Laboratory Investigation (2016) 96, 186–196

PATHOBIOLOGY IN FOCUS

Wnt signaling in cartilage development and diseases: lessons from animal studies

Yu Usami[1,2], Aruni T Gunawardena[1], Masahiro Iwamoto[1] and Motomi Enomoto-Iwamoto[1]

Cartilage not only plays essential roles in skeletal development and growth during pre- and postnatal stages but also serves to provide smooth movement of skeletons throughout life. Thus, dysfunction of cartilage causes a variety of skeletal disorders. Results from animal studies reveal that β-catenin-dependent canonical and independent non-canonical Wnt signaling pathways have multiple roles in regulation of cartilage development, growth, and maintenance. β-Catenin-dependent signaling is required for progression of endochondral ossification and growth of axial and appendicular skeletons, while excessive activation of this signaling can cause severe inhibition of initial cartilage formation and growth plate organization and function in mice. In contrast, non-canonical Wnt signaling is important in columnar organization of growth plate chondrocytes. Manipulation of Wnt signaling causes or ameliorates articular cartilage degeneration in rodent osteoarthritis models. Human genetic studies indicate that Wnt/β-catenin signaling is a risk factor for osteoarthritis. Accumulative findings from analysis of expression of Wnt signaling molecules and *in vivo* and *in vitro* functional experiments suggest that Wnt signaling is a therapeutic target for osteoarthritis. The target tissues of Wnt signaling may be not only articular cartilage but also synovium and subchondral bone.
Laboratory Investigation (2016) **96,** 186–196; doi:10.1038/labinvest.2015.142; published online 7 December 2015

Cartilage is largely composed of a single type of cells, called chondrocytes and various types of extracellular matrix proteins including highly sulfated macromolecule proteoglycans, small proteoglycans, collages including collagen 2, 6, 9, 10, and 11, and others. Cartilage can be structurally and functionally classified in different manners, but is generally subdivided into three classes by histological feature: hyaline, elastic, and fibrous cartilage. Hyaline cartilage is composed of predominantly collagen 2 and highly sulfated proteoglycans enriched in glycosaminoglycans. Hyaline cartilage is the most common cartilage in the body and can be further divided into two groups: permanent cartilage and transient cartilage. Both are essential components for axial and appendicular skeletons, but their functions are quite distinct. The former is essential for skeletal formation, patterning, and growth at embryonic and postnatal stages, while the latter is required for smooth movement of skeletons throughout life.

Skeletal formation has received much attention from the research filed of developmental biology because this biological event is a suitable and enthusiastic model to study morphogenesis using *in ovo* chick embryos and *in vivo* transgenic mouse systems. Studies of Wnts on skeletal formation starts with the research of limb morphogenesis, which shows that Wnt signaling, together with other morphogenetic signaling pathways, are essential for specification of three-dimensional axes: posterior-anterior, proximal-distal and ventral-dorsal axes.[1,2] Further, human genetic studies on skeletal disorders have accelerated the Wnt research on cartilage and bone.[3,4] Continuous and extensive efforts have been made and have revealed that Wnt signaling has a multitude of tasks in regard to regulation of cartilage and bone development/function via control of specification of cell lineages of skeletogenic stem/progenitor cells and differentiation of chondrocytes and osteoblasts. Aberrant Wnt signaling would cause or be closely associated with skeletal disorders such as dwarfism, deformity of skeletons, degenerative joint disorders, osteoporosis, and high bone-mass syndrome.

In this review, we will summarize the findings obtained from the studies of Wnt signaling on cartilage development and discuss the roles of Wnt signaling in regulation of chondrocyte function during long bone formation and growth. We will also discuss involvement of Wnt signaling in pathogenesis of cartilage disorders, especially osteoarthritis (OA).

[1]Translational Research Program in Pediatric Orthopaedics, Division of Orthopaedic Surgery, Department of Surgery, The Children's Hospital of Philadelphia, Philadelphia, PA, USA and [2]Clinical Laboratory, Osaka University Dental Hospital, Osaka University, Osaka, Japan
Correspondence: Dr M Enomoto-Iwamoto, PhD, DDS, Translational Research Program in Pediatric Orthopaedics, Division of Orthopaedic Surgery, Department of Surgery, The Children's Hospital of Philadelphia, 3615 Civic Center Boulevard, ARC 902, Philadelphia, PA 19104, USA.
E-mail: iwamotom1@email.chop.edu

Received 10 July 2015; revised 30 September 2015; accepted 30 September 2015

ESSENTIAL ROLES OF CARTILAGE IN THE FORMATION, GROWTH, AND FUNCTION OF LONG BONES

The skeleton provides an organism with the framework in which tissues and organs can attach, ultimately providing both shape and support. The main component of the skeleton is bone, a vascularized skeletal tissue consisting of several types of cells and a mineralized extracellular matrix.[5] Bone formation begins in embryogenesis and continues during postnatal growth and further throughout life coupling with bone resorption. During embryogenesis there are two processes in which bone is formed: intramembranous formation and endochondral formation. Intramembranous formation creates the craniofacial bones, while endochondral formation is responsible for most axial and appendicular skeletons. The process of endochondral ossification begins by forming a cartilage template and ends at its replacement to bone. Number, size, and shape of cartilage templates will produce a variety of bones including long bones (eg, femur and humerus), short bones (eg, wrist and ankle), flat bones (eg, ribs), and irregular bones (eg, vertebrae).

In detail, endochondral ossification is comprised of the following steps: mesenchymal condensation, formation of cartilage primodium with chondrogenic differentiation of mesenchymal cells, growth plate organization with a series of chondrocyte differentiation, and replacement to bone. During mesenchymal condensation, cells that originated from the mesoderm congregate into clusters at prospective skeleton sites and form a distinct outer boundary that limits the chondrogenic region (Figure 1a). Mesenchymal condensation is visualized as dense packaging of cells and characterized by upregulation of versican, tenascin, syndecan, neural cell adhesion molecule 1 (N-CAM), N-cadherin, and other molecules. The cells of the condensed mesenchyme undergo lineage restriction to chondrocytes and form cartilage primodium (Figure 1b). This is accompanied by synthesis of extracellular matrix proteins such as collagens (collagen 2, 9, and 11) and highly sulfated proteoglycans.

Chondrocytes proliferate and mature to begin the formation of the growth plate (Figure 1c). They create morphologically distinct zones: the resting zone, proliferating zone, prehypertrophic zone, and the hypertrophic zone (Figure 2a). The resting zone is composed of round chondrocytes, and may function as a reservoir of chondrocyte progenitors. The proliferating zone is characterized with dividing and flattening cells into organized columns in the orthogonal plane in the direction of the bone's longitudinal axis. The resulting daughter cells intercalate, forming a stack. The chondrocytes of the prehypertrophic zone exit from the cell cycle and enter hypertrophic differentiation. Chondrocytes of the hypertrophic zone of the upper region increase in size, while chondrocytes of the lower region induce matrix mineralization. Once the chondrocytes become hypertrophic, they express unique proteins such as collagen 10 and matrix metalloproteinase 13 (*Mmp13*). The calcified zone of growth plate is invaded by vessels and osteoblasts, and eventually is replaced by bone (Figure 1d). During skeletal development, chondrocytes are persevered in the region between the primary and secondary ossification centers and organize growth plate (Figure 1e). Growth plate is the sole region that allows longitudinal growth of long bones, at adulthood this area will become ossified and longitudinal bone growth will cease in humans. Chondrocytes present in the inner layer of the perichondrium differentiate into osteoblasts and form the bone collar.

Cartilage development and endochondral ossification are highly regulated processes, which involve an array of systemic and local factors, transcription factors, and extracellular matrix proteins.[6–9] Systemic and local factors include growth hormone, sex hormones, thyroid hormone, vitamins, insulin-like growth factors (IGFs), parathyroid hormone-related protein (PTHrP), fibroblast growth factors (FGFs), platelet-derived growth factors (PDGFs), transforming growth factor β (TGFβ)/bone morphogenetic protein (BMP) superfamily, Indian hedgehog, and Wnts. Endocrine, paracrine, and/or autocrine actions of these factors govern proliferation, differentiation, and matrix mineralization of chondrocytes, and replacement of cartilage to bone via transactivation and posttranslational modification of a variety molecules in chondrocytes and other cells such as chondroblasts/osteoclasts, endothelial cells, and osteoprogenitors (see reviews[8–10]). If imbalance of this regulatory system occurs due to genetic or acquired causes, it leads to skeletal malformation and defects.[7,9] For example, achondroplasia represents severe impairment of cartilage growth and growth plate organization, which strongly impacts axial and appendicular skeletal growth, shape, and size, leading to dwarfism and deformity of skeletons.[11] Mutations of FGF receptor 3 are the most common cause of achondroplasia. Studies have shown that FGFR3 mutations activate FGF signaling, affecting chondrocyte function and attenuating endochondral ossification.[12]

There are various transcription factors that regulate cartilage development and endochondral ossification.[6,7] Homeobox genes, in particular Hoxa11, Hoxa13, and Hoxd13 coordinate initial patterning of cartilage rudiments in the limbs. SOX trio members—SOX5, SOX6, and SOX 9—are essential not only for initiation and progression of embryonic cartilage formation and but also for postnatal growth plate formation and function.[13–16] Runx2 is a transcription factor that has been identified as a master gene for bone formation.[17] Runx2 also exerts an indispensible role for stimulation of chondrocyte hypertrophy and induction of matrix calcification.[18]

Long bones are articulated by a synovial joint, which is composed of several distinct tissues. One of the components is articular cartilage that resides at both ends of long bones. Articular cartilage plays a significant role in maintaining joint function by providing tissue resilience. It persists throughout life and does not express terminal differentiation phenotype that growth plate chondrocytes do. Articular cartilage development is tightly synchronized with the development

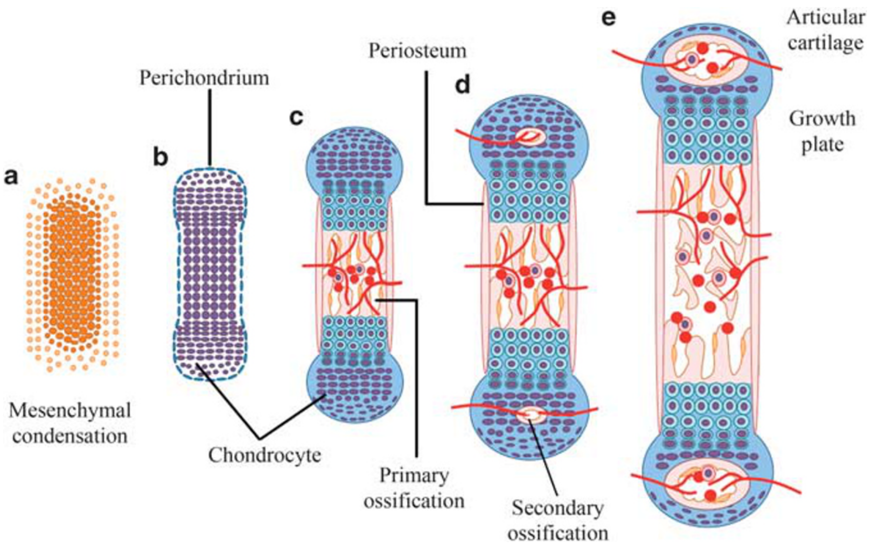


Figure 1 Cartilage development and endochondral ossification. (**a**) The process of endochondral ossification begins from mesenchymal condensation of bipotential osteochondroprogenitors at prospective skeleton sites. These mesenchymal progenitors undergo chondrogenic differentiation, and makes cartilage primodium. (**b**) The primordial cartilage continues to grow, forming an avascular cartilaginous template surrounded by the perichondrium. Chondrocyte in the center part of the primodium that initiate a growth plate, and undergo hypertrophy. (**c**) Hypertrophic chondrocytes are calcified and invaded by microvessels forming primary ossification. (**d**) Vessels invade the epiphyses and form secondary ossification center with osteoblasts and bone marrow. (**e**) The growth plate contributes to long bone growth. Articular cartilage provides resilience and smooth movement to the joint.

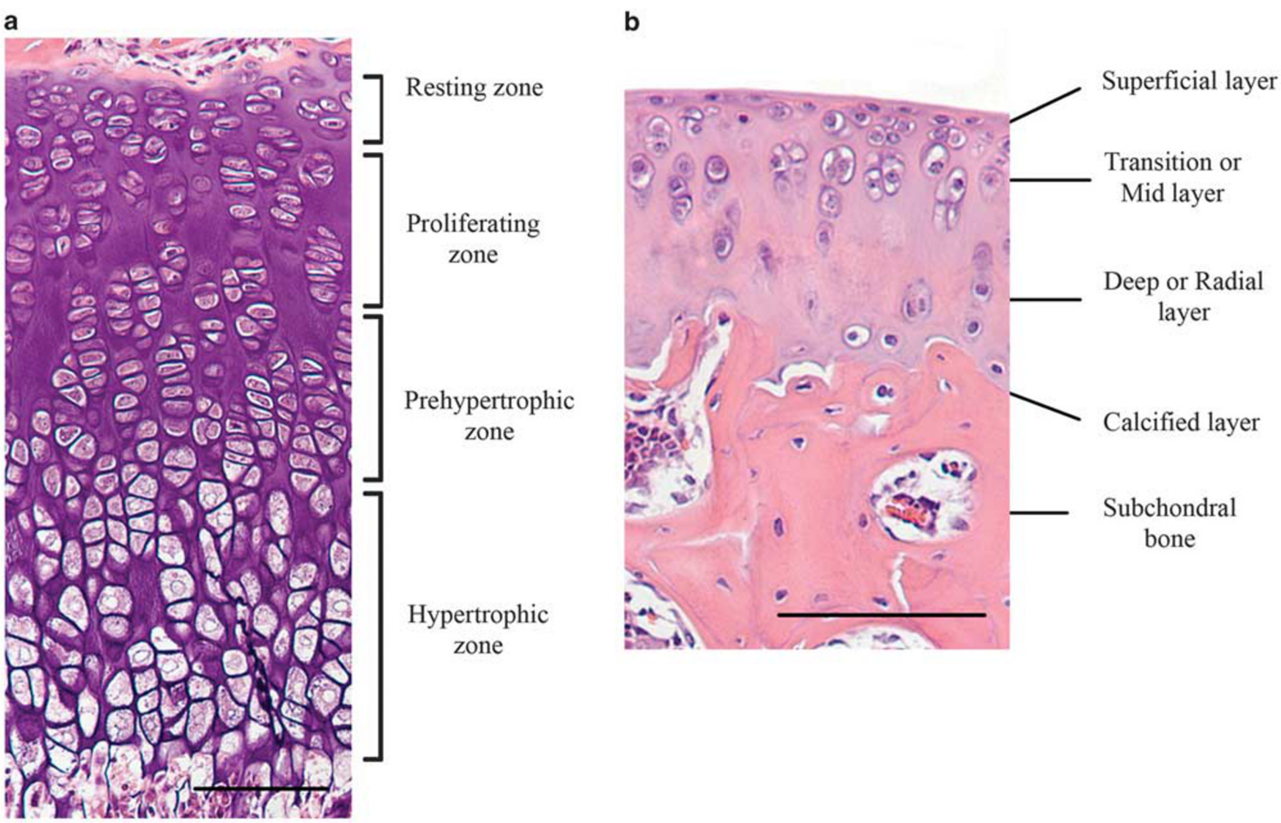


Figure 2 Histology of growth plate (**a**) and articular cartilage (**b**) of the proximal tibia in 4-week (**a**) and 8-week (**b**) old mice. (**a**) Growth plate is characterized by several morphologically distinct zones: resting zone, proliferating zone, prehypertrophic zone, and hypertrophic zone. (**b**) Mature articular cartilage constitutes zonal organization: superficial layer, transition or mid layer, deep or radial layer, and calcified layer that is lined by subchondral bone. Scale bars, 100 μm.

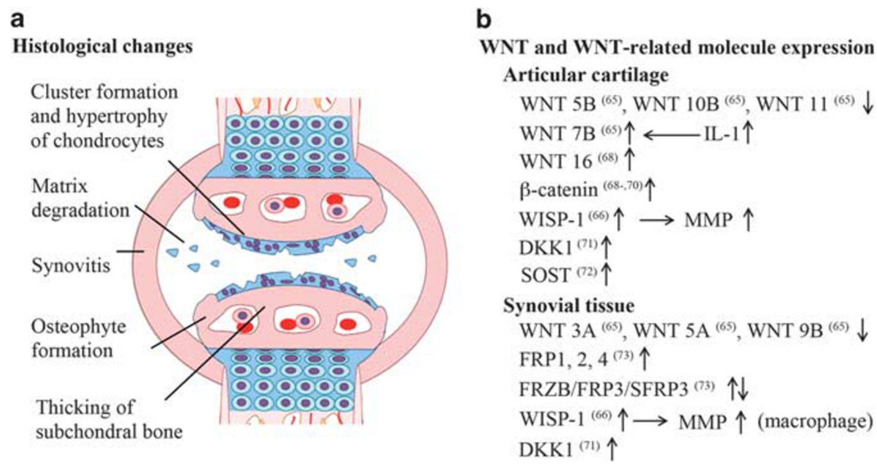


Figure 3 Pathological features of OA and dysregulation of Wnt and Wnt-related molecule expression in human OA. (**a**) Affected articular cartilage represents matrix degradation, including loss of cartilage matrix, fissure formation, erosion, and loss of cartilage structure. Inflammatory changes in the synovium (synovitis), osteophyte formation, and thickening of subchondral bone are also observed. (**b**) Wnts and Wnt-related genes/proteins that are upregulated or downregulated in the articular cartilage and synovial tissues.

of other synovial joint structures.[19–21] Synovial joints are originated by a specific population of mesenchymal cells, called interzone cells,[20,21] and thereby the cell population making articular cartilage is likely to be different from that of the growth plate. The mature articular cartilage constitutes zonal organization that are divided into the superficial layer, the transition or mid layer, the deep or radial layer, and the calcified layer in order to form the surface of articular cartilage, and is lined by subchondral bone[22–24] (Figure 2b). Unfortunately, articular cartilage has limited regenerative capacity and can proceed toward degeneration once damaged. Most common conditions affecting articular cartilage are OA and rheumatoid arthritis (RA).[25,26] OA is a multifactorial disorder that can be caused or associated with aging and other environmental risk factors including obesity, repetitive joint injury, and joint malalignment. OA is characterized by many pathological features (Figure 3a). The affected articular cartilage represents loss of cartilage matrix and defects in structure such as fissure formation, erosion, and loss. The articular chondrocytes may proliferate making clusters and acquire the hypertrophic phenotype. Although articular cartilage is a major target tissue in OA, inflammatory changes in the synovium (the lining tissue of the joint capsule), osteophyte formation, and thickening of subchondral bone underneath articular cartilage are also observed in OA, and may contribute to initiation or progression of OA. RA is a chronic and progressive autoimmune disease of unknown etiology. T and B cells are activated at the earliest phases of the disease and inflammatory cytokines have a considerable importance in the hierarchy of the RA pathogenesis. In RA, histological changes are apparent in the synovium, which are characterized by proliferation of synoviocytes and infiltration of inflammatory cells and active angiogenesis. The hypertrophized synovium, called pannus invade covering articular cartilage surface and erodes articular cartilage and bone.

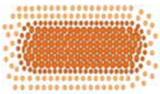
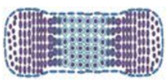
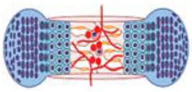
Wnt SIGNALING IN GROWTH PLATE DURING LONG BONE DEVELOPMENT AND GROWTH

Discovery of Wnt Roles in Developing Cartilage

Wnt signaling pathways can be classified into two modes through dependency of β-catenin: the β-catenin-dependent pathway is called a canonical Wnt/β-catenin signaling and the other independent pathway is collectively called the non-canonical pathway.[2,27,28] The former pathway is initiated by binding of Wnts to the Wnt receptor (frizzled)/co-receptor (lipoprotein receptor-related protein-LRP-5/6) complex, triggering a conformational change in the downstream molecule complex that consist of Dishevelled, adenomatosis polyposis coli (APC), axin, glycogen synthase kinase 3β (GSK3β), β-catenin, and other proteins. Through sequential changes in interaction and phosphorylation status of these proteins, phosphorylation of the amino-terminal domain of β-catenin is disturbed, leading to stabilization and nuclear translocation of β-catenin. The nuclear-translocated β-catenin stimulates transcription of the target genes with co-transcription factors such as T-cell factor/lymphoid-enhancing factor (TCF/LEF) family. The non-canonical Wnt pathway is as important as the canonical pathway. The intercellular signaling molecules involved in the non-canonical pathway are many, including inositol triphosphate (IP3)-intercellular calcium, MAPKs, and G-protein RhoA/Rho-associated kinase (Rock). The receptors/co-receptors that mediate these signaling include frizzled, receptor tyrosin kinase (Ryk), and tyrosine-protein kinase transmembrane receptor (Ror2).

Direct participation of Wnt signaling in cartilage development was initially indicated by studies looking for expression

Table 1 Role of Wnts and Wnt-related molecules in cartilage development

	Role in cartilage development	
Developmental events	Stimulation or requirement	Inhibition
 Chondrogenesis	Wnt 5a,[30,36] Wnt 5b,[36] and Frzb/Frp3/Sfrp3[30–32,37]	Wnt 1,[29,36] Wnt 4,[30,36] Wnt 8a,[37] Wnt 9a,[33,39,40] Wnt 11,[36] and Wnt/β-catenin[14,33,39,40]
 Growth plate assembly columnar formation	Wls,[49,60] Wnt 5a,[30,63] and Ror2[63]	Excess Wnt/β-catenin[14,39,40]
 Hypertrophy mineralization	Wnt 9a,[30,39] Wnt 4,[30] Wnt/β-catenin,[33,39,41,43–45] and Lrp5/6[46]	Frzb/Frp3/Sfrp3,[37] Wnt 5a,[30,60] and ICAT[50]
 Perichondrial bone formation	Wnt/β-catenin[43,45]	

A number of Wnts and Wnt-related molecules have been implicated in the regulation of cartilage development. The regulation of Wnt signaling is not only dependent on the presence of ligands but also on the action of endogenous antagonists of Wnt signaling.

patterns of Wnