Osteochondromas are cartilage-capped tumors that arise near growing physes and are the most common benign bone tumor in children. Osteochondromas can lead to skeletal deformity, pain, loss of motion, and neurovascular compression. Currently, surgery is the only available treatment for symptomatic osteochondromas. Osteochondroma mouse models have been developed to understand the pathology and the origin of osteochondromas and develop therapeutic drugs. Several cartilage regulatory pathways have been implicated in the development of osteochondromas, such as bone morphogenetic protein, hedgehog, and WNT/β-catenin signaling. Retinoic acid receptor-γ is an important regulator of endochondral bone formation. Selective agonists for retinoic acid receptor-γ, such as palovarotene, have been investigated as drugs for inhibition of ectopic endochondral ossification, including osteochondromas. This review discusses the signaling pathways involved in osteochondroma pathogenesis and their possible interactions with the retinoid pathway. (Am J Pathol 2021, 191: 2042–2051; https://doi.org/10.1016/j.ajpath.2021.08.003)
which is characterized as an excess endochondral bone formation that accumulates within connective and muscle tissues. In addition, independent studies have reported RAR-γ inhibitory action using different heterotopic ossification animal models. More importantly, palovarotene, a synthetic agonist for RAR-γ, strongly inhibits osteochondroma formation in the mouse model. These preclinical studies led to a clinical trial of pediatric patients to evaluate the efficacy and safety of palovarotene for the systemic treatment of HME and the prevention of disease progression (https://clinicaltrials.gov; trial number NCT03442985). Elucidation of the precise molecular and cellular mechanism of RAR-γ agonists on osteochondromas and ectopic endochondral formation will provide useful information to develop effective pharmacologic therapies.

This review provides an overview of available murine models, useful for basic and translational osteochondroma studies. Signaling pathways that have been demonstrated to be implicated in pathogenesis of osteochondromas are discussed with the possible interaction of these pathways with RAR-γ signaling in which pharmacologic activation would result in inhibition of osteochondromas.

**Molecular Pathology of Multiple Osteochondromas**

Multiple formation of osteochondromas is a characteristic of HME, which is a pediatric, autosomal-dominant disorder with reported incidences of approximately 1 per 50,000 individuals worldwide. Patients with HME have autosomal dominant functional mutations in the EXT1 and/or EXT2 genes, triggering the development of multiple osteochondromas. EXT mutations include frame shift, missense, and splice-site mutations. The human EXT1 and EXT2 genes encode endoplasmic reticulum—localized type II transmembrane glycoproteins that are tightly associated with glycosyltransferase activities. Exostosin 1 (EXT1) and exostosin 2 (EXT2) glycosyltransferases form a stable hetero-oligomeric complex that accumulates in the Golgi apparatus and are involved in heparan sulfate proteoglycan (HSPG) biosynthesis.

HSPGs consist of extracellular core proteins in which a tetrasaccharide linker is synthesized on conserved serine residues of the core protein of HSPGs. EXT1 and EXT2 allow the elongation of heparan sulfate (HS) chains by sequentially adding alternating units of N-acetylgalactosamine and glucuronic acid. As HS chains are created, deacetylation, sulfation, and epimerization occur. Four HSPG isoform families have been identified: syndecan, glypican, perlecan, and CD44. Glypicans and syndecans that carry long HS chains specifically bind and interact with signaling proteins, plasma proteins, and growth factors. Therefore, these HSPGs affect a variety of neighboring cell responses, such as cell differentiation, cell-to-cell interaction, receptor trafficking, and control tissue morphogenesis and gradients of growth factors, such as fibroblast growth factors, bone morphogenetic proteins (BMPs), hedgehogs (HHs), and Wnts in the extracellular matrix. The pathogenesis of osteochondroma development has not been clearly elucidated; however, HS synthesis deficiency most likely underlies the molecular mechanism of osteochondroma formation.

**Current Mouse Models of Osteochondromas**

Because osteochondromas are located close to growth plate, osteochondroma may presumably arise from growth plate or neighboring connective mesenchymal cells potentially caused by misregulation of chondrogenesis and/or endochondral ossification during skeletal development and growth. Several mouse models have been generated by systemic or cartilage- or perichondrium-specific inactivation of Ext1 and/or Ext2, and have been used to study pathology and pharmacotherapy of osteochondromas (Table 1).

