runx family of transcription factors contains three members, and they share RHD, AD and ID domains, and C-terminus VWRPY motif. The transcriptional functions of Runx1 are enhanced with the combination of other molecules.

Table 1. The major functions of Runx1. Runx1 is an essential transcriptional factor, playing a critical role in many systems. In addition to those listed in the table, Runx1 has other functions that require be discovering and exploring.

System		Major function	References
Circulatory system	Hematopoiesis	Runx1 is essential for both definitive embryonic and adult hematopoiesis.	[52, 53, 54]
	Leukemia	More than 50 Runx1 mutations, including translocations and point mutations, have been identified as causative factors in multiple leukemias.	[19, 55]
	Immune system	Runx1 is involved at multiple stages of the complex cell-fatedecisions of T lymphocyte development in the thymus, and is important for basophil development and lymphoid tissue inducer cells.	[35]
	Angiogenesis	Runx1 regulated the expression of angiogenic and adhesion molecules in endothelial cells (ECs), and increased the angiogenic activity of ECs.	[56, 57]
Nervous system		Runx1 is crucial for the diversification of sensory neuron lineages, including sensory neurons in skin epidermis and hair follicles, PNS nociceptive neuron, the neuronal tissue of the olfactory system the homeostasis of glial populations of the CNS and the PNS.	[25, 58]
Tumorigenesis		Runx1 has indirect and direct biological functions in modulating cancer metastasis, proliferation, angiogenesis, cancer stemness and chemoresistance to anticancer drugs.	[59, 60]
Cartilage and bone		Runx1 is vital for the early stages of chondrogenesis and osteogenesis, and the homeostasis of skeleton system.	[61, 62, 70]
Hair follicles		Runx1 controls timely emergence of hair follicle stem cells and proper maturation during embryogenesis.	[63]
Mammary gland		Runx1 is required for the differentiation of luminal cells and is highly expressed in the epithelium of virgin females and post-lactation, but gradually decreases throughout pregnancy.	[64]

3. Impacts of Runx1 on joint and osteoarthritis

3.1. The process of chondrogenesis and osteogenesis

Chondrogenesis is a vital process through people's lives. During adulthood, chondrogenesis can be seen in fracture healing, and osteoarthritis influences the differentiation and maintenance of articular cartilage [65]. The process starts with the aggregation and condensation of loose mesenchyme; that is, mesenchymal stem cells (MSCs) migrate and gather to the place where the cartilage or skeleton will be formed [66]. MSCs, which are now recognized as chondroblasts, produce extracellular matrix components such as Col2a1, aggrecan, and Sox9 [3, 65, 67]. Then, chondroblasts differentiate into proliferative chondrocytes, and some become mature chondrocytes forming cartilage while some gradually initiate the process of hypertrophic differentiation, instigating endochondral ossification [65, 67].

Osteogenesis has two distinct processes. Intramembranous ossification is achieved by the differentiation of MSCs into osteoblasts, which mature into osteocytes and eventually form bone [25]. Alternatively, during endochondral ossification, hypertrophic chondrocytes express components such as Col10a1, alkaline phosphatase (ALP), and MMP13, which lead to calcification and degradation of the cartilage matrix [68, 69]. Ultimately, osteoclasts and mesenchymal cells invade together with blood vessels, chondrocytes undergo apoptosis, and the remaining cartilage matrix becomes the foundation of bone formation and turnover [65].

3.2. Impacts of Runx1 on skeletal development

Runx1 contributes to skeletal development by mediating the onset of mesenchymal cell differentiation toward chondrogenesis [3, 22]. Research has shown that Runx1 is expressed in the early stage of cartilage formation [2, 3], especially in chondrocytes in superficial zones [12]. At E12.5, Runx1 is observed in emerging cartilage, and by E14.5, Runx1 is expressed in the perichondrium and the periosteum [2]. With the formation of future bone growth plates, the expression of Runx1 remains in immature chondrocytes of the resting zone and perichondrium [2]. The phenotype of mesenchymal-cell-specific Runx1-deficient mice (the bone fails to develop where Runx1 is knocked out) cooperates with the finding that cartilage development in mice whose Runx1 is knocked out in committed chondrocytes was normal although delayed [67, 70] revealed that Runx1 is involved in mesenchymal condensations, but has little role in the maturation of chondrocytes. Research has indicated that this condensation process may be related to Sox proteins [67]. Moreover, the examination of bovine articular cartilage demonstrated the highest Runx1 level in superficial zones and the lowest Runx1 level in deep zones [12]. In addition, Runx1 is expressed in chondrocytes with permanent cartilage structure [2].

Runx1 impacts bone formation. Runx1 is expressed in the osteogenic cell compartments in the craniofacial bones and the bone collar of long bones, and its expression decreases upon terminal differentiation of osteoblasts [71]. In mesenchymal-cell-specific Runx1-deficient mice (Runx1$^{f/f}$/Prx1-Cre), marrow adipocytes show a significant increase compared to the control, sternum fails to form [67], and lower bone density, reduced bone volume and trabecular bone number can be seen [72]. Similarly, chondrocyte-specific Runx1 knockout mice (Runx1$^{f/f}$/Col2a1-Cre) are shorter in length, lighter in weight and lower in bone density than wild-type mice, revealing Runx1's impact on skeletal development [70, 72]. Moreover, the osteogenic activity in the hypertrophic zone was shown by ALP staining to be decreased in 3- and 6-month-old Runx1$^{f/f}$/Col2a1-Cre mouse femurs [72]. Mice specifically deleting the Runx1 gene in the osteoblast lineage (Runx1$^{f/f}$/Osx-Cre) have decreased ossification and bone density and mandibular defects, showing an osteoporotic phenotype [4]. Additionally, deletion of Runx1 impairs fracture healing [73, 74].

3.3. Impacts of Runx1 on skeletal muscle

Knee osteoarthritis (KOA) can be caused by reduced joint stability due to impaired quadriceps strength [75, 76], and muscle activity differs in OA patients compared with normal subjects [75], but whether muscle weakness facilitates disease progression is controversial. Additionally, aged mice lost joint afferents before developing OA [77], and anterior cruciate ligament sensory loss may contribute to OA [78], indicating that denervation of muscle can result in OA. Studies have shown that the expression of Runx1 is increased in skeletal myocytes under pathological conditions [42, 79, 80], and Runx1 is poorly expressed in innervated muscle, but strongly induced in muscle shortly after denervation [81]. The deficiency of Runx1 leads to worsened fiber atrophy, excessive autophagy and structural abnormalities, showing a positive effect on skeletal muscle, although opposite to cardiac muscle [42, 81]. Overexpression of Runx1 inhibits myogenic differentiation but promotes the proliferation of myoblasts [82]. Therefore, Runx1 can protect the joints by regulating the response of myocytes when injured.

3.4. Impacts of Runx1 on osteoarthritis

Runx1 attenuates the damage of OA to the joint. The synovium is vital for the joints because articular cartilage has no blood vessels to provide nutrition and synovitis is one of the causes of osteoarthritis [83, 84]. Runx1 supports cell proliferation through MSCs, and the markers of MSCs and Runx1 have the same trend during OA progression in both cartilage and synovium [12, 84, 85]. These results indicate that Runx1 can promote the repair of joints by synovium and that Runx1 is a compensatory response to cartilage damage, protecting articular cartilage maintenance. Moreover, endochondral ossification has been considered the most important process of osteophytes, which implies irreversible OA [9]. Runx1 enhances cartilage matrix production and suppresses hypertrophic differentiation by regulating molecules such as Smad, Sox, and Bapx [5, 14], thus contributing to articular cartilage maintenance and inhibiting osteophyte formation.

The expression of Runx1 in cartilage changes during OA. Normally, Runx1 is expressed in chondrocytes in the superficial zone of articular cartilage and diminishes through two deeper zones [12]. As shown in the mouse model, Runx1 is depleted due to cell loss in progressing OA lesions, but is induced in chondrocytes at the OA lesion boundaries [12]. In OA tissue samples of varus knee patients, the medial side has more Runx1 than the lateral side, and compressed bovine cartilage elevates Runx1 expression compared to normal cartilage [12]. These results demonstrate that mechanical compression results in the upregulation of Runx1. Thus, we hypothesized that Runx1 is produced in response to cartilage damage, but as OA lesions progress, tissue loss occurs, and Runx1 expression decreases.

3.5. Current promising treatment for osteoarthritis related to Runx1

Researchers are dedicated to identifying disease-modifying OA drugs (DMOADs) and several candidates for DMOAD targeting Runx1 have been found. TD-198946 [14] and kartogenin (KGN) [86] function via Runx1 induction. In TD-198946-treated mice, Runx1, Sox proteins and Col2a1 are upregulated, while Col10a1 and matrix metalloproteinases-13 (MMP-13) are downregulated [14]. KGN can inhibit matrix breakdown by matrix metalloproteinases, and Runx1 may be the downstream effector of KGN [86]. However, none of these methods have yet been added to the therapeutic arsenal because the research was not conducted thoroughly and some problems remained unsolved [85]. Furthermore, some treatments are under investigation, such as the intraarticular injection of polyplex nano micelles containing Runx1 mRNA in OA mice [87].

4. The mechanisms of Runx1

4.1. Runx1 can interact with the promoters of genes directly

As a transcription factor, Runx1 can interact with the promoters of genes directly. Research has found that the -293/-288 site in the Col2a1 promoter contains Runx motifs and is the core responsive element of Runx1 [14] (Figure 2). The Sox9 promoter, between nucleotides -1705 and -1700 and between nucleotides -1047 and -1042, were identified as Runx-responsive elements. Similarly, the Sox5 promoters (between nucleotides -1824 and -1818) and Sox6 promoters (between nucleotides -2923 and -2918 as well as between nucleotides -1123 and -1119) have Runx1 -binding sites [88] (Figure 2). Additionally, the promoters of OCN [4] (Figure 2), Runx2 [4] (Figure 2), Ihh [70] (Figure 2), miR-455 and miR-210 [84], BMP7, Alk3 and Atf4 [72] (Figure 2) have Runx protein binding sites.

