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REVIEW

Mechanisms of synovial joint and articular cartilage development

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Abstract

Articular cartilage is formed at the end of epiphyses in the synovial joint cavity and permanently contributes to the smooth movement of synovial joints. Most skeletal elements develop from transient cartilage by a biological process known as endochondral ossification. Accumulating evidence indicates that articular and growth plate cartilage are derived from different cell sources and that different molecules and signaling pathways regulate these two kinds of cartilage. As the first sign of joint development, the interzone emerges at the presumptive joint site within a pre-cartilage tissue. After that, joint cavitation occurs in the center of the interzone, and the cells in the interzone and its surroundings gradually form articular cartilage and the synovial joint. During joint development, the interzone cells continuously migrate out to the epiphyseal cartilage and the surrounding cells influx into the joint region. These complicated phenomena are regulated by various molecules and signaling pathways, including GDF5, Wnt, IHH, PTHrP, BMP, TGF-β, and FGF. Here, we summarize current literature and discuss the molecular mechanisms underlying joint formation and articular development.

Keywords Articular cartilage · Joint · Interzone · Chondrocyte

Abbreviations

BMP	Bone morphogenic protein
CDMP1	Cartilage-derived morphogenetic protein 1
cKO	Conditional knockout
FGF	Fibroblast growth factor
FGFR3	Fibroblast growth factor receptor 3
GAG	Glycosaminoglycan
GDF5	Growth differentiation factor 5
IHH	Indian hedgehog
MAPK	Mitogen-activated protein kinase
NICD	Notch intracellular domain
PTHrP	Parathyroid hormone-related protein
Prg4	Progeoglycan 4
SFZ	Superficial zone
TGF-β	Transforming growth factor-β
Tgfbr2	TGF-β type II receptor
UDPGD	Uridine diphosphoglucose dehydrogenase

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Introduction

Osteoarthritis, a representative degenerative joint disease, is threatening the quality of life and daily activities of many elderly people. Many studies have been performed to understand its pathophysiology and develop disease-modifying drugs over decades. However, neither of these aims have yet been successfully achieved. However, an increasing number of developmental biology studies have revealed various kinds of molecules and signaling pathways involved in skeleton formation. In particular, achievements of cell biology and regenerative medicine research have enabled the induction of chondrocytes from pluripotent and somatic stem cells in vitro [1–7]. Moreover, recent studies indicate that chondrocytes are generated through several different biological steps according to their localization during skeleton formation [8, 9].

Articular cartilage is a highly specialized tissue that anatomically caps the end of epiphyses in the synovial joint cavity. Matured articular cartilage is also referred to as hyaline cartilage because of its translucent appearance that reflects its unique constituents, such as type II collagen, glycosaminoglycans (GAGs), and low cellularity [10]. In addition, articular cartilage does not have blood vessels, lymphatic vessels, or nerves [10]. Articular chondrocytes produce extracellular matrices and maintain their environment with

very little or no cell turnover [11, 12]. GAGs, including chondroitin sulfate and hyaluronic acid, bind to each other via core proteins in cooperation with longitudinally orientated type II collagen [10, 13]. These protein complexes further form the intricate network of extracellular matrices, which are responsible for the distribution and absorption of mechanical forces loaded on the articular cartilage [14, 15]. Another structural feature of articular cartilage is the lubricated smooth surface composed of lubricin and horizontally oriented collagens, which attenuates friction generated through skeletal motion [15, 16]. Thus, articular cartilage consists of multiple layers that have differently oriented matrices and cell populations.

In vertebrate animals, most skeletal elements develop from transient cartilage by a biological process well known as endochondral ossification [17–19]. In the initial step, cartilage anlagen is formed through mesenchymal condensation at the presumptive site for bone. Cartilage anlagen grow to form a cartilage template, accompanied by chondrocyte proliferation and differentiation, which has a similar shape to future bones. At the center of the cartilage, chondrocytes undergo hypertrophic differentiation and then apoptosis, resulting in vascular invasion and ossification by osteoblasts. This sequential event longitudinally spreads to metaphysis. Later, another ossification site, termed as the secondary ossification center, is newly formed at the epiphysis and radially spreads within it. Part of the cartilage between the two ossification centers remains as a growth plate physis during skeletal growth, and other parts between the joint cavity and the secondary ossification center permanently remain as articular cartilage. Accumulating evidence indicates that articular and growth plate cartilage is derived from different cell sources and that different molecules and signaling pathways regulate these two kinds of cartilage [8, 9]. In this review, we introduce several crucial factors defining the inception of joint generation and development of articular cartilage.