### Col2-rTA-Cre;Ext1e2neo/flx/e2neoflo with Doxycycline

The dominant inheritance of HME might indicate that heterozygous mutations in the EXT1 or EXT2 gene would result in systemic reduction of HS levels, leading to osteochondroma formation. However, haploinsufficiency might not always trigger osteochondroma formation in a number of patients with HME. A loss of heterozygosity model has been proposed to explain the molecular pathogenesis of multiple osteochondromas. The EXT1 single-allele mutation experiences a second somatic mutation in a normal EXT allele in osteochondromas occurring at multiple lesions in patients with HME. Jones et al developed the transgenic mice that harbor an inducible loss-of-function allele of Ext1 (Ext1e2neo/flox or Ext1e2flo) in which exon 2 was flanked by head-to-head loxP sites and determined the necessity for loss of heterozygosity in osteochondroma formation. They demonstrated that clones of proliferating chondrocytes in the osteochondroma tissue are homozygous for the inverted Ext1 allele and are negative for HS staining. Thus, this study indicates that loss of heterozygosity is critical for osteochondroma formation.

### Col2CreER;Ext1fl/fl without Tamoxifen

Matsumoto et al created conditional compound mouse mutants bearing a standard floxed Ext1 gene (Ext1fl/fl) and Col2CreERT transgene that directs tamoxifen-inducible Cre recombinase expression under the Col2a1 promoter/enhancer control. Col2-CreERT;Ext1fl/fl mice developed multiple osteochondromas in long bones and other HME-associated skeletal defects (short stature, bowing deformity of the forearm, subluxation or dislocation of the radius, and scoliosis) without tamoxifen treatment caused by a low level of stochastic leakiness of the Cre recombinase activity.
Osteochondromas arise with a 100% penetrance in long bones. Growth plate of the femur and tibia were stained with X-gal and showed a low frequency of lacZ+ cells that form clusters that correspond to the columnar organization of chondrocytes in the growth plate. No lacZ+ cells were found in the perichondrium, including the area known as the groove of Ranvier, the perichondrium adjacent to growth plate. The study suggests that the cellular origin of osteochondromas is within the growth plate and that biallelic inactivation of Ext1 is important in the initiation of osteochondroma formation but might not be necessary for growing osteochondromas in mice.

### Ext1+/− and Ext2+/− Heterozygotes

Efforts to recapitulate the human HME genotypes that involve heterozygous mutations of the Ext1 and Ext2 allele have been investigated. Zak et al16 have created compound Ext1+/−;Ext2+/− heterozygote mice and compared the phenotype of either Ext1 and/or Ext2 null allele mice with double heterozygote mice by monitoring ectopic cartilage development located in the ribs and long bones. The Ext1+/−;Ext2+/− heterozygote mice revealed 71.4% penetrance, whereas the single Ext1 and Ext2 heterozygote had 36.5% to 44.4% penetrance, respectively. In addition to rib outgrowths, the compound Ext1+/−;Ext2+/− mutants have exhibited osteochondromas in the tibia and femur. Histologic analysis has shown that the periosteal border is interrupted by the developing osteochondromas that consisted of growth plate—like arrangement of chondrocytes. This model might be suitable for the study on cellular and molecular mechanisms underlying initiation of osteochondromas because osteochondromas in other mouse models are mostly induced by deletion of both Ext1 alleles. It has also been demonstrated that Ext2+/− mice have formed multiple osteochondromas in ribs with an approximate 1:3 ratio and represented abnormality in endochondral ossification with 100% penetrance.23

### Col2CreER;Ext1fl/fl with Tamoxifen

The same Col2-CreERT;Ext1fl/fl system was used but was performed with tamoxifen induction.17 On tamoxifen injection at postnatal day 5, Col2-CreERT;Ext1fl/fl mice developed ectopic cartilaginous outgrowths on the outer region surrounding the mutant growth plate as early as 1 week after tamoxifen induction. The histologic findings of cartilage deformation were similar to other reports that mimic osteochondroma development.14,15 In addition, developmental delay of the secondary ossification center in long bones and abnormality of articular chondrocytes, including ectopic expression of collagen X and matrix metalloproteinase 13 and hypertrophy in shape, were
observed. The changes on these diverse cell populations suggest additional function of Ext1 in chondrocytes.