4.2. Runx1 and CBFβ in bones

Core binding factor β (CBFβ) is an essential binding partner for Runx1, which stabilizes Runx1 by forming heterodimers, without which Runx1 can barely be detected [89]. CBFβ provides Runx proteins with an inhibitory domain and enhances their binding to DNA [35, 90], while it is a nonDNA binding factor [91] (Figure 3a). In general, CBFβ plays an important role in skeletal development, impacting the differentiation of osteoblasts and chondrocytes.

CBFβ has an α/β structure consisting of two three-stranded β-sheets packed on one another in a sandwich arrangement, with four peripheral α-helices [92] and has two functional isoforms (CBFβ1 and CBFβ2), which have different but redundant functions in skeletal development [93].

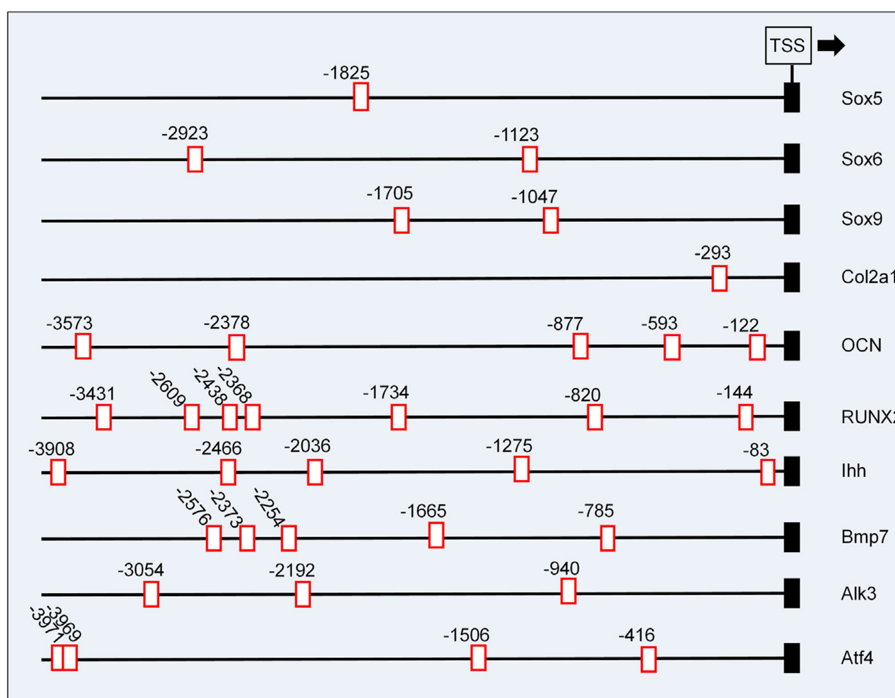


Figure 2. The binding sites of Runx1 on the promoter of genes. Runx1 can directly bind to the promoter of other genes, regulating their expression. Some genes have more than one Runx binding sites. The white box indicates the binding sites on the promoter while the black box indicates the coding sequence.

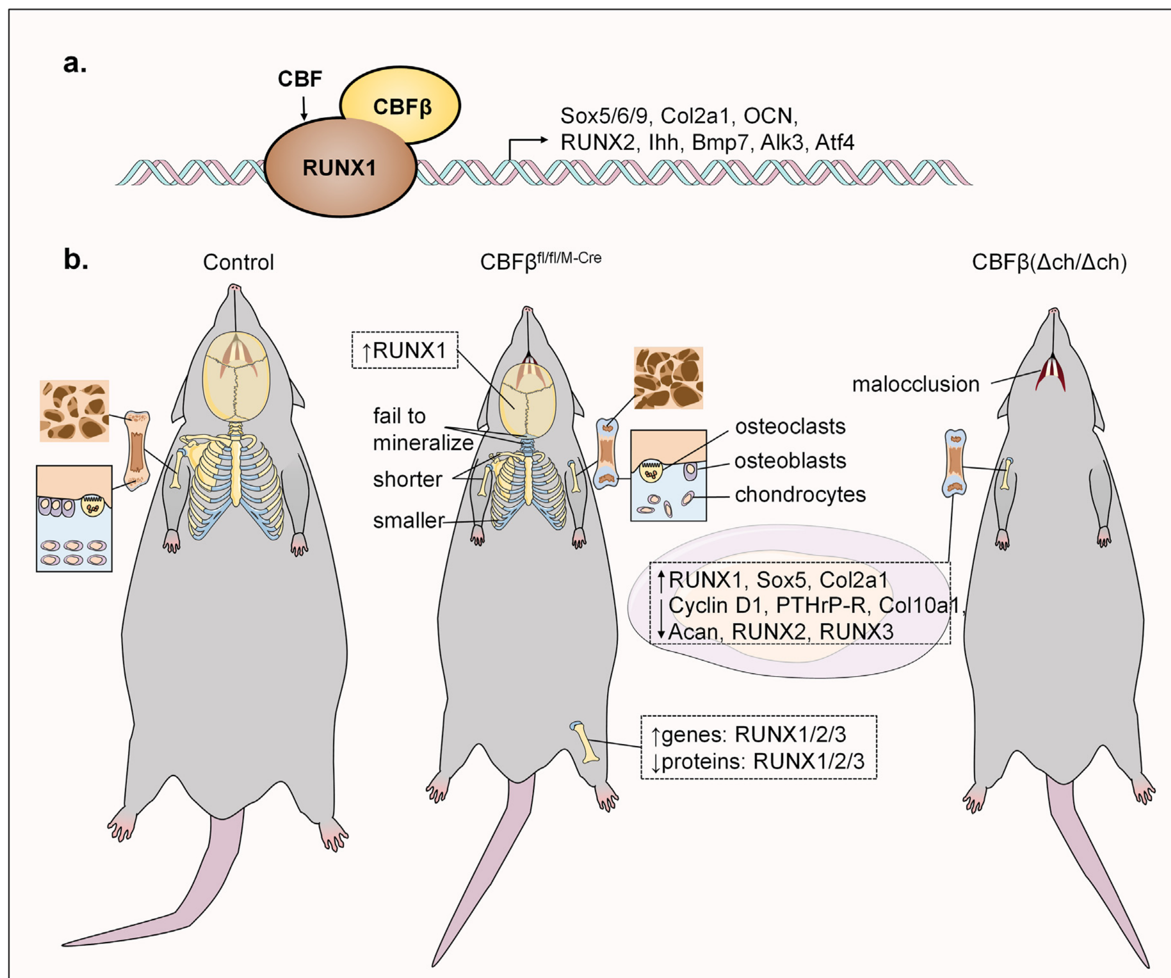


Figure 3. Runx1 and CBFβ. a. Runx1 protein and core binding factor β (CBFβ) form the heterodimerization of core-binding factor (CBF) and regulate the promoter of other genes. Runx1 protein is the DNA-binding subunit and CBFβ plays the role of stablizing Runx1 and enhancing the DNA-binding function of Runx1. **b.** Comparison among the control group, $CBF\beta^{fl/fl/M-Cre}$ group, and CBFβ(Δch/Δch) group. $CBF\beta^{fl/fl/M-Cre}$, mesenchymal-cell-specific CBFβ-deficient mice including Prrx1-Cre and Twist-Cre; CBFβ(Δch/Δch), chondrocyte-specific CBFβ-deficient mice including Sox9-Cre and Col2a1-Cre. Compared with the control group, $CBF\beta^{fl/fl/M-Cre}$ group and CBFβ (Δch/Δch) group both have fewer and thinner trabecular bones, delayed bone development and ossification, and fewer osteoblasts. In $CBF\beta^{fl/fl/M-Cre}$ group, the ossification reduces, the growth plates shorten, the cartilage resting zones elongate, the chondrocytes shape abnormally, and the number of osteoblasts are significantly reduced, while that of osteoclasts remain unchanged. In CBFβ(Δch/Δch) group, the proliferative chondrocytes increase, with no hypertrophic zones.

Mice deficient in CBFβ die of a lack of fetal liver hematopoiesis before birth, showing its role in skeletal development [94]. In mesenchymal-cell-specific CBFβ-deficient mice, dwarfism, skeletal malformation, reduced calcification, and delayed ossification occur, with multiple bone defects [94–96]. Mutant mice have smaller thoracic cages and shorter clavicles and limbs than control mice [94, 95], and reduced ossification can be seen in the mandible, hyoid bone, vertebrae, forelimbs, clavicles, hindlimbs and feet [95, 96]. The growth plate in mutant mice is shorter and showed an elongated resting zone and shorter proliferative and hypertrophic zones, indicating a delay in chondrocyte maturation [95, 96]. Cartilage formation of the airway has defects while occipital bones and cervical vertebrae fail to mineralize in embryos with CBFβ knockout [94–96]. Furthermore, the number and thickness of trabecular bones and the number of osteoblasts are significantly reduced, while osteoclasts are unchanged in newborn knockout mice and the femurs from the mutant mice have irregularly arranged and abnormally shaped chondrocytes [95, 96]. Ihh-cyclin D1 and the Ihh-PTHrP negative feedback loop may contribute to these effects [95]. In chondrocyte-specific CBFβ-deficient (CBFβ(Δch/Δch)) mice, the phenotype is similar to that of $CBF\beta^{fl/M-Cre}$ mice. Mice also have dwarfism with shortened limbs and sternal bodies, delayed ossification (observed mainly in ribs, vertebrae, hyoid bone, and thyroid cartilage), lower bone density, and elongated but deformed growth plates [97]. Moreover, endochondral bone development is delayed, proliferative chondrocytes increased, and hypertrophic zones can hardly be seen till E14 [98]. Interestingly, malocclusion is witnessed in the teeth of $CBF\beta^{fl/fl/M-Cre}$ mice [97] (Figure 3b).

CBFβ is vital for Runx1 in mesenchymal cells and can stabilize Runx proteins and promote osteoblast differentiation [94]. $CBF\beta^{fl/fl/M-Cre}$ hindlimbs have slightly higher expression of the Runx1, 2 & 3 genes but significantly lower expression of the Runx1, 2 and 3 proteins, while the expression of Sox5 and Sox6 is upregulated at E15.5 [94]. Col10a1, Osterix (Osx), MMP-13, secreted phosphoprotein-1 (SPP-1), and Indian hedgehog (Ihh) are suppressed in $CBF\beta^{fl/fl/M-Cre}$ mice [94, 96]. Moreover, in calvarial cells, only Runx1 expression is observed to be reduced in $CBF\beta^{fl/fl/M-Cre}$ mice and CBFβ knockout has less impact [95]. According to research, the expression of Sox5, Col2a1 and Runx1 increases in chondrocytes deprived of CBFβ while that of Ihh, Cyclin D1, PTHrP-R, Col10a1, ACAN, Runx2 and Runx3 decreases [97, 98], indicating the compensatory role of Runx1 (Figure 3b).

4.3. The interplay among Runx proteins in bones

A given target gene may in fact be regulated by different Runx proteins in different tissues [27]. There is auto and cross-regulation among the Runx proteins and genes. In immature cartilage, only Runx1 is

expressed while Runx2 is mainly found in hypertrophic chondrocytes, and Runx3 is expressed in proliferating and prehypertrophic chondrocytes [99, 100].

The Runx2 protein regulates MMP, growth factor and extracellular matrix protein genes [12], thus playing an essential role in chondrocyte differentiation, maturation, matrix degradation and subsequent bone formation [85, 101–103]. Its expression can be seen in hypertrophic chondrocytes, the perichondrium and osteoblasts [84]. Ectopic hypertrophy and bone formation occur if Runx2 is expressed in nonhypertrophic chondrocytes [101]. Moreover, Runx2-deficient mice (Runx2$^{-/-}$) have problems forming mature cartilage while the overexpression of Runx2 in the chondrocytes of Runx2$^{-/-}$ mice restore chondrocyte maturation [104]. The heterozygous mutation of Runx2 causes cleidocranial dysplasia (CCD), featuring hypoplastic clavicles, open fontanelles, and supernumerary teeth [94]. Runx2 was expressed in the mesenchymal condensates of bones and tooth formation [101].

Runx1 and Runx2 interact during bone development. mRNA Levels of Runx1 and Runx2 are inversely correlated in skeletal development; Runx2 interacts with its own promoter and the Runx1 promoter, and small interfering RNA (siRNA)-mediated depletion of Runx2 increases Runx1 activity [105]. Runx2 may compensate for the functions of Runx1 in sternum formation when Runx1 is deficient [3, 67], although it was also discovered that Runx2 expression is downregulated in chondrocytes deprived of Runx1 [70, 88]. In Runx2$^{-/-}$ mice, Runx1 expression increases around the upper tooth germs, Meckel's cartilage and mandibular bone at E14.5 [71]. Runx1 can cooperate with Runx2 and promote the commitment of mesenchymal cells to the chondrocyte lineage by inducing the expression of Sox5 and Sox6, and Runx2 is essential for osteochondroprogenitor cell development [67].

Runx3 is vital for chondrocyte maturation. The expression of Runx3 increases in prehypertrophic chondrocytes and decreases in hypertrophic chondrocytes. Research has shown that chondrocytes in Runx2$^{-/-}$ mice mature, although delayed, while Runx2-and Runx3-deficient (Runx2$^{-/-}$3$^{-/-}$) mice have no mature chondrocytes, indicating that Runx3 is involved in chondrocyte maturation. Additionally, Runx2$^{-/-}$3$^{-/-}$ mice have no expression of Ihh and are shorter in limb length [102].

4.4. The signaling pathway of Runx1 related to chondrogenesis and osteogenesis

In Runx1$^{f/f}$/Col2a1-Cre mice, chondrogenic genes (Col2a1, Sox9, ACAN) decline while hypertrophic genes (Col10a1, MMP-13) and the expression of osteogenic genes (Runx2, osteocalcin (OCN), osteopontin (OPN), activating transcription factor 4 (Atf4), Osx) also decreases in osteogenesis period [4], and the mRNA levels of Ihh, parathyroid hormone-related peptide receptor (PTH1R) and cyclinD1, which promotes cell proliferation, showed downward trends, but CBFβ and Runx3 remained unchanged [70, 72] (Figure 4a). These results suggest that Runx1 promotes the differentiation from BMSCs to chondrocyte and osteoblast lineage commitment. Runx1 deficiency downregulates TGFβ/BMP signaling, with a significant decrease in the phosphorylation ratio and the total amount of Smad1/5/8 and Smad2/3 [72] (Figure 4b).

If Runx1 is overexpressed in chondrocytes, the protein levels of Col2a1, Sox9, ACAN, papillary thyroid carcinoma (PTC), PTH1R and Bapx1 can be enhanced, while Sox6 is unchanged and Col10a1 and MMP-13 are downregulated [3, 5, 14, 70]. Therefore, cartilage matrix formation is promoted, and chondrocyte hypertrophy and osteogenesis are inhibited. Interestingly, adipocyte differentiation was found in chondrocytes deficient in Runx1 [70], which may be the reason for osteoporosis (Figure 4a).

There are other pathways related to Runx1 and cartilage. Protein phosphatase 5 (PP5) can downregulate Runx1 and Runx2 by phosphorylating peroxisome proliferator-activated receptor γ (PPARγ) Ser-112 and reducing the transcriptional activity of PPARγ, thus decreasing the expression of ACAN, Col2a1, Col10a1, OPN, and OCN [106]. Furthermore, it was found that all-trans retinoic acid (ATRA), which is vital for chondrocyte proliferation and differentiation, can regulate cartilage development by the BMP2-WNT4-Runx1 pathway [107, 108]. Moreover, in chondrocytes of antler cartilage, insulin-like growth factor 1 (IGF1) was discovered to promote the proliferation activity of chondrocytes through insulin receptor substrate 1 (IRS1) and IRS2, whose downstream targets were Runx1 [109] (Figure 4b).

Among these factors, Sox protein is one of the most important in chondrogenesis, but it is not fully understood. Some research infers that Sox9 is genetically upstream of Sox5 and Sox6, as a decrease in Sox5 and Sox6 expression was observed when Sox9 was knocked out [67]. The comparison of mesenchymal condensation in mesenchymal-specific Sox9-deficient mice and mesenchymal-specific Runx1-and Runx2-deficient mice revealed that Runx1 and Runx2 are located between Sox9 and Sox5/6 in the genetic cascade, although it is not clear whether Runx1 and Runx2 are direct targets of Sox9 [67]. Researchers discovered that Runx1 and Runx2 can induce Sox5, Sox6 and Sox9 proteins [14, 67], while deficiency of Runx1 downregulates Sox9 [70]. Sox9 is upregulated by Runx1 overexpression, while Sox6 is unchanged [5]. Additionally, a specific protein-protein interaction between Runx1 and Sox proteins is confirmed by coimmunoprecipitation (Co-IP) [5]. Therefore, we suspect that there are complex interplays among Runx and Sox proteins, which may vary during different developmental periods (Figure 4c).

Runx1 impacts osteogenesis. Bone morphogenetic proteins (BMPs), members of the transforming growth factor β (TGF-β) superfamily, have been shown to regulate embryonic development, differentiation and cell function [110, 111], induce osteogenic differentiation of mouse multilineage cells and promote bone formation during bone remodeling [112]. To date, more than 20 kinds of BMPs have been identified, among which BMP2, BMP6 and BMP7 play an important role in MSC differentiation into osteogenesis [113]. In osteoblasts, Runx1/Bmp7/Alk3/Smad1/5/8/Runx2/ATF4 is a signaling pathway that functions in bone formation [72], and Runx1 may affect the phosphorylation of Smad1/5/8 induced by BMP9 [114]. Moreover, RNA-sequencing analysis shows that Runx1 promotes osteogenesis and inhibits adipogenesis by orchestrating canonical BMP signaling, noncanonical BMP/ERK signaling, and WNT/β-catenin signaling [43, 72]. Furthermore, Runx1 expression in preosteoclasts negatively regulates osteoclast formation and activity [44].