Early stages of articular cartilage differentiation

Interzone emergence

The development of the synovial joint precedes articular cartilage formation. The timing of joint development depends on its site: forelimb and proximal joint formation generally precede hindlimb and distal joint formation, respectively [20–22]. Mice nascent limb joints are observed at around E12.5–E15.5, whereas articular cartilage is identified after birth [19]. The substantial morphological appearance of articular cartilage is observed in 2–4-week-old mice [12, 23, 24] and approximately 1-month-old rabbits [25].

Notably, most parts of articular cartilage derive from different lineages from the growth plate cartilage. The first signs of joint development are presented by the appearance of condensed flattened cells at the presumptive joint site within a pre-cartilage tissue [26, 27] known as the interzone, the origin of the joint (Fig. 1). Removal of the interzone from a chick embryo leads to an uninterrupted long bone lacking joints [28], indicating that the interzone provides segmentation of skeletal elements in limbs. The interzone arises from mesenchymal/pre-cartilaginous tissue in which the cells initially express chondrocyte marker genes such as type II collagen, aggrecan, and matrillin-1 [11, 12, 24, 29–32]. Instead of the decreased expression of these chondrogenic markers, the interzone cells acquire the expression of growth differentiation factor 5 (*Gdf5*), formerly known as bone morphogenetic protein 14 (*BMP14*), or cartilage-derived morphogenetic protein 1 (*CDMP1*). Gdf5 is a representative marker for the interzone during early joint development [24, 29, 30, 33]. In addition to *Gdf5*, *Wnt4*, *Wnt9a* (formerly known as *Wnt14*), *Wnt16*, *Erg*, doublecortin, and *Gli* are also expressed in the interzone [34].

Joint cavitation

Joint cavitation is one of the most remarkable events specific to synovial joints and is a necessary step toward articular cartilage development (Fig. 1). Joint cavities are identified at around the same time as hypertrophic chondrocytes are observed in the center of adjacent cartilage templates. Previous studies have proposed that the cavity is generated through the apoptosis of the cells in the center of the interzone termed as the intermediate zone [35–38]. However, cell death is sparsely observed within a thin intermediate zone [36, 37]. Instead, recent literature suggests that the joint cavity develops by the filling of the fluidic extracellular matrix, particularly hyaluronan [11, 24, 31, 39–41]. Hyaluronan synthases, hyaluronan binding proteins, and the activity of uridine diphosphoglucose dehydrogenase (UDPGD) were specifically up-regulated at the intermediate zone before and during the detachment of cell–cell adhesion [11, 24, 31, 39, 40]. Indeed, mutant mice for hyaluronan synthetase 2 exhibit severe deformity of the joints [41]. These events are possibly regulated by mitogen-activated protein kinase (MAPK) signaling including p38 and Erk1/2, which are activated at the intermediate zone before the expression of hyaluronan related factors, and directly stimulate hyaluronan synthesis in the interzone cells in vitro [42, 43]. An extrinsic mechanical stimulus may be a potent candidate for an upstream of these pathways. Skeletal muscle paralysis in chick embryos causes joint cavitation failure [44–46]. While interzone generation and *Gdf5* expression were not altered in this model [47], the activation of MAPK signaling and hyaluronan synthesis were decreased at the intermediate zone [42, 48].