CtskCre;Pttn1fl/fl

Yang et al.18 generated a mouse model with a conditional deletion of Pttn11, a tyrosine phosphatase called SHP2 in the cells where cathepsin K promoter activity is activated, and demonstrated that this mouse represents metachondromatosis, characterized by the development of endochondromas and osteochondromas. Interestingly, most cells in the groove of Ranvier are affected in this mouse system and form chondromas. These findings indicate that the perichondrial cells in the groove of Ranvier can potentially be the origin of osteochondromas. However, growth plate chondrocytes should not be excluded as the source of osteochondroma because lineage studies trace some growth plate chondrocytes as descenders of cathepsin K lineage cells.18

AggrecanCreER;Ext1e2neofl/e2neofl with Tamoxifen

Sinha et al.13 generated the AggrecanCreER;Ext1e2neofl/e2neofl osteochondroma model and observed osteochondroma-like outgrowths in the cranial base on tamoxifen injection. Formation of osteochondromas in the cranial base has been found in a number of HME patients.19 A combination with reporter mice has demonstrated that the responding cells are the bulk of synchondrosis growth plates and the cells located along the chondroperichondrial border, showing similar pathologic findings elicited by Col2-CreER;Ext1e2neofl/e2neofl with and without the tamoxifen system.15,17 These mutant mice have developed osteochondromas in both long bones and ribs at 100% penetrance on tamoxifen induction. This study demonstrated that Smad-mediated BMP activation is critical for osteochondroma formation in this model.

Fsp1Cre;Ext1fl/fl

Inubushi et al.20 performed a perichondrium-targeted conditional knockout of Ext1 using fibroblast-specific protein 1 (Fsp1) that was expressed in the perichondrium. This model involves the deletion of the Ext1 gene in progenitor cells in the perichondrium that undergo aberrant differentiation into chondrocytes but not chondrocytes in the growth plate. Fsp1Cre;Ext1fl/fl mice developed osteochondromas that are similar to those seen in cartilage-targeted conditional knockout of Ext1. 14,15,17,19 Fsp1Cre;Ext1fl/fl mutants not only develop multiple osteochondromas at 100% penetrance in the long bones and ribs but also grow normally without any growth retardation and survived for longer than 1 year. The observations seen in this mouse model indicate that cell origin of osteochondromas can be additional progenitor cells in the perichondrium and that inhibition of BMP signaling can effectively suppress osteochondroma formation.

Signaling Pathways Involved in Osteochondromas

Endochondral ossification is an essential process that creates bone from cartilaginous templates in appendicular and axial skeletons. Initially, skeletal progenitors condense and differentiate into chondrocytes in programmed regions at embryonic stages. Cartilage development and endochondral ossification are controlled by an interplay of intricate signaling networks that involve the surrounding matrix, secreted morphogens, and growth factors, such as BMPs, Indian hedgehog (IHH), Wnts, and fibroblast growth factors.24,25 Studies have indicated that dysregulation of those signaling pathways can cause or are associated with pathology of osteochondroma formation.12,26

BMP Signaling

BMPs contain a group of growth factors.27,28 BMP signaling gradients exist across the growth plate and regulate bone formation and homeostasis, development, or morphogenesis of cartilage. BMPs bind to BMP receptor type I (BMPR-I) and BMP receptor type II (BMPR-II) transmembrane receptor proteins. Once this binding and receptor dimerization of BMPR-I and BMPR-II occurs, BMPR-II phosphorylates the GS domain of BMPR-I, causing the phosphorylation of signal-transduction proteins Smad 1, 5, or 8 (R-Smads) in the cell. Smad 6 and 7 (I-Smads) inhibit and modulate the phosphorylation of R-Smads. In addition, R-Smad interacts with Smad 4 (co-Smad) to form a complex that translocates to the nucleus and activates the transcription of BMP-responsive genes.27,28 More than 15 BMPs have been identified, and among them, BMP-2, BMP-4, and BMP-7 regulate the growth and maturation of chondrocytes in vitro, whereas BMP-2, BMP-4, BMP-6, and BMP-7 induce bone and cartilage formation in vivo.28 Dysregulation of the BMP signaling pathway is involved in the disease fibrodysplasia ossificans progressiva (FOP), which is a rare autosomal dominant disorder with mutations of ACVR1 that result in progressive heterotopic ossification of skeletal muscle and soft connective tissue.29 These findings indicate a close relation of BMP signaling with ectopic cartilage formation.