4.5. The signaling pathway of Runx1 related to osteoarthritis

In cartilage with OA, Runx1 can enhance cartilage matrix production and promote chondrocyte differentiation from MSCs at the same time [3]. The synovial fluid microenvironment can be changed by the Runx1 protein and promote cartilage regeneration. TGF-β1 is an important molecule in the joint and is overexpressed in inflammation. Deletion of TGF-β receptor type II, whose ligand can be TGF-β1, in articular chondrocytes resulted in an osteoarthritis-like phenotype in mice [115]. It has been discovered that the Runx1 protein in synoviocytes is stimulated in KOA by transforming growth factor β1 (TGF-β1) through Smad2/3 signaling [42, 84]. Runx1 activates relevant genes, thus upregulating the expression of miR-455 and miR-210 in synoviocytes whose overexpression could increase chondrogenic gene expression and reduce hypertrophic gene expression. However, as a pluripotent cytokine, TGF-β1 functions variously if injected or in healthy joints, making it unlikely to be an ideal KOA therapeutic target [84]. Furthermore, the interaction of the Runx1 protein with Sox5, Sox6, and Sox9 was proven to be another way to stimulate matrix production. The levels of Col2a1 mRNA and glycosaminoglycan (GAG) are further enhanced when Sox5, Sox6, and Sox9 are co-overexpressed with Runx1 compared with the overexpression of Runx1 alone [5]. Additionally, researchers have discovered that parathyroid hormone (PTH) can activate Runx1 by the PKA signaling pathway and promote the differentiation from MSCs to chondrocytes [116]. However, no valid evidence has shown that PKA signaling pathway is the only pathway between PTH and Runx1, and

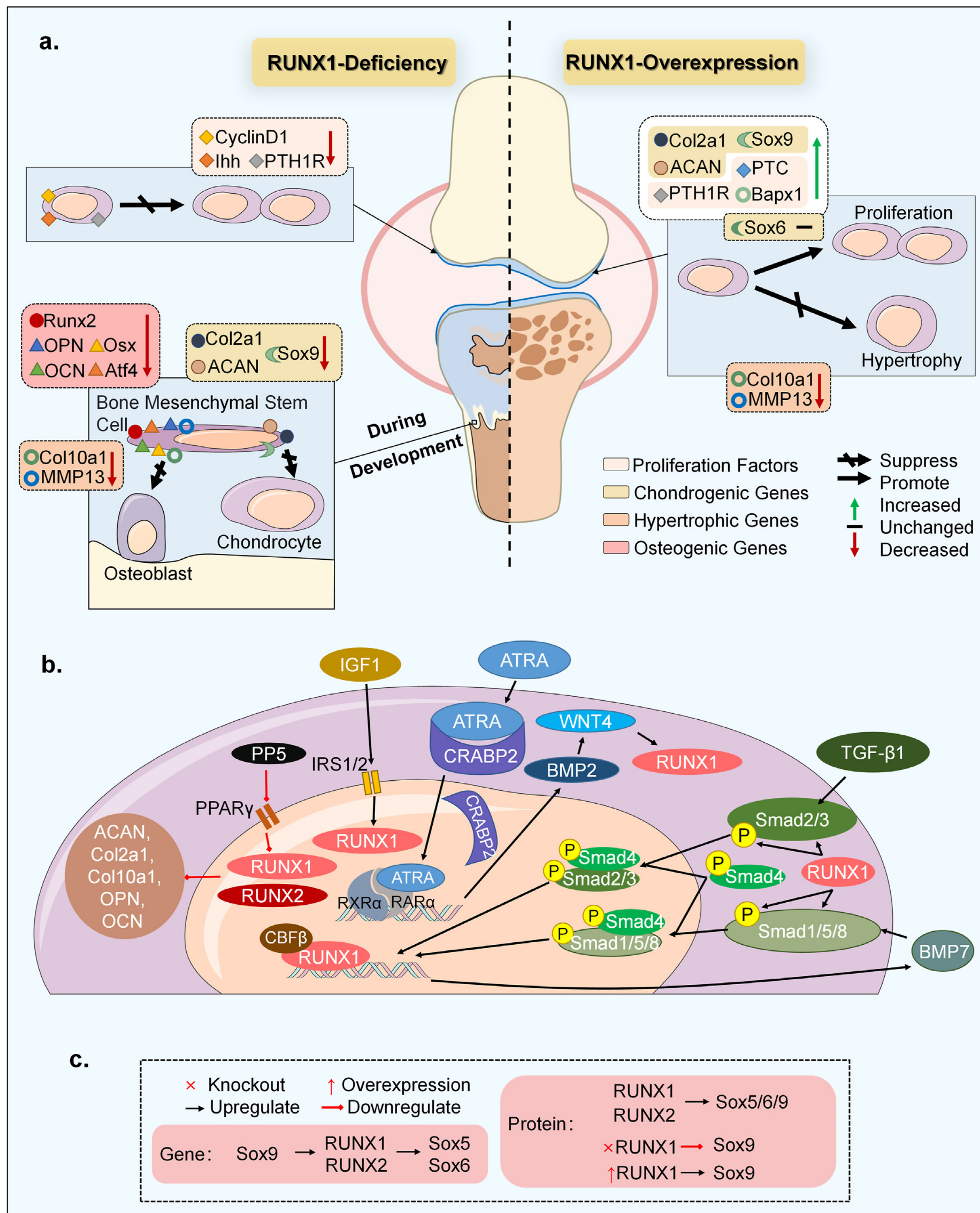


Figure 4. The signaling pathway of Runx1 related to chondrogenesis and osteogenesis. a. The difference in bone or chondrocytes when Runx1 is deficient or overexpressed. In mice deficient of Runx1, proliferation factors in chondrocytes decrease, and the differentiation of bone marrow mesenchymal cells into osteoblasts and chondrocytes is inhibited through low expression of related genes. In mice overexpressed with Runx1, the proliferation factors and chondeogenic genes increase, thus inhibiting cell hypertrophy and promoting cell proliferation. Ihh, Indian hedgehog; PTH1R, parathyroid hormone-related peptide receptor; OCN, osteocalcin; OPN, osteopontin; Osx, Osterix; Atf4, activating transcription factor 4; Col2a1, collagen type II; Col10a1, collagen type X; MMP-13, matrix metalloproteinase-13; ACAN, aggrecan; Sox9, sex-determining region Y-type high mobility group box protein 9. **b.** Other signaling pathways related to Runx1 and bone. Transforming growth factor-beta1 (TGF-β1) and bone morphogenetic proteins (BMPs) regulate chondrogenesis via Smad-dependent signal pathways and Runx1 helps to phosphorylate Smad2/3. Moreover, all-trans retinoic acid (ATRA), insulin-like growth factor 1 (IGF1), protein phosphatase 5 (PP5) can increase the expression of Runx1 through different signal pathways. Runx1 can then upregulate the expression of ACAN, Col2a1, Col10a1, OPN and OCN. **c.** The interplay of Runx1 and Sox proteins is complex. Runx1 protein and Sox9 protein influence each other mutually. Sox5 and Sox6 are the downstream targets of Runx1 and Sox9.

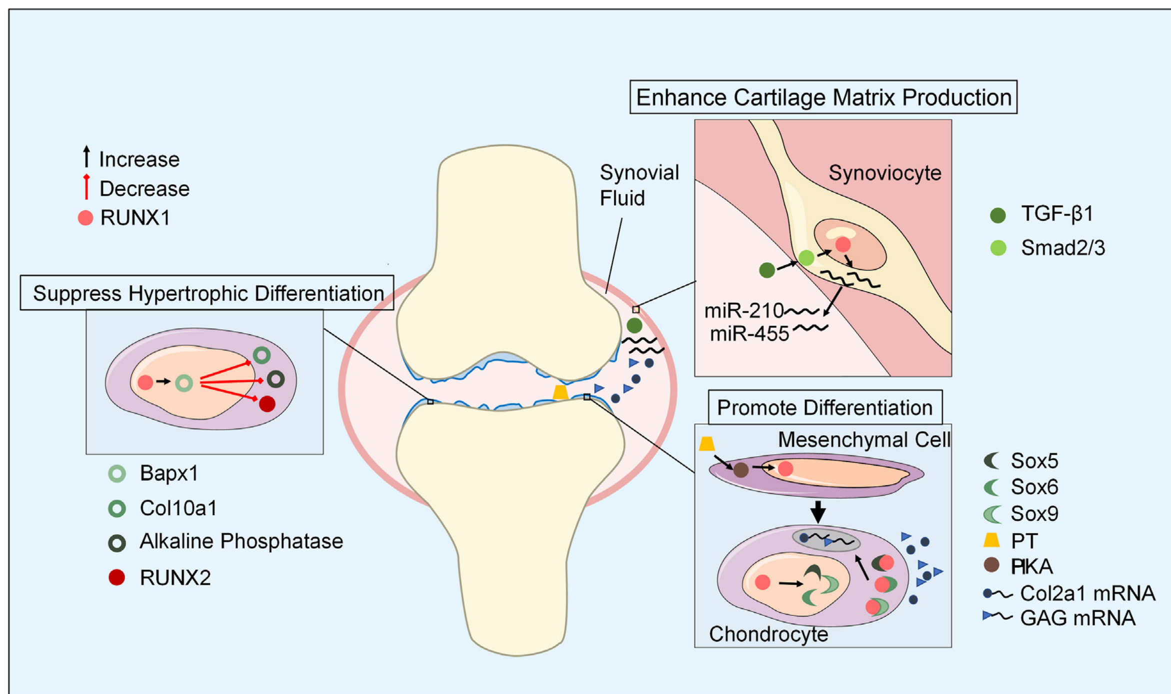


Figure 5. The signaling pathway of Runx1 related to osteoarthritis.

the molecular mechanism how PKA regulates Runx1 needs further exploration (Figure 5).

Runx1 enhances cartilage matrix production, promotes differentiation, and supresses hepertrophic differentiation in cartilage with OA. Transforming growth factor β1 (TGF-β1) stimulates Runx1 in synoviocytes, thus upregulating the expression of miR-455 and miR-210 and promoting cartilage matrix production. Parathyroid hormone (PTH) can activate Runx1 by the PKA signaling pathway and promote the differentiation from MSCs to chondrocytes. Moreover, the interaction of the Runx1 protein with Sox5, Sox6, and Sox9 can stimulate matrix production. Additionally, Runx1 can induce the expression of Bapx1, thus supressing hepertrophic differentiation.

In cartilage with OA, Runx1 can suppress hypertrophic differentiation [5, 14]. Bapx1, also known as Nkx3.2, is a suppressive factor of chondrocyte hypertrophy [117]. Runx1 can induce the expression of Bapx1, although the possibility that Runx1 and Bapx1 function individually cannot be excluded [5] (Figure 5). Our lab recently found that overexpression of Runx1 in articular cartilage showed a protective effect by slowing the destruction of osteoarthritis in cartilage in early osteoarthritis and alleviating the pathological progression of growth plate cartilage in late osteoarthritis [121]. Runx1 could interact with transmembrane anterior posterior transformation 1 (TAPT1), protein RIC1 homolog (RIC1), fibroblast growth factor 20 (FGF20) to protects against the pathological progression of OA.

5. Conclusions and perspectives

Accumulating evidence supports that Runx1 is essential for chondrogenesis and osteoarthritis, and the increased Runx1 is helpful to attenuate OA, thus demonstrating that Runx1 can be a promising target in treating OA.

However, the molecular mechanisms of Runx1 are not yet fully understood. Research has shown that all-trans retinoic acid (ATRA) can regulate cartilage development by the BMP2-WNT4-Runx1 pathway [107, 108], but whether this pathway contributes to the recovery of OA remains unknown. Additionally, previous studies have shown that PTH, which acts as a promoter of chondrogenic differentiation [116], can regulate many signaling molecules, such as protein kinase C (PKC), mitogen-activated protein kinases (MAPK), and Wnt signaling. However, whether and how these molecules function during chondrogenic or osteogenic differentiation needs further study. Sox protein is another vital molecule of chondrocytes, and the interplay of Sox proteins and Runx1 is mysterious. Some researchers discovered that Sox9 can mediate Runx1, which induces Sox5 and Sox6 [67]. However, it was also revealed that the deficiency or overexpression of Runx1 influences the expression of Sox9 [5].

Despite of much unknown information of Runx1 beyond the above, Runx1 is still a promising molecule for DMOAD. Agents targeting precise diseases based on the mechanisms of transcription factors have been successfully applied, including modulating receptor ligand binding domains, essential protein–protein interactions, transcription factor-DNA binding with DNA binding compounds [118]. The modulation of the oestrogen receptor (ER) and androgen receptor (AR) are currently used for treatment of breast cancer and prostate cancer respectively, and an inhibitor (AI-10-49) that disrupts the protein-protein interaction between CBFβ-SMMHC and Runx1 has been licensed to Systems Oncology for clinical development [119]. Additionally, Histone deacetylase (HDAC) inhibitors that restored RA-dependent transcriptional activation, is a potential transcriptional/differentiation therapy for AML [120]. Therefore, as a transcription factor that is essential for the homeostasis of joint cartilage and can decelerate the development of OA, Runx1 has the potential to be a modulating target for OA. The exploration of modulation can be conducted to upregulate the expression of Runx1, increase the downstream molecule of Runx1, activate the receptor of Runx1 signal pathway, and so on.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

Funding statement

Demao Zhang and Dr. Jing Xie were supported by National Natural Science Foundation of China [82001062 & 81771047].

Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no competing interests.

Additional information

No additional information is available for this paper.

References

[1] H. Miyoshi, K. Shimizu, T. Kozu, N. Maseki, Y. Kaneko, M. Ohki, t(8;21) breakpoints on chromosome 21 in acute myeloid leukemia are clustered within a limited region of a single gene, AML1, Proc. Natl. Acad. Sci. U.S.A. 88 (1991) 10431–10434.

[2] C.A. Yoshida, T. Komori, Role of Runx proteins in chondrogenesis, Crit. Rev. Eukaryot. Gene Expr. 15 (2005) 243–254.

[3] Y. Wang, R.M. Belflower, Y.F. Dong, E.M. Schwarz, R.J. O'Keefe, H. Drissi, Runx1/AML1/Cbfa2 mediates onset of mesenchymal cell differentiation toward chondrogenesis, J. Bone Miner. Res. 20 (2005) 1624–1636.

[4] J. Tang, J. Xie, W. Chen, C. Tang, J. Wu, Y. Wang, X.D. Zhou, H.D. Zhou, Y.P. Li, Runt-related transcription factor 1 is required for murine osteoblast differentiation and bone formation, J. Biol. Chem. 295 (2020) 11669–11681.

[5] F. Yano, S. Ohba, Y. Murahashi, S. Tanaka, T. Saito, U.I. Chung, Runx1 contributes to articular cartilage maintenance by enhancement of cartilage matrix production and suppression of hypertrophic differentiation, Sci. Rep. 9 (2019) 7666.

[6] J. Xie, D. Zhang, Y. Lin, Q. Yuan, X. Zhou, Anterior cruciate ligament transection-induced cellular and extracellular events in menisci: implications for osteoarthritis, Am. J. Sports Med. 46 (2018) 1185–1198.

[7] C. Zhou, M. Duan, D. Guo, X. Du, D. Zhang, J. Xie, Microenvironmental stiffness mediates cytoskeleton re-organization in chondrocytes through laminin-FAK mechanotransduction, Int. J. Oral Sci. 14 (2022) 15.

[8] H. Chen, J. Li, D. Zhang, X. Zhou, J. Xie, Role of the fibroblast growth factor 19 in the skeletal system, Life Sci. 265 (2021) 118804.

[9] [a] H. Chen, Y. Cui, D. Zhang, J. Xie, X. Zhou, The role of fibroblast growth factor 8 in cartilage development and disease, J. Cell Mol. Med. 26 (2022) 990–999; [b] Z. Xu, T. Chen, J. Luo, S. Ding, S. Gao, J. Zhang, Cartilaginous metabolomic study reveals potential mechanisms of osteophyte formation in osteoarthritis, J. Proteome Res. 16 (2017) 1425–1435.

[10] B. Ruaro, A. Casabella, S. Paolino, C. Pizzorni, E. Alessandri, C. Seriolo, G. Botticella, L. Molfetta, P. Odetti, V. Smith, M. Cutolo, Correlation between bone quality and microvascular damage in systemic sclerosis patients, Rheumatology 57 (2018) 1548–1554.

[11] L.A. Mandl, Osteoarthritis year in review 2018: clinical, Osteoarthritis Cartilage 27 (2019) 359–364.

[12] K.T. LeBlanc, M.E. Walcott, T. Gaur, S.L. O'Connell, K. Basil, C.P. Tadiri, A. Mason-Savas, J.A. Silva, A.J. van Wijnen, J.L. Stein, G.S. Stein, D.C. Ayers, J.B. Lian, P.J. Fanning, Runx1 activities in superficial zone chondrocytes, osteoarthritic chondrocyte clones and response to mechanical loading, J. Cell. Physiol. 230 (2015) 440–448.

[13] J. Li, H. Chen, D. Zhang, J. Xie, X. Zhou, The role of stromal cell-derived factor 1 on cartilage development and disease, Osteoarthritis Cartilage 29 (2021) 313–322.

[14] S. Kan, M. Duan, Y. Liu, C. Wang, J. Xie, Role of mitochondria in physiology of chondrocytes and diseases of osteoarthritis and rheumatoid arthritis, Cartilage 13 (2021) 1102S–1121S.

[15] D. Zhang, X. Li, C. Pi, L. Cai, Y. Liu, W. Du, W. Yang, J. Xie, Osteoporosis-decreased extracellular matrix stiffness impairs connexin 43-mediated gap junction intercellular communication in osteocytes, Acta Biochim. Biophys. Sin. 52 (2020) 517–526.

[16] M. Duan, Q. Wang, Y. Liu, J. Xie, The role of TGF-β2 in cartilage development and diseases, Bone Joint Res. 10 (2021) 474–487.

[17] Y. Ito, Oncogenic potential of the RUNX gene family: 'overview, Oncogene 23 (2014) 4198–4208.

[18] T. Yamashiro, T. Aberg, D. Levanon, Y. Groner, I. Thesleff, Expression of Runx1, -2 and -3 during tooth, palate and craniofacial bone development, Mech. Dev. 119 (2002) S107–S110.

[19] R. Sood, Y. Kamikubo, P. Liu, Role of RUNX1 in hematological malignancies, Blood 129 (2017) 2070–2082.

[20] J.P. Gergen, B.A. Butler, Isolation of the Drosophila segmentation gene runt and analysis of its expression during embryogenesis, Genes Dev. 2 (1988) 1179–1193.

[21] C. Nüsslein-Volhard, E. Wieschaus, Mutations affecting segment number and polarity in Drosophila, Nature 287 (1980) 795–801.

[22] D. Hong, A.J. Fritz, J.A. Gordon, C.E. Tye, J.R. Boyd, K.M. Tracy, S.E. Frietze, F.E. Carr, J.A. Nickerson, A.J. Van Wijnen, A.N. Imbalzano, S.K. Zaidi, J.B. Lian, J.L. Stein, G.S. Stein, RUNX1-dependent mechanisms in biological control and dysregulation in cancer, J. Cell. Physiol. 234 (2019) 8597–8609.

[23] Y. Ito, S.C. Bae, L.S. Chuang, The RUNX family: developmental regulators in cancer, Nat. Rev. Cancer 15 (2015) 81–95.

[24] X. Zhao, V. Jankovic, A. Gural, G. Huang, A. Pardanani, S. Menendez, J. Zhang, R. Dunne, A. Xiao, H. Erdjument-Bromage, C.D. Allis, P. Tempst, S.D. Nimer, Methylation of RUNX1 by PRMT1 abrogates SIN3A binding and potentiates its transcriptional activity, Genes Dev. 22 (2008) 640–653.

[25] R. Mevel, J.E. Draper, M. Lie-A-Ling, V. Kouskoff, G. Lacaud, RUNX transcription factors: orchestrators of development, Development 146 (2019), dev148296.

[26] C. Bonifer, E. Levantini, V. Kouskoff, G. Lacaud, Runx1 structure and function in blood cell development, Adv. Exp. Med. Biol. 962 (2017) 65–81.

[27] F. Otto, M. Lübbert, M. Stock, Upstream and downstream targets of RUNX proteins, J. Cell. Biochem. 89 (2003) 9–18.

[28] S.K. Zaidi, A. Javed, J.Y. Choi, A.J. van Wijnen, J.L. Stein, J.B. Lian, G.S. Stein, A specific targeting signal directs Runx2/Cbfa1 to subnuclear domains and contributes to transactivation of the osteocalcin gene, J. Cell Sci. 114 (2001) 3093–3102.

[29] G.S. Stein, J.B. Lian, A.J. van Wijnen, J.L. Stein, A. Javed, M. Montecino, J.Y. Choi, D. Vradii, S.K. Zaidi, J. Pratap, D. Young, Organization of transcriptional regulatory machinery in nuclear microenvironments: implications for biological control and cancer, Adv. Enzym. Regul. 47 (2007) 242–250.

[30] S.K. Zaidi, A.J. Sullivan, A.J. van Wijnen, J.L. Stein, G.S. Stein, J.B. Lian, Integration of Runx and Smad regulatory signals at transcriptionally active subnuclear sites, Proc. Natl. Acad. Sci. U. S. A 99 (2002) 8048–8053.

[31] J.A. Coffman, Runx transcription factors and the developmental balance between cell proliferation and differentiation, Cell Biol. Int. 27 (2003) 315–324.

[32] K.E. Elagib, F.K. Racke, M. Mogass, R. Khetawat, L.L. Delehanty, A.N. Goldfarb, RUNX1 and GATA-1 coexpression and cooperation in megakaryocytic differentiation, Blood 101 (2003) 4333–4341.

[33] D. Wotton, J. Ghysdael, S. Wang, N.A. Speck, M.J. Owen, Cooperative binding of Ets-1 and core binding factor to DNA, Mol. Cell Biol. 14 (1994) 840–850.

[34] D.E. Zhang, C.J. Hetherington, S. Meyers, K.L. Rhoades, C.J. Larson, H.M. Chen, S.W. Hiebert, D.G. Tenen, CCAAT enhancer-binding protein (C/EBP) and AML1 (CBF alpha2) synergistically activate the macrophage colony-stimulating factor receptor promoter, Mol. Cell Biol. 16 (1996) 1231–1240.

[35] W. Seo, I. Taniuchi, The roles of RUNX family proteins in development of immune cells, Mol. Cell. 43 (2020) 107–113.

[36] L.S. Chuang, K. Ito, Y. Ito, RUNX family: regulation and diversification of roles through interacting proteins, Int. J. Cancer 132 (2013) 1260–1271.

[37] C.P. Koh, C.Q. Wang, C.E. Ng, Y. Ito, M. Araki, V. Tergaonkar, G. Huang, M. Osato, RUNX1 meets MLL: epigenetic regulation of hematopoiesis by two leukemia genes, Leukemia 27 (2013) 1793–1802.

[38] H. Guo, A.D. Friedman, Phosphorylation of RUNX1 by cyclin-dependent kinase reduces direct interaction with HDAC1 and HDAC3, J. Biol. Chem. 286 (2011) 208–215.

[39] Y.H. Jin, E.J. Jeon, Q.L. Li, Y.H. Lee, J.K. Choi, W.J. Kim, K.Y. Lee, S.C. Bae, Transforming growth factor-beta stimulates p300-dependent RUNX3 acetylation, which inhibits ubiquitination-mediated degradation, J. Biol. Chem. 279 (2004) 29409–29417.

[40] J.H. Kim, J.W. Jang, Y.S. Lee, J.W. Lee, X.Z. Chi, Y.H. Li, M.K. Kim, D.M. Kim, B.S. Choi, J. Kim, H.M. Kim, A. van Wijnen, I. Park, S.C. Bae, RUNX family members are covalently modified and regulated by PIAS1-mediated sumoylation, Oncogenesis 3 (2014), e101.

[41] N. Li, H. Chen, J. Wang, DNA damage and repair in the hematopoietic system, Acta Biochim. Biophys. Sin. 54 (2022) (2022) 847–857.

[42] A. Riddell, M. McBride, T. Braun, S.A. Nicklin, E. Cameron, C.M. Loughrey, T.P. Martin, RUNX1: an emerging therapeutic target for cardiovascular disease, Cardiovasc. Res. 116 (2020) 1410–1423.

[43] Y. Luo, Y. Zhang, G. Miao, Y. Zhang, Y. Liu, Y. Huang, Runx1 regulates osteogenic differentiation of BMSCs by inhibiting adipogenesis through Wnt/β-catenin pathway, Arch. Oral Biol. 97 (2019) 176–184.

[44] Y. Soung do, J. Kalinowski, S.K. Baniwal, C.E. Jacome-Galarza, B. Frenkel, J. Lorenzo, H. Drissi, Runx1-mediated regulation of osteoclast differentiation and function, Mol. Endocrinol. 28 (2014) 546–553.

[45] C.S. Hoi, S.E. Lee, S.Y. Lu, D.J. McDermitt, K.M. Osorio, C.M. Piskun, R.M. Peters, R. Paus, T. Tumbar, Runx1 directly promotes proliferation of hair follicle stem cells and epithelial tumor formation in mouse skin, Mol. Cell Biol. 30 (2010) 2518–2536.

[46] N. Kanaykina, K. Abelson, D. King, A. Liakhovitskaia, S. Schreiner, M. Wegner, E.N. Kozlova, In vitro and in vivo effects on neural crest stem cell differentiation by conditional activation of Runx1 short isoform and its effect on neuropathic pain behavior, Ups, J. Med. Sci. 115 (2010) 56–64.

[47] E.S. Sokol, S. Sanduja, D.X. Jin, D.H. Miller, R.A. Mathis, P.B. Gupta, Perturbation-expression analysis identifies RUNX1 as a regulator of human mammary stem cell differentiation, PLoS Comput. Biol. 11 (2015), e1004161.

[48] K.B. Umansky, Y. Gruenbaum-Cohen, M. Tsoory, E. Feldmesser, D. Goldenberg, O. Brenner, Y. Groner, Runx1 transcription factor is required for myoblasts proliferation during muscle regeneration, PLoS Genet. 11 (2015), e1005457.

[49] J.W. Wang, S. Stifani, Roles of Runx genes in nervous system development, Adv. Exp. Med. Biol. 962 (2017) 103–116.

[50] M. Yoshikawa, M. Hirabayashi, R. Ito, S. Ozaki, S. Aizawa, T. Masuda, K. Senzaki, T. Shiga, Contribution of the Runx1 transcription factor to axonal pathfinding and muscle innervation by hypoglossal motoneurons, Dev. Neurobiol. 75 (2015) 1295–1314.

[51] M.S. Recouvreux, E.N. Grasso, P.C. Echeverria, L. Rocha-Viegas, L.H. Castilla, C. Schere-Levy, J.M. Tocci, E.C. Kordon, N. Rubinstein, RUNX1 and FOXP3