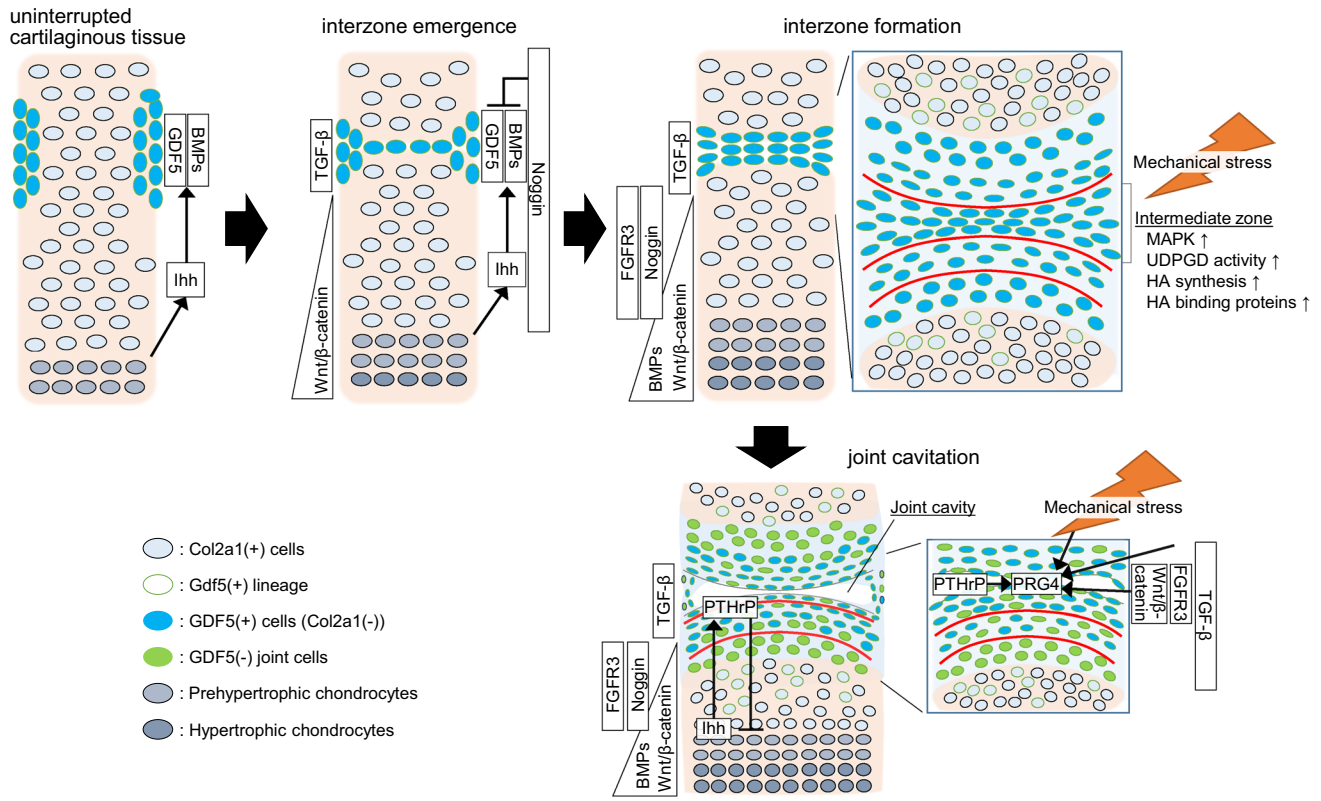

Fig. 1 Joint formation and articular cartilage development in the early stage. The interzone emerges at the presumptive joint site within a pre-cartilage tissue. After that, joint cavitation occurs in the center of the interzone, and the cells in the interzone and its surroundings gradually form articular cartilage and synovial joints. During joint development, the interzone cells continuously migrate to the epiphyseal cartilage and the surrounding cells influx into the joint region. The width of each box indicates the area, where a particular molecule is expressed. *GDF5* growth differentiation factor 5, *BMP* bone morphogenetic protein, *Ihh* Indian hedgehog, *FGFR* fibroblast growth factor receptor, *TGF-β* transforming growth factor-β, *MAPK* mitogen-activated protein kinase, *UDPGD* uridine diphosphoglucose dehydrogenase, *HA* hyaluronic acid, *PTHrP* parathyroid hormone-related protein, *PRG4* proteoglycan 4, *Col2a1* type II collagen, (+) positive, (−) negative

Similar or severer phenotypes are observed in muscle-less mice (known as splotched-delay mutants) [49, 50]. Rolfe et al. carried out microarray analyses of muscle-less mice and showed associations of molecule signaling pathways including the transforming growth factor (TGF)-β superfamily, fibroblast growth factors (FGFs), hedgehogs, and *Wnt* [50]. Although little is known about how mechanical stimuli are transduced to intracellular signals, these findings indicate that movement, myogenesis, and muscle contraction play essential roles in cavitation and the healthy development of joints through these representative signaling pathways.

Regulators in the early stages

Growth differentiation factor 5

GDF5, a member of the TGF-β superfamily, was first identified as the gene responsible for brachypodism in mice that show altered skeletal morphology, in particular in distal joints [51]. Mutations of the human *GDF5* gene causes skeletal malformations including brachydactyly [52] and chondrodysplasia [53–55], and mutations of its receptor BMPR1B also cause brachydactyly [56, 57]. These outcomes suggest that GDF5 is essential for healthy joint development. In the early stages, *Gdf5* mRNA is faintly detected surrounding the pre-cartilage area, then restricted within the interzone with reinforced expression [35, 58–60]. In mice with brachypodism, *Gdf5* is strongly expressed throughout the cartilage anlagen, out of the interzone, which results in the fusion of digit joints [59]. These data suggest the role of *Gdf5* in the generation and maintenance of the interzone. While *Gdf5* is detected in most synovial joints of limbs, some proximal joints like elbow and knee joints are not fused in brachypodism mice [61]. This eventuality is likely due to compensation by *Gdf6*, another member of the GDF family which is dominantly expressed in proximal joints [62]. Indeed, double mutations of *Gdf5* and *Gdf6* cause severe joint deformity which is not observed in each mutant [63, 64].

Several studies using *Gdf5-Cre* transgenic mice have shown that *Gdf5*-expressing cell lineage gives rise to all mature joint structures including articular cartilage, meniscus, ligaments, and synovium [65]. Therefore, interzone cells have been considered as progenitors for joints over the decades. Interestingly, the *Gdf5*-lineage progeny cells are also detected in subchondral bone as osteoblasts and osteocytes [9, 66], while *Gdf5* expression is gradually down-regulated during joint development and diminished until birth [35, 36, 58]. Tsumaki et al. reported that *Col11a2-Gdf5* transgenic mice, which continuously express *Gdf5* in chondrocytes, display joint fusion with cartilage hyperplasia [67], as shown in brachypodism mice. Taken together with the transient expression of *Gdf5*, cell tracking based on the *Gdf5-Cre* system may not reflect the substantial fates of the interzone progeny. Recently, *Gdf5-Cre*^{ERT2} and Cre-dependent reporter mice, in which *Gdf5*-positive ((+)) cells can be labeled by tamoxifen administration, have provided novel findings [9, 12, 33]. Shwartz et al. demonstrated the embryonic stage-specific localization of *Gdf5* (+) cells and their fate from E10.5 to E18.5 [33]. *Gdf5* (+) cells continuously influx into the joint region from the surrounding tissues, and contrarily, the interzone/early joint cells migrate out to the epiphyseal cartilage losing *Gdf5* expression [33]. Surprisingly, most cells that initially form the interzone do not give rise to articular cartilage but to transient cartilage, ligaments, and meniscus [33]. Decker et al. demonstrated the spatiotemporal distribution of *Gdf5* (+) cells up to adulthood, and also observed the reciprocal cell migration between the interzone and the surrounding tissues [9, 12], as well as the previous report by Shwartz et al. [33]. It is now accepted that joint components are formed by the integration of peripheral cells in joint development. Epiphyseal chondrocytes migrate into the interzone at early stages [68], and the external regions of joints such as the synovium/joint capsule [69] and outer parts of the meniscus [30] are mainly composed of lately integrated cells. Thus, the fate of embryonic interzone cells, the surrounding cells, and their progeny cells may be determined by their spatiotemporally environment.

Although *Gdf5* signaling has chondrogenic effects in vivo [67] and in vitro [58, 70–72], *Gdf5* is unlikely to contribute directly to articular cartilage development, because the diminishment of *Gdf5* expression is significant before articular cartilage appearance and some joints in brachypodism mice have normal articular cartilage [33, 61]. In adulthood, *Gdf5* may be associated with homeostasis of the articular cartilage, because genome-wide association studies have revealed that GDF5 is one of the susceptible genes for osteoarthritis [73–75]; however, its role in adult articular cartilage remains unclear.

Wnt signaling

Wnt4, *Wnt9a*, and *Wnt16* are expressed in the interzone and flanking areas at the early stages, before or simultaneously with *Gdf5* expression in the interzone [31, 37, 49, 60, 76, 77]. The canonical Wnt signaling potently suppresses chondrogenesis of the limb bud mesenchymal cells in vitro [60, 70, 77, 78]. Several gain-of-function studies suggest that the canonical Wnt signaling provides cells with the interzone phenotype upstream of *Gdf5* by suppressing chondrogenesis [60, 76]. Meanwhile, loss-of-function studies indicate that joint development is achieved in Wnt ligands knockout mice [77–79]. Spater et al. report that the deletion of *Wnt9a* does not affect the expression of joint markers, but causes ectopic chondrogenesis-like synovial chondromatosis in some joints, which is enhanced by the additional deletion of *Wnt4* [77, 78]. The conditional knockout (cKO) of β-catenin using *Col2a1-Cre* or *Gdf5-Cre* slightly affects joint development [70, 80–82], while Guo et al. report the fusion of the wrist or knee in cKO using *Col2a1-Cre* or *Dermo1-Cre*, respectively [76]. These data should be carefully discussed, because alteration of the canonical Wnt signaling itself impairs chondrocyte differentiation and endochondral ossification [23, 77–79, 83].

Actual activity of the canonical Wnt signaling can be monitored using TOPGAL, a reporter containing a β-galactosidase gene under the control of LEF/TCF and β-catenin inducible promoter [84]. Yamagami et al. demonstrate that signaling activity is not detected in the interzone of the elbow and shoulder at E12.5–E14.5, whereas *Wnt4* transcripts are detected there [85]. Kahn et al. show that *Wnt4* and *Wnt9a* are predominant in the interzone of the elbow at E13.5, but TOPGAL activity is obscure [49]. The strong TOPGAL signal is observed in the cartilage anlagen at this stage, rather than in the interzone [49, 70, 85]. Although it is still controversial, WNT4 possibly inhibits canonical Wnt signaling [86, 87]. The role of Wnt in joint development is conflicting amongst reports, because Wnt ligand expression, their target cells, and their signaling pathways are complicated.