HSPGs are responsible for sequestering BMPs at the cell surface or in extracellular matrix while also enhancing BMP activity by continuously serving BMPs to their signaling receptors on the cell surface.30 Gene expression of BMP2 and BMPRIB and nuclear localization of phospho-Smad1/5/8 have been observed in the cartilage caps of human osteochondroma specimens,31 suggesting that enhanced BMP signaling might play a role in osteochondroma
formation. Cellular pSmad1/5/8 levels have been increased in the perichondrium by Ext1 deficiency in organ culture and mouse osteochondroma model. Pharmacologic inhibition of BMP signaling by the LDN-193189 inhibitor reduced osteochondroma formation in osteochondroma mouse models. These findings support the idea that activation or dysregulation of BMP signaling in growth plate chondrocytes and/or perichondrium cells can underlie osteochondroma progression and formation.

IHH Signaling

The core components of the HH pathway is composed of three different HH ligands (Sonic HH, Desert HH, and IHH), a 12-pass transmembrane receptor patched1 (PTCH1), and a G-protein–coupled receptor–like seven-pass transmembrane protein smoothened (SMO). In the absence of HH ligand, PTCH1 sequesters SMO, inhibiting SMO activation. However, when HH is present, this repressive action is released. SMO forms a complex with the Ellis-van Creveld (EVC) syndrome protein and EVC2, triggering downstream trafficking and processing of GLI transcription factors. Three transcription factors (GLI1, GLI2, and GLI3) mediate HH signaling.

HHs contain the HS/heparin binding domain that mediates interaction with HS proteoglycans and include a consensus Cardin-Weintraub motif. IHH is one of the main regulators of chondrocyte differentiation in the normal growth plate. It is produced by prehypertrophic chondrocytes and controls proliferation and the onset of hypertrophic differentiation of chondrocytes and osteoblast differentiation. The analysis of achondroplasia seen in brachymorphic mice have revealed that abnormal distribution of Ihh proteins and a decrease in Ihh signaling in the growth plate are associated with undersulfation of the chondroitin sulfate proteoglycans. This finding suggests that undersulfated HS might affect the diffusion of Ihh proteins and Ihh signaling.

Koziel et al found that HS negatively regulates the range of hedgehog signaling in a concentration-dependent manner in the growth plate and that reduced HS biosynthesis caused by hypomorphic mutation on Ext1 resulting in increased chondrocyte proliferation and delayed onset of hypertrophy via elevated Ihh signaling in embryonic bone formation in mice. Ext1<sup>+/−</sup> mouse embryos have also represented an enhanced Ihh diffusion in the tibia with increased proliferation and delayed hypertrophy of growth plate chondrocytes. These findings lead to the theory that the development of osteochondromas might be attributed to the inability of HS to regulate the distribution of IHH. However, haploinsufficiency of Ihh does not change the development of osteochondromas as determined by tumor size and number, whereas HH signaling activation by deletion of an allele of Ptc1, reduces the number of tumors. The same study found that in a subset of osteochondromas, Ihh activation increased the cellularity and inhibited chondrocyte maturation, raising concern about whether changes in HH signaling activity might predispose to individuals to osteochondroma transformation. Of note, data from humans indicate that increased HH activation is linked to chondrosarcoma development, even though the study found that a decrease in IHH signaling is associated with malignant transformation toward secondary peripheral chondrosarcoma in human specimens. Evidence that supports the role of HH signaling in osteochondromas is still limited and should be further studied.

Wnt/β-Catenin Signaling

The Wnt family is a group of highly conserved proteins responsible for cell development and homeostasis. The Wnt pathway includes 19 Wnt molecules and 15 frizzled receptors and co-receptors, such as low density lipoprotein receptor-related protein cell surface markers. In addition, the pathway is also regulated by extracellular and intercellular inhibitors, including secreted Frizzled-related proteins, Wnt inhibitor factor-1, Dickkopfs, and axis inhibition proteins. Wnt/β-catenin signaling is a major signal transduction pathway of Wnts. Once Wnt proteins bind to the cell surface receptor complexes Frizzleds and LRP5/6, downstream intercellular protein Disheveled is activated, resulting in the inhibition of glycogen synthase-3β kinase and β-catenin phosphorylation. This inhibition then allows β-catenin to escape the ubiquitin proteasome degradation pathway, allowing β-catenin accumulation in the cytoplasm. Stabilized nonphosphorylated β-catenin is then able to translocate to the nucleus and interacts with multiple transcription factors, activating transcription of Wnt-targeted genes. Wnt/β-catenin signaling also strongly influences development and organization of growth plates. Research on the roles of this signaling pathway in limb development and growth have been performed using various transgenic mice. The findings support that proper control of the signaling activity is essential for joint formation, growth plate function, and skeletal formation.

Wnt/β-catenin signaling is also modulated by HS, like HH signaling. HSPGs have been implicated in regulating the distribution and receptor binding of several members of Wnt at the cell surface and thereby support the solubility of hydrophobic Wnt and its activity. The importance of HS proteoglycans to regulate Wnt activity has been initially addressed in Drosophila and later in vertebrate models. In general, in a variety of cancers, Wnt/β-catenin
pathways are known to be upregulated, causing part of the pathogenesis, but this scenario might not be the same for osteochondroma pathogenesis. Interestingly, β-catenin conditional deletion induces ectopic chondroma-like cartilage formation in mice during cartilage development.45 When an β-catenin signaling stimulator is present, apoptotic and cell death levels are elevated in cultured wild-type chondrocytes. In addition, immunohistochemical analysis of hereditary multiple exostoses specimens revealed that the amount of detectable β-catenin in cartilage cells of osteochondromas was much lower than that in hypertrophic chondrocytes in normal human growth plates. These findings suggest that inhibition of Wnt/β-catenin signaling is related to the development of osteochondroma formation. Moreover, Wnt/β-catenin signaling was found to be an essential fate determinant of mesenchymal cells to osteoblasts or chondrocytes and strongly suggests involvement of this signaling in the pathogenesis of osteochondroma. Wnt/β-catenin signaling activation regulates both cartilage development and homeostasis and shows diverse effects on chondrocyte differentiation, proliferation, and survival.11 This finding might explain why both genetic stimulation and reduction of Wnt/β-catenin signaling suppressed osteochondroma formation in mice.39 Future studies are needed to evaluate the mechanism behind Wnt/β-catenin signaling in the reduction of osteochondroma formation.

Interaction of RAR-γ Signaling with Signaling Pathways in Osteochondroma Pathogenesis

Retinoid Signaling and Endochondral Ossification

Retinols enter the cell through retinol binding protein receptor (stimulated by retinoic acid 6). Retinol is bound to cellular retinol binding protein and is converted into retinaldehyde by retinol dehydrogenase, which is then subsequently converted into retinoic acid by retinaldehyde dehydrogenase.46 Retinoic acids exert their functions...
through two specific classes of receptors: RARs and retinoic X receptors (RXRs). Each class contains three subtypes: α, β, and γ. The expression of these receptors is regulated by the receptors themselves, by nuclear receptors, or by other subtypes in the same family. On the nuclear binding of retinoic acid to RARs, conformational changes cause the ligand-binding domain to expose a binding site for coactivators, including CBP/p300, p300/CBP-associated factor, and p160 family members, which then recruit proteins that contain histone acetyltransferase activity. RARs and RXRs form heterodimeric complexes to function as ligand-dependent transcription factors and bind to retinoic acid response elements located at the enhancer regions of target genes. Unliganded receptors are responsible for repressing target gene expression. In the absence of ligands, the coactivators of RARs/RXRs are replaced by corepressors, which include nuclear receptor co-repressor 1/2 (silencing mediators of RARs and thyroid hormone receptors), mSin3A, and histone deacetylases. Together this complex results in transcriptional repression of the target genes of RAR/RXR heterodimers.

Retinoic acid is a negative regulator of growth plate chondrogenesis and can be influenced by the expression and/or the activity of SOX9, a major chondrogenic driver. Retinoid signaling strongly inhibits growth plate chondrocyte proliferation as shown in the retinoic acid–treated metatarsal organ cultures and in Cyp26b1-deficient mice. Furthermore, retinoic acid suppresses cartilage matrix synthesis in growth plate chondrocyte cultures. In cephalic chick chondrocyte cultures, retinoic acid stimulates chondrocyte hypertrophy and mineralization. Genes encoding RAR-α, RAR-β, and RAR-γ display spatiotemporal patterns of expression during cartilage formation. RAR-α is expressed throughout the limb mesenchyme early in limb development, decreases as cells begin to differentiate into chondrocytes, and then remains low in cartilaginous tissues. RAR-β is expressed in regions of the developing limb that are not destined to form cartilage, whereas RAR-γ is expressed preferentially in both prechondrogenic mesenchymal condensations and newly differentiated chondrocytes before chondrocytes hypertrophy, with respect to skeletal development in the limb.

In limb bud cells and chondrocytes, RAR-γ–mediated retinoic acid action strongly inhibits chondrogenesis and cartilage matrix synthesis.

Synthetic retinoid agonists specific for RAR-γ, such as palovarotene, function as potent inhibitors of osteochondromas in rodent models. The RAR-γ agonist can exert antitumor function on human osteochondromas by inhibiting matrix synthesis, promoting cartilage matrix degradation, and stimulating cell death. The inhibitory action of RAR-γ signaling on osteochondromas might be mediated by interaction of RAR-γ signaling with the multiple signaling pathways that are important for cartilage development and endochondral ossification.

Interaction of Retinoid Signaling with BMP, HH, and Wnt/β-Catenin Signaling Pathways

Heterotopic ossification involves induction of ectopic endochondral ossification and can be triggered by uncontrolled activation of BMP signaling, as observed in diseases such as FOP, which are associated with genetic mutations in BMP type I receptor ACVR1. RAR-γ agonists inhibit phosphorylation of Smad1/5 and stimulate degradation of Smad1/5 proteins. BMP inhibition can be an attribute to chondrogenic inhibition by RAR-γ agonists, which typically requires a decrease in retinoid signaling while inversely upregulating BMP signaling. A similar inhibition of Smad1/5/8 phosphorylation by the RAR-γ agonists is addressed in the mouse osteochondroma model. In addition, pharmacologic interference of RAR-γ stimulates Smad1/5/8 phosphorylation. Therefore, it is very likely that inhibition of BMP signaling is an important mechanism in which RAR-γ agonists suppress ectopic cartilage formation in osteochondromas.

RAR-γ transcripts are detected in prehypertrophic chondrocytes, overlapping with Ihh gene expression in E18 mandibular condyle. Shimo et al studied the cross-interaction between retinoid and Ihh signaling pathways using chick cephalic chondrocyte culture system. The authors found that retinoic acid stimulates Ihh gene expression, which requires de novo protein synthesis and activation of p38 mitogen-activated protein kinase, which is important for retinoic acid–induced Ihh gene expression, and that retinoic acid inhibits proliferation of proliferating chondrocytes but not that of hypertrophic chondrocytes. Yoshiida et al found that the 5′ flanking region of the human Ihh gene contains a retinoic acid response element and that retinoic acid treatment upregulates Ihh and Ptcched1 gene expression in high-density cultures of rabbit growth-plate chondrocytes. Conversely, Wu et al reported that Shh and Ihh are expressed in growth plate by quantitative PCR and in situ hybridization and that retinoic acid stimulates gene expression of Shh but inhibits Ihh expression. These findings suggest that retinoid signaling can alter HH signaling in both positive and negative ways. Considering the finding that Ihh potentiates BMP signaling and induces ectopic cartilage formation, the RAR-γ agonist might inhibit HH signaling when it inhibits ectopic cartilage formation and osteochondromas, but the interaction between retinoid and HH signaling in osteochondromas can vary, depending on the stage of tumor development and the microenvironment of where and how tumors grow.

Wnt/β-catenin signaling molecules interact with the nuclear receptor family members in diverse types of cells, tissues, and organs and are developmentally and clinically important. Cross-talking among Wnt, retinoid, HH, BMP, and fibroblast growth factor signaling pathways sophisticatedly regulates limb morphogenesis. Spatiotemporal expression of the ligands, receptors, and regulatory
molecules of these signaling pathways coordinates signaling gradients and specifies the anterior-posterior axis and the proximo-distal pattern." Retinoic acid plays a role in specification of the proximal part of the limb, Wnt3 and Wnt5a contribute to the distal growth, and Wnt7a contributes to the ventralization. However, the molecular interaction between retinoid and Wnt/β-catenin signaling during limb morphogenesis is not yet elucidated.

Retinoic acid treatment increases gene expression of the Wnt5a (Wnt2b, Wnt4, Wnt5a, Wnt5b, and Wnt14) and receptors and coreceptors (Fzd8, Lrp5, and Lrp6) and enhances Wnt/β-catenin signaling. In conditions where retinoid ligands are not available in normal chondrocytes, unliganded RAR-γ is associated with β-catenin via the amino-terminal domain of RAR-γ and inhibits the β-catenin transcriptional activity with lymphoid enhancer factor/T-cell factor transcription factors. Therefore, alterations in RAR-γ signaling can change Wnt/β-catenin activity in chondrogenesis, endochondral ossification, and possibly in osteochondromas. Wnt/β-catenin signaling can be stimulated by treatment with the RAR-γ agonists, leading to inhibition of growth of osteochondromas (Figure 1).

Palovarotene

Two clinical trials of palovarotene were conducted as potential treatments for FOP (https://clinicaltrials.gov, trial number NCT03312634) and for multiple osteochondromas (https://clinicaltrials.gov, trial number NCT03442985). The US Food and Drug Administration placed both trials on hold in response to cases of early plate closure in FOP pediatric patients. The multiple osteochondromas trial was later terminated (Ipsen Pharma, https://www.ipsen.com/websites/Ipsen_Online/wp-content/uploads/2020/03/09112219/Ipsen-Palovarotene-Update-26-March-2020.pdf, last accessed July 14, 2021), and the FOP study was refined to enroll children 14 years and older and resumed only for those patients >14 years of age.

The multiple osteochondromas clinical trial evaluated two daily dosage regimens (2.5 and 5.0 mg) in 194 pediatric patients. The FOP clinical trial evaluated different dosage regimens of 5, 10, or 20 mg in 54 pediatric patients. It has not been concluded which doses of palovarotene cause disorders of growth plate in pediatric patients. The negative effect of palovarotene treatment on skeletal tissues has also been observed in preclinical studies. When palovarotene is administered daily via intraperitoneal injections in mice from postnatal day 14, the treatment disrupts growth plate and synovial joint morphology in juvenile FOP mice. Palovarotene-treated wild-type and FOP mice had growth plate loss and shortened body lengths, spine lengths, and tibial lengths compared with untreated control mice. Imbushi et al also detected inhibition of skeletal growth with oral gavage of palovarotene starting from postnatal day 14, but deformity was not detected when treatment was initiated from postnatal day 21 with the same dose of palovarotene. The findings from the animal experiments suggest that susceptibility of the growth plate to palovarotene might reduce at older ages and that the route of palovarotene administration seems to be an important risk for skeletal deformity. Additional studies are required to evaluate the skeletal toxicity, including growth inhibition and joint degeneration, to determine the therapeutic potential of palovarotene.

Conclusions

Local application of RAR-γ agonists abolished growth of transplanted human chondrosarcoma cells in a xenograft mouse model, suggesting that RAR-γ agonists can be locally applied. A local treatment would be an alternative option of RAR-γ agonist treatment, which might lower the risk of the drug adverse effects but should be investigated intensively. Currently, the clinical approach is a careful follow-up for small lumps of osteochondromas until the masses grow to meet surgery application criteria. Drug therapy delivery, however, might effectively inhibit tumor progression if applied at the beginning stage when osteochondromas are small masses.

Palovarotene, an RAR-γ selective agonist, was originally investigated as a possible drug for emphysema and is currently proposed as a potent inhibitor drug for FOP, suggesting potential therapeutic benefits in various types of heterotopic ossification. Pharmacologic activation of RAR-γ signaling must alter various signaling pathways in cartilage tissues (articular cartilage and growth plate) as described here. Understanding the interaction of RAR-γ signaling with these important signaling pathways is vital to elucidate not only the pharmacologic action of RAR-γ agonist drugs on heterotopic ossification and osteochondromas but also the physiologic roles of RAR-γ-mediated retinoid signaling in endochondral ossification.

References