interplay regulates expression of breast cancer related genes, Oncotarget 7 (2016) 6552–6565.
[52] M.J. Chen, T. Yokomizo, B.M. Zeigler, E. Dzierzak, N.A. Speck, Runx1 is required for the endothelial to haematopoietic cell transition but not thereafter, Nature 457 (2009) 887–891.
[53] M. Kurokawa, AML1/Runx1 as a versatile regulator of hematopoiesis: regulation of its function and a role in adult hematopoiesis, Int. J. Hematol. 84 (2006) 136–142.
[54] T. Okuda, J. van Deursen, S.W. Hiebert, G. Grosveld, J.R. Downing, AML1, the target of multiple chromosomal translocations in human leukemia, is essential for normal fetal liver hematopoiesis, Cell 84 (1996) 321–330.
[55] T. Zhen, Y. Cao, G. Ren, L. Zhao, R.K. Hyde, G. Lopez, D. Feng, L. Alemu, K. Zhao, P.P. Liu, RUNX1 and CBFβ-SMMHC transactivate target genes together in abnormal myeloid progenitors for leukemia development, Blood 136 (2020) 2373–2385.
[56] S. Kaur, P. Rawal, H. Siddiqui, S. Rohilla, S. Sharma, D.M. Tripathi, S. Baweja, M. Hassan, S. Vlaic, R. Guthke, M. Thomas, R. Dayoub, C. Bihari, S.K. Sarin, T.S. Weiss, Increased expression of RUNX1 in liver correlates with NASH activity score in patients with non-alcoholic steatohepatitis (NASH), Cells 8 (2019) 1277.
[57] K. Iwatsuki, K. Tanaka, T. Kaneko, R. Kazama, S. Okamoto, Y. Nakayama, Y. Ito, M. Satake, S. Takahashi, A. Miyajima, T. Watanabe, T. Hara, Runx1 promotes angiogenesis by downregulation of insulin-like growth factor-binding protein-3, Oncogene 24 (2005) 1129–1137.
[58] F.M. Theriault, H.N. Nuthall, Z. Dong, R. Lo, F. Barnabe-Heider, F.D. Miller, S. Stifani, Role for Runx1 in the proliferation and neuronal differentiation of selected progenitor cells in the mammalian nervous system, J. Neurosci. 25 (2005) 2050–2061.
[59] T.C. Lin, RUNX1 and cancer, Biochim. Biophys. Acta, Rev. Cancer 1877 (2022) 188715.
[60] Q. Li, Q. Lai, C. He, Y. Fang, Q. Yan, Y. Zhang, X. Wang, C. Gu, Y. Wang, L. Ye, L. Han, X. Lin, J. Chen, J. Cai, A. Li, S. Liu, RUNX1 promotes tumour metastasis by activating the Wnt/β-catenin signalling pathway and EMT in colorectal cancer, J. Exp. Clin. Cancer Res. 38 (2019) 334.
[61] N. Smith, Y. Dong, J.B. Lian, J. Pratap, P.D. Kingsley, A.J. van Wijnen, J.L. Stein, E.M. Schwarz, R.J. O'Keefe, G.S. Stein, M.H. Drissi, Overlapping expression of Runx1(Cbfa2) and Runx2(Cbfa1) transcription factors supports cooperative induction of skeletal development, J. Cell. Physiol. 203 (2005) 133–143.
[62] J.B. Lian, E. Balint, A. Javed, H. Drissi, R. Vitti, E.J. Quinlan, L. Zhang, A.J. Van Wijnen, J.L. Stein, N. Speck, G.S. Stein, Runx1/AML1 hematopoietic transcription factor contributes to skeletal development in vivo, J. Cell. Physiol. 196 (2003) 301–311.
[63] K.M. Osorio, K.C. Lilja, T. Tumbar, Runx1 modulates adult hair follicle stem cell emergence and maintenance from distinct embryonic skin compartments, J. Cell Biol. 193 (2011) 235–250.
[64] M.P. van Bragt, X. Hu, Y. Xie, Z. Li, RUNX1, a transcription factor mutated in breast cancer, controls the fate of ER-positive mammary luminal cells, Elife 3 (2014), e03881.
[65] Y. Yang, J. Wei, J. Li, Y. Cui, X. Zhou, J. Xie, Lipid metabolism in cartilage and its diseases: a concise review of the research progress, Acta Biochim. Biophys. Sin. 53 (2021) 517–527.
[66] B.K. Hall, T. Miyake, All for one and one for all: condensations and the initiation of skeletal development, Bioessays 22 (2000) 138–147.
[67] A. Kimura, H. Inose, F. Yano, K. Fujita, T. Ikeda, S. Sato, M. Iwasaki, T. Jinno, K. Ae, S. Fukumoto, Y. Takeuchi, H. Itoh, T. Imamura, H. Kawaguchi, U.I. Chung, J.F. Martin, S. Iseki, K. Shinomiya, S. Takeda, Runx1 and Runx2 cooperate during sternal morphogenesis, Development 137 (2010) 1159–1167.
[68] D. Nurminsky, S. Shanmugasundaram, S. Deasey, C. Michaud, S. Allen, D. Hendig, A. Dastjerdi, P. Francis-West, M. Nurminskaya, Transglutaminase 2 regulates early chondrogenesis and glycosaminoglycan synthesis, Mech. Dev. 128 (2011) 234–245.
[69] J. Bernhard, J. Ferguson, B. Rieder, P. Heimel, T. Nau, S. Tangl, H. Redl, G. Vunjak-Novakovic, Tissue-engineered hypertrophic chondrocyte grafts enhanced long bone repair, Biomaterials 139 (2017) 202–212.
[70] C.Y. Tang, W. Chen, Y. Luo, J. Wu, Y. Zhang, A. McVicar, M. McConnell, Y. Liu, H.D. Zhou, Y.P. Li, Runx1 up-regulates chondrocyte to osteoblast lineage commitment and promotes bone formation by enhancing both chondrogenesis and osteogenesis, Biochem. J. 477 (2020) 2421–2438.
[71] T. Yamashiro, X.P. Wang, Z. Li, S. Oya, T. Aberg, T. Fukunaga, H. Kamioka, N.A. Speck, T. Takano-Yamamoto, I. Thesleff, Possible roles of Runx1 and Sox9 in incipient intramembranous ossification, J. Bone Miner. Res. 19 (2004) 1671–1677.
[72] C.Y. Tang, M. Wu, D. Zhao, D. Edwards, A. McVicar, Y. Luo, G. Zhu, Y. Wang, H.D. Zhou, W. Chen, Y.P. Li, Runx1 is a central regulator of osteogenesis for bone homeostasis by orchestrating BMP and WNT signaling pathways, PLoS Genet. 17 (2021), e1009233.
[73] Y. Shintaku, T. Murakami, T. Yanagita, N. Kawanabe, T. Fukunaga, K. Matsuzaki, S. Uematsu, Y. Yoshida, H. Kamioka, T. Takano-Yamamoto, K. Takada, T. Yamashiro, Sox9 expression during fracture repair, Cells Tissues Organs 194 (2011) 38–48.
[74] D.N. Paglia, M.E. Diaz-Hernandez, J.L. Roberts, J. Kalinowski, J. Lorenzo, H. Drissi, Deletion of Runx1 in osteoclasts impairs murine fracture healing through progressive woven bone loss and delayed cartilage remodeling, J. Orthop. Res. 38 (2020) 1007–1015.
[75] S.K. Sharma, S.L. Yadav, U. Singh, S. Wadhwa, Muscle activation profiles and co-activation of quadriceps and hamstring muscles around knee joint in indian primary osteoarthritis knee patients, J. Clin. Diagn. Res. 11 (2017) RC09–RC14.
[76] S. Takagi, G. Omori, H. Koga, K. Endo, Y. Koga, A. Nawata, N. Endo, Quadriceps muscle weakness is related to increased risk of radiographic knee OA but not its progression in both women and men: the Matsudai Knee Osteoarthritis Survey, Knee Surg. Sports Traumatol. Arthrosc. 26 (2018) 2607–2614.
[77] Y. Liu, M. Duan, D. Guo, S. Kan, L. Zhang, M. Aili, D. Zhang, W. Du, J. Xie, PDGF-AA promotes cell-to-cell communication in osteocytes through PI3K/Akt signaling pathway, Acta Biochim. Biophys. Sin. 53 (2021) 1640–1649.
[78] J. Wei, Y. Yang, D. Guo, S. Xu, H. Huang, D. Zhang, J. Xie, X. Zhou, Osteoblasts induce glucose-derived ATP perturbations in chondrocytes through noncontact communication, Acta Biochim. Biophys. Sin. 54 (2022) 625–636.
[79] I.Y. Shadrin, A. Khodabukus, N. Bursac, Striated muscle function, regeneration, and repair, Cell. Mol. Life Sci. 73 (2016) 4175–4202.
[80] T.L. Russo, S.M. Peviani, J.L. Durigan, D. Gigo-Benato, G.B. Delfino, T.F. Salvini, Stretching and electrical stimulation reduce the accumulation of MyoD, myostatin and atrogin-1 in denervated rat skeletal muscle, J. Muscle Res. Cell Motil. 31 (2010) 45–57.
[81] X. Wang, C. Blagden, J. Fan, S.J. Nowak, I. Taniuchi, D.R. Littman, S.J. Burden, Runx1 prevents wasting, myofibrillar disorganization, and autophagy of skeletal muscle, Genes Dev. 19 (2005) 1715–1722.
[82] M. Bao, S. Liu, X.Y. Yu, C. Wu, Q. Chen, H. Ding, C. Shen, B. Wang, S. Wang, Y.H. Song, Y. Li, Runx1 promotes satellite cell proliferation during ischemia - induced muscle regeneration, Biochem. Biophys. Res. Commun. 503 (2018) 2993–2997.
[83] F.W. Roemer, A. Guermazi, D.T. Felson, J. Niu, M.C. Nevitt, M.D. Crema, J.A. Lynch, C.E. Lewis, J. Torner, Y. Zhang, Presence of MRI-detected joint effusion and synovitis increases the risk of cartilage loss in knees without osteoarthritis at 30-month follow-up: the MOST study, Ann. Rheum. Dis. 70 (2011) 1804–1809.
[84] X. Zhao, F. Meng, S. Hu, Z. Yang, H. Huang, R. Pang, X. Wen, Y. Kang, Z. Zhang, The synovium attenuates cartilage degeneration in KOA through activation of the Smad2/3-Runx1 cascade and chondrogenesis-related miRNAs, Mol. Ther. Nucleic Acids 22 (2020) 832–845.
[85] F.J. Blanco, C. Ruiz-Romero, New targets for disease modifying osteoarthritis drugs: chondrogenesis and Runx1, Ann. Rheum. Dis. 72 (2013) 631–634.
[86] K. Johnson, S. Zhu, M.S. Tremblay, J.N. Payette, J. Wang, L.C. Bouchez, S. Meeusen, A. Althage, C.Y. Cho, X. Wu, P.G. Schultz, A stem cell-based approach to cartilage repair, Science 336 (2012) 717–721.
[87] H. Aini, K. Itaka, A. Fujisawa, H. Uchida, S. Uchida, S. Fukushima, K. Kataoka, T. Saito, U.I. Chung, S. Ohba, Messenger RNA delivery of a cartilage-anabolic transcription factor as a disease-modifying strategy for osteoarthritis treatment, Sci. Rep. 6 (2016) 18743.
[88] Y. Soung do, L. Talebian, C.J. Matheny, R. Guzzo, M.E. Speck, J.R. Lieberman, N.A. Speck, H. Drissi, Runx1 dose-dependently regulates endochondral ossification during skeletal development and fracture healing, J. Bone Miner. Res. 27 (2012) 1585–1597.
[89] G. Huang, K. Shigesada, K. Ito, H.J. Wee, T. Yokomizo, Y. Ito, Dimerization with PEBP2beta protects RUNX1/AML1 from ubiquitin-proteasome-mediated degradation, EMBO J. 20 (2001) 723–733.
[90] T.H. Tahirov, T. Inoue-Bungo, H. Morii, A. Fujikawa, M. Sasaki, K. Kimura, M. Shiina, K. Sato, T. Kumasaka, M. Yamamoto, S. Ishii, K. Ogata, Structural analyses of DNA recognition by the AML1/Runx-1 Runt domain and its allosteric control by CBFbeta, Cell 104 (2001) 755–767.
[91] A. Beghini, Core binding factor leukemia: chromatin remodeling moves towards oncogenic transcription, Cancers 11 (2019) 1973.
[92] X. Huang, J.W. Peng, N.A. Speck, J.H. Bushweller, Solution structure of core binding factor beta and map of the CBF alpha binding site, Nat. Struct. Biol. 6 (1999) 624–627.
[93] Q. Jiang, X. Qin, T. Kawane, H. Komori, Y. Matsuo, I. Taniuchi, K. Ito, S. Izumi, T. Komori, Cbfb2 isoform dominates more potent Cbfb1 and is required for skeletal development, J. Bone Miner. Res. 31 (2016) 1391–1404.
[94] X. Qin, Q. Jiang, Y. Matsuo, T. Kawane, H. Komori, T. Moriishi, I. Taniuchi, K. Ito, Y. Kawai, S. Rokutanda, S. Izumi, T. Komori, Cbfb regulates bone development by stabilizing Runx family proteins, J. Bone Miner. Res. 30 (2015) 706–714.
[95] F. Tian, M. Wu, L. Deng, G. Zhu, J. Ma, B. Gao, L. Wang, Y.P. Li, W. Chen, Core binding factor beta (Cbfβ) controls the balance of chondrocyte proliferation and differentiation by upregulating Indian hedgehog (Ihh) expression and inhibiting parathyroid hormone-related protein receptor (PPR) expression in postnatal cartilage and bone formation, J. Bone Miner. Res. 29 (2014) 1564–1574.
[96] M. Wu, C. Li, G. Zhu, Y. Wang, J. Jules, Y. Lu, M. McConnell, Y.J. Wang, J.Z. Shao, Y.P. Li, W. Chen, Deletion of core-binding factor β (Cbfβ) in mesenchymal progenitor cells provides new insights into Cbfβ/Runxs complex function in cartilage and bone development, Bone 65 (2014) 49–59.
[97] M. Wu, Y.P. Li, G. Zhu, Y. Lu, Y. Wang, J. Jules, M. McConnell, R. Serra, J.Z. Shao, W. Chen, Chondrocyte-specific knockout of Cbfβ reveals the indispensable function of Cbfβ in chondrocyte maturation, growth plate development and trabecular bone formation in mice, Int. J. Biol. Sci. 10 (2014) 861–872.
[98] N.R. Park, K.E. Lim, M.S. Han, X. Che, C.Y. Park, J.E. Kim, I. Taniuchi, S.C. Bae, J.Y. Choi, Core binding factor β plays a critical role during chondrocyte differentiation, J. Cell. Physiol. 231 (2016) 162–171.
[99] D. Levanon, Y. Groner, Structure and regulated expression of mammalian RUNX genes, Oncogene 23 (2004) 4211–4219.
[100] S. Stricker, R. Fundele, A. Vortkamp, S. Mundlos, Role of Runx genes in chondrocyte differentiation, Dev. Biol. 245 (2002) 95–108.
[101] S. Sato, A. Kimura, J. Ozdemir, Y. Asou, M. Miyazaki, T. Jinno, K. Ae, X. Liu, M. Osaki, Y. Takeuchi, S. Fukumoto, H. Kawaguchi, H. Haro, K. Shinomiya, G. Karsenty, S. Takeda, The distinct role of the Runx proteins in chondrocyte

differentiation and intervertebral disc degeneration: findings in murine models and in human disease, Arthritis Rheum. 58 (2008) 2764–2775.
[102] C.A. Yoshida, H. Yamamoto, T. Fujita, T. Furuichi, K. Ito, K. Inoue, K. Yamana, A. Zanma, K. Takada, Y. Ito, T. Komori, Runx2 and Runx3 are essential for chondrocyte maturation, and Runx2 regulates limb growth through induction of Indian hedgehog, Genes Dev. 18 (2004) 952–963.
[103] C.G. Chen, D. Thuillier, E.N. Chin, T. Alliston, Chondrocyte-intrinsic Smad3 represses Runx2-inducible matrix metalloproteinase 13 expression to maintain articular cartilage and prevent osteoarthritis, Arthritis Rheum. 64 (2012) 3278–3289.
[104] T. Komori, Regulation of skeletal development by the Runx family of transcription factors, J. Cell. Biochem. 95 (2005) 445–453.
[105] M. van der Deen, J. Akech, D. Lapointe, S. Gupta, D.W. Young, M.A. Montecino, M. Galindo, J.B. Lian, J.L. Stein, G.S. Stein, A.J. van Wijnen, Genomic promoter occupancy of runt-related transcription factor RUNX2 in Osteosarcoma cells identifies genes involved in cell adhesion and motility, J. Biol. Chem. 287 (2012) 4503–4517.
[106] J. Wang, Y. Cao, B. Qiu, J. Du, T. Wang, C. Wang, R. Deng, X. Shi, K. Gao, Z. Xie, W. Yong, Ablation of protein phosphatase 5 (PP5) leads to enhanced both bone and cartilage development in mice, Cell Death Dis. 9 (2018) 214.
[107] H.L. Zhang, B. Guo, Z.Q. Yang, C.C. Duan, S. Geng, K. Wang, H.F. Yu, Z.P. Yue, ATRA signaling regulates the expression of COL9A1 through BMP2-WNT4-RUNX1 pathway in antler chondrocytes, J. Exp. Zool. B Mol. Dev. Evol. 328 (2017) 575–586.
[108] H.L. Zhang, Z.Q. Yang, C.C. Duan, S. Geng, K. Wang, H.F. Yu, Z.P. Yue, B. Guo, WNT4 acts downstream of BMP2 to mediate the regulation of ATRA signaling on RUNX1 expression: implications for terminal differentiation of antler chondrocytes, J. Cell. Physiol. 233 (2018) 1129–1145.
[109] Z.Q. Yang, H.L. Zhang, C.C. Duan, S. Geng, K. Wang, H.F. Yu, Z.P. Yue, B. Guo, IGF1 regulates RUNX1 expression via IRS1/2: implications for antler chondrocyte differentiation, Cell Cycle 16 (2017) 522–532.
[110] D. Chen, M. Zhao, G.R. Mundy, Bone morphogenetic proteins, Growth Factors 22 (2004) 233–241.
[111] B.L. Hogan, Bone morphogenetic proteins: multifunctional regulators of vertebrate development, Genes Dev. 10 (1996) 1580–1594.
[112] H.H. Luu, W.X. Song, X. Luo, D. Manning, J. Luo, Z.L. Deng, K.A. Sharff, A.G. Montag, R.C. Haydon, T.C. He, Distinct roles of bone morphogenetic proteins in osteogenic differentiation of mesenchymal stem cells, J. Orthop. Res. 25 (2007) 665–677.
[113] S.S. Hakki, B. Bozkurt, E.E. Hakki, S.A. Kayis, G. Turac, I. Yilmaz, E. Karaoz, Bone morphogenetic protein-2, -6, and -7 differently regulate osteogenic differentiation of human periodontal ligament stem cells, J. Biomed. Mater. Res. B Appl. Biomater. 102 (2014) 119–130.
[114] C. Ji, X. Liu, L. Xu, T. Yu, C. Dong, J. Luo, RUNX1 plays an important role in mediating BMP9-induced osteogenic differentiation of mesenchymal stem cells line C3H10T1/2, murine multi-lineage cells lines C2C12 and MEFs, Int. J. Mol. Sci. 18 (2017) 1348.
[115] J. Shen, J. Li, B. Wang, H. Jin, M. Wang, Y. Zhang, Y. Yang, H.J. Im, R. O'Keefe, D. Chen, Deletion of the transforming growth factor β receptor type II gene in articular chondrocytes leads to a progressive osteoarthritis-like phenotype in mice, Arthritis Rheum. 65 (2013) 3107–3119.
[116] J. Wang, X. Wang, J.D. Holz, T. Rutkowski, Y. Wang, Z. Zhu, Y. Dong, Runx1 is critical for PTH-induced onset of mesenchymal progenitor cell chondrogenic differentiation, PLoS One 8 (2013), e74255.
[117] H. Akazawa, I. Komuro, Y. Sugitani, Y. Yazaki, R. Nagai, T. Noda, Targeted disruption of the homeobox transcription factor Bapx1 results in lethal skeletal dysplasia with asplenia and gastroduodenal malformation, Gene Cell. 5 (2000) 499–513.
[118] J.H. Bushweller, Targeting transcription factors in cancer-from undruggable to reality, Nat. Rev. Cancer 19 (2019) 611–624.
[119] A. Illendula, J.A. Pulikkan, H. Zong, J. Grembecka, L. Xue, S. Sen, Y. Zhou, A. Boulton, A. Kuntimaddi, Y. Gao, R.A. Rajewski, M.L. Guzman, L.H. Castilla, J.H. Bushweller, Chemical biology. A small-molecule inhibitor of the aberrant transcriptionfactor CBFβ-SMMHC delays leukemia in mice, Science 347 (2015) 779–784.
[120] F.F. Ferrara, F. Fazi, A. Bianchini, F. Padula, V. Gelmetti, S. Minucci, M. Mancini, P.G. Pelicci, F. Lo Coco, C. Nervi, Histone deacetylase-targeted treatment restoresretinoic acid signaling and differentiation in acute myeloid leukemia, Cancer Res. 61 (2001) 2–7.
[121] C. Zhou, Y. Cui, Y. Yang, D. Guo, D. Zhang, Y. Fan, X. Li, J. Zou, J. Xie, Runx1 protects against the pathological progression of osteoarthritis, Bone Res 9 (2021) 50.