Besides these effects in the early stages of joint development, canonical Wnt signaling plays another role in specifying the superficial zone (SFZ) of the articular cartilage during the late phase. The SFZ is responsible for joint lubrication by producing lubricin (encoded by the Proteoglycan 4 (*Prg4*) gene) [88]. Koyama et al. report that flattened cells in the SFZ disappear and *Prg4* expression is decreased in β-catenin cKO mice in *Col2a1* (+) or *Gdf5* (+) lineages [70]. Yasuhara et al. also showed a reduced number of SFZ cells and Prg4 expression in the articular cartilage of 7-week-old *Col2a1-Cre*^{ERT}*;β-catenin*$^{fl/fl}$ mice which received tamoxifen administration at P7 [81]. The articular cartilage of the cKO mice has no stratified structure and is homogenously

composed of chondrocyte-like round cells [81]. Yamagami et al. monitored TOPGAL activity in joints during development up to adulthood [85]. After joint cavitation, surface cells facing the cavity exhibit strong signal activity, which is observed until P7. The LacZ-positive cells are reduced in the articular and epiphyseal cartilage at P10 and eventually, are rarely found there at P50. Taken together, canonical Wnt signaling is less essential for the interzone/early joint development but orchestrates the integrity of joint formation through anti-chondrogenic effects in the SFZ of the articular cartilage.

Indian hedgehog—parathyroid hormone-related protein

Cartilage templates and early joints influence each other via paracrine signaling. Indian hedgehog (IHH) and parathyroid hormone-related protein (PTHrP) signaling have been intensively studied. IHH is widely involved in both the proliferation and differentiation of chondrocytes during endochondral development [89–91]. IHH is produced from pre-hypertrophic chondrocytes and up-regulates PTHrP expression in peri-articular chondrocytes [92, 93]. PTHrP inhibits the differentiation of proliferating chondrocytes into pre-hypertrophic chondrocytes [94–96]. This feedback loop determines the length of long bones [97]. Before the feedback loop, pre-hypertrophic chondrocytes around the center of anlagen are associated with interzone generation through the secretion of IHH. The loss of IHH causes not only dwarfism but also joint fusion in distal limb joints [89, 98]. In *Ihh* deficient mice, the interzone is absent or markedly hypoplastic [98]. *Gdf5* (+) cells are observed at prospective joint sites in mutants, but they flank and surround uninterrupted joint sites [98]. The authors conclude that Ihh is indispensable for the recruitment and immigration of flanking cells into the interzone [99].

Ihh expression is initially detected in the ossification center, where cells first have pre-hypertrophic phenotypes and then undergo the endochondral ossification process. Although *Ihh* itself is not expressed in the presumptive joint sites [78, 89], Patched-1, a receptor of the IHH ligand, has been detected around the interzone and cartilage anlagen [78, 89, 100]. *Gli1* and *Gli3*, major downstream transcription factors of IHH signaling, are expressed around the interzones and joints in the early stages [57, 59, 80, 98, 100, 101]. *Gli3* knockout mice exhibit a malformation of phalanges and irregular joint shapes [101, 102]. Thus, the IHH signaling pathway regulates joint morphogenesis [103], and the cartilage anlagen contribute to development of the interzones and joints through secretion of IHH.

In addition to these spatial features of IHH-related molecules, the regulation of joint formation by IHH signaling is probably transient, since Patched-1 expression around the interzone is observed only from E12.5 to E13.5 in the elbow joints [89]. When IHH signaling is continuously activated in chondrocytes, *Gdf5* (+) cells do not migrate into the interzone and joints are fused [80]. Furthermore, excessive IHH signaling activity in the interzone progeny induces ectopic cartilage formation in the knee [104]. Notably, joint morphology is not changed even when IHH signaling is disrupted in the interzone progeny (*Gdf5-Cre;Smo*$^{fl/fl}$) [104], contrary to the severe deformity of forelimb joints in *Col2a1-Cre;Smo*$^{fl/fl}$ [91]. Zhou et al. demonstrate that *Ihh* deletion in adult articular cartilage does not alter joint phenotype. Instead, it attenuates osteoarthritis progression [105]. Other studies have also shown the association of IHH with osteoarthritis [106–108]. Taken together, the data suggest that IHH signaling is possibly less essential after interzone specification during joint development.

Unlike in endochondral ossification, IHH and PTHrP seem to be independent in interzone generation and joint development. The genetic alteration of PTHrP causes the impairment of endochondral ossification, but no severe changes in joints [94–96, 109, 110]. Even after IHH signaling becomes silent, PTHrP-expressing cells exist in articular cartilage over a lifetime [110–112]. Recombinant human PTH (1–34) suppresses osteoarthritis development [113], and PTH/PTHrP signaling induces lubricin [114]. PTHrP possibly contributes to the postnatal development and homeostasis of articular cartilage.

Bone morphogenic protein signaling—Noggin

BMP signaling plays a central role in both chondrogenesis and osteogenesis [115]. Mice lacking Smad1, 5, and 8 canonical mediators of BMPs display severe chondrodysplasia both in appendicular and vertebral skeletons [116]. In addition to *Gdf5*, *Bmp2* and *Bmp4* are expressed in the interzone [34, 38, 65, 80, 117]. Although it has not been fully revealed, BMP and IHH signaling possibly regulate each other in the early stages [80, 89, 91, 116, 118]. Once the interzone is specialized, BMP signaling is negatively regulated. The phosphorylation of Smads are not observed in the presumptive joint site before cavitation, unlike in the adjacent epiphyseal cartilage [68, 119, 120]. Noggin and chordin, the BMP antagonists, contribute to this process. They directly bind to BMP2 [121], BMP4 [122], and GDF5 [35]. The expression pattern of these antagonists depends on the location of joints and species [31, 118, 123]. The mRNA of noggin is detected in the cartilage anlagen and temporally in the interzone [31, 37, 38, 69, 117, 124]. Chondrocyte-specific noggin transgenic mice display a marked impairment of skeleton formation [67], and noggin knockout causes the remarkable hyperplasia of cartilage templates lacking in articular cartilage [125, 126]. Similar phenotypes are observed in *Col2a1-Cre; Noggin*$^{fl/fl}$ mice or

noggin antibody-injected chick embryos [68]. In humans, missense mutations of GDF5 cause synostosis, because the GDF5 mutants become insensitive to noggin [127]. Thus, noggin in the interzone contributes to the joint formation by antagonizing the chondrogenic effect of GDF5 [35, 36, 58].

As described above, the loss of embryo movement causes joint fusion. Notably, phosphorylated Smad1/5/8-positive cells are detected in the fused regions of paralyzed chick embryos and muscle-less mice [68, 119]. Considering that BMP signaling is regulated by the balance between ligands and antagonists, it is expected that the up-regulation of BMP ligands or down-regulation of BMP signaling by noggin or chordin may occur here. However, in the fused joints of muscle-less mice, noggin expression is up-regulated [49]. Singh et al. show that noggin expression is not altered and *Bmp4* expression is down-regulated both in immobilized chicks and mice [119]. The interaction between mechanical loading and BMP signaling is not currently revealed.

After joint cavitation, noggin expression is converged within the epiphyseal cartilage and prevents diffusion of the BMP ligands from the hypertrophic chondrocytes and ossification center to the joint region [38, 68, 70, 117, 119]. Additionally, another BMP antagonist, gremlin 1, is associated with the regulation of BMP signaling after birth to adulthood [128–130]. BMP signaling and its various modulators are involved in the development, homeostasis, and pathophysiology of joints.

Transforming growth factor-β

TGF-β signaling plays a substantial role in the homeostasis of articular cartilage [131–133]. TGF-β ligands bind to TGF-β type II receptor (Tgfbr2), which leads to TGFβ type I receptor recruitment. This heterodimer complex activates intercellular signaling cascades, such as Smad2/3 or Smad-independent pathways. Age-related decreases of TGF-β signaling in chondrocytes, partially caused by the decreased expression of their receptors, is associated with cartilage degeneration [132, 134]. Currently, the intraarticular administration of human chondrocytes transduced with a viral vector containing the gene for Tgf-β1 transcription is undergoing a clinical trial for the treatment for osteoarthritis [135].

The role of TGF-β signaling in joint development is also prominent [136, 137]. The deletion of Tgfbr2 in the early mesenchyme (*Prx1-Cre*) results in the inhibition of interzone appearance in phalanges [36, 37]. Accordingly, the *Gdf5* (+) lineage cannot enter into putative sites for the interzone in *Prx1-Cre;Tgfbr2* $^{fl/fl}$ mice [36, 37], similar to *Ihh* null mice [98]. Furthermore, TGF-β signaling exerts suppressive effects against chondrogenesis in limb bud culture [36] as well as Wnt/β-catenin signaling [76, 80]. As mentioned previously, the generation of the interzone requires the inhibition of chondrogenesis within the pre-cartilage anlagen, where BMP signaling is activated. BMP and TGF-β signaling share a co-mediator, Smad4, which triggers the nuclear translocation of the Smad complex. Therefore, this competition may be significant in interzone formation. Smad4 deletion in the early mesenchyme causes the severe impairment of limb development, including joint creation [138, 139], whereas in chondrocytes, it predominantly affects endochondral ossification accompanied with less alteration of the joints [140].

Expression analyses have also provided significant evidence. Spagnoli et al. show that *Tgfbr2* positive cells were detected explicitly in the interzone from E12.5 to E16.5 using *Tgfbr2* reporter transgenic mice [37]. Apart from the transient expression in the interzone, *Tgfbr2* expression is sustained in the joints and surrounding tissues until adulthood [69]. Joint intima including the perichondrium, synovium, enthesis, and articular cartilage surfaces in knees are expressed in neonates, while the epiphyseal cartilage is not [69]. These findings may support the hypothesis that articular and growth plate cartilage are derived from different cell sources [70].

Fibroblast growth factor 18/fibroblast growth factor receptor 3 signaling

FGF18 is among the FGF family members involved in articular cartilage. *Fgf18* null mice die in the early neonatal period and display impaired skeletal development [141–143]. Mutations of its receptor FGFR3 also cause severe dwarfism and achondroplasia in mice and humans [144–146]. Thus, it is evident that FGF18/FGFR3 signaling is indispensable for skeletogenesis. FGFR3 expression is found in differentiating chondrocytes [142, 147–149], whereas FGF18 is secreted from the perichondrium [142, 150]. This interaction is one of the characteristic findings indicating that the morphology of the cartilage template is determined by the perichondrium [151, 152]. FGF18/FGFR3 signaling induces chondrogenesis from the limb mesenchyme in vitro [153], suppresses the proliferation and hypertrophy of growth plate chondrocytes in vivo [142, 143, 154, 155], and partially regulates subsequent osteogenesis [142–144, 154, 156].

FGF18 transcripts can be detected in the interzone cells [142, 143]; however, its role is unknown, because most *Fgf18* null limb joints are unaffected [141–145]. Generally, FGF18/FGFR3 signaling seems to be deeply associated with IHH-PTHrP and the canonical Wnt signaling. Gain-of-function of FGFR3 signaling leads to the decreased expression of *Ihh* ligands and *Pthrp* receptors [93, 157, 158]. Postnatal chondrocyte-specific *Fgfr3* deletion induces multiple chondroma-like lesions adjacent to the disordered growth plates by the up-regulation of Ihh signaling [159]. On the other hand, the constitutive activation of IHH signaling decreases *Fgf18* expression and subsequent ectopic cartilage

hyperplasia in joints [104]. Furthermore, the hedgehog-induced phenotypes are rescued by the stabilization of β-catenin or treatment with FGF18 [104]. Interestingly, *Fgf18* is a direct transcriptional target of canonical Wnt signaling [160, 161]. Considering these findings, FGF18 may be involved in joint generation by mutually affecting the Ihh/PTHrP and the canonical Wnt signaling.

Adult articular chondrocytes also express FGF18 and FGFR3 [153, 162]. Mori et al. report that *Fgf18* is dominantly expressed in articular cartilage compared with growth plate cartilage, both in infant and adult rats [163]. *Fgfr3* knockout results in early osteoarthritis [164] with enhanced Ihh signaling [165, 166]. It is currently accepted that FGFR3 signaling exerts anabolic effects in healthy articular cartilage, while FGFR1 signaling exerts catabolic effects in articular chondrocytes [167]. Indeed, FGF2, a representative FGFR1 ligand, is up-regulated [168], and FGFR3/FGF18 is down-regulated in osteoarthritis cartilage [162, 169]. The therapeutic effects of FGF18 have been validated in various studies [163, 166, 170–172], and a clinical trial using recombinant human FGF18 for osteoarthritis is ongoing [135].

Late stages of articular cartilage differentiation

Interzone and joint development are regulated by various factors and signaling pathways as described above. Some of them may be dispensable during the specification of articular cartilage. Indeed, *Gdf5*, *Wnt9a*, *Ihh*, *Bmp*, and noggin disappear in the late stages of joint development [24, 34, 70]. Instead, the expression of structural proteins such as lubricin, tenascin-C, CD44, and type II collagen, which contribute to smooth movement and loading, becomes marked during differentiation toward adult articular cartilage [34, 70]. TGF-βs, FGF18, and PTHrP are continuously expressed from the interzone cells to matured articular chondrocytes, implying their extensive roles in articular cartilage. Currently, they all are considered as potential therapeutic agents for osteoarthritis [135].

Kozhemyakina et al. identify *Prg4* (+) cells in the SFZ as articular cartilage progenitors [173]. When *Prg4* (+) cells are labeled at E17.5, their progeny cells compose all layers of articular cartilage in adulthood [173]. Even in 1-month-old mouse cartilage, *Prg4* (+) cells slowly expand to the entire cartilage layers above the tidemark in 1 year [173]. Meanwhile, Decker et al. recently showed that articular cartilage is thickened mainly by zone-specific increases in cell volume in the late stage, and that cell proliferation or death plays a minor role [12]. Neonatal peri-articular chondrocytes actively proliferate, but underneath chondrocytes do not [12]. Although it is widely known that cell turnover is markedly suppressed and much less essential for the homeostasis of articular cartilage, it is surprising that the proliferation of articular chondrocytes is almost undetected in 2-week-old mice [12]. The peri-articular cartilage at this age contains fewer glycosaminoglycans, which are abundant in the underneath cartilage templates [12, 23, 110, 174]. These data may indicate that articular cartilage is not a residual of a cartilage template, rather it is newly formed by articular chondrocytes. Although whether the articular chondrocytes are derived from the interzone or the cartilage template is still controversial, joint components are probably constructed by the influx and efflux of cells during development [12, 33].

Regulators in the late stage

Lubricin

Lubricin, encoded by *Prg4*, is one of the major components of the synovial fluid, which is produced from synoviocytes and articular chondrocytes [175, 176]. Lubricin is responsible for joint lubricity [177, 178], and its expression is decreased in osteoarthritis [179, 180]. Exogenous lubricin injection is a promising treatment for osteoarthritis [181–183]. *Prg4* expression is observed at the inception of joint cavitation and becomes intense during cavitation [88, 184]. Even after development, lubricin is dominantly expressed in the surface cells of the synovium and articular cartilage [88, 185]. Several factors, including mechanical loading, PTHrP, and TGF-β have been identified as upstream regulators of *Prg4* [114, 186, 187]. The transient activation of the canonical Wnt signaling up-regulates SFZ cell growth and *Prg4* expression, and its deletion impairs SFZ development along with *Prg4* down-regulation [70, 81]. Meanwhile, the deletion of *Prg4* in mice does not alter skeletal development in the neonatal period, and gradually causes abnormality at the surface of the articular cartilage with the deposition of an acellular layer with aging [88, 177, 188]. Although *Prg4* is detected at the joint cavitation, it is likely less critical during joint formation and contributes to articular cartilage homeostasis.

Notch

Notch signaling regulates many asymmetric cellular developments via binding cell surface ligands (Jagged1, 2, Delta-like 1, 3, 4) and receptors (Notch1–4), whereby Notch intracellular domain (NICD) translocates into the nucleus and activates their downstream genes including Hes/Hey family members in concert with co-transcriptional regulator RBPjκ [189, 190]. In development, cells interfere with neighbor cells via Notch signaling, which yields cell diversity from a homogenous population. Notch signaling inhibits chondrogenesis in the early stage mesenchyme. *Rbpjκ* deletion in

the limb mesenchyme enhances chondrogenesis, and NICD overexpression in chondrocytes severely impairs skeletal development [191, 192]. Moreover, notch signaling also regulates the survival, proliferation, and differentiation of chondrocytes during the endochondral ossification process via the RBPjκ-independent pathway [152].

In contrast to its robust role in endochondral ossification, Notch signaling is dispensable for joint formation and articular cartilage development. Joint structure and articular cartilage are almost normal in *Rbpjκ* cKO mice using *Prx1-Cre* and *Col2a1-Cre* [192, 193]. In the maturation and homeostasis of articular cartilage, the role of Notch signaling is controversial. Mirando et al. show that the chondrocyte-specific deletion of *Rbpjκ* at 1 month of age leads to a progressive osteoarthritis-like pathology in the subsequent course with aging [193], while Hosaka et al. demonstrate that the chondrocyte-specific deletion of *Rbpjκ* at 7 weeks suppresses osteoarthritis development in a surgically induced mouse model [194]. Furthermore, the up-regulation of Notch signaling in adult articular cartilage induces osteoarthritis [195]. Notably, Notch expression is detected in the SFZ cells of articular cartilage, which are considered a cartilage progenitor [184, 196, 197]. Notch expression and positive cells respond to osteoarthritic change with activation and altered distribution [184, 194, 198]. Considering that Notch is expressed in the SFZ and that articular cartilage homeostasis is disturbed when *Rbpjκ* is deleted at 2–4 weeks [193, 199], Notch signaling may be involved in the final differentiation or maturation of articular cartilage.

Conclusion

In this review, we introduced crucial factors involved in joint and articular cartilage development. Molecular mechanisms underlying endochondral ossification and joint specification have been well studied over decades. Additionally, articular cartilage homeostasis and the pathophysiology of osteoarthritis have been a research focus in recent years. On the other hand, molecules, signaling pathways, and cells that regulate the late stage differentiation and maturation of articular cartilage remain obscure. Furthermore, articular cartilage development in humans may be entirely different from mice, where articular cartilage is thinner and multilayered. These issues may be obstacles to clinical application of the findings mentioned in this review.

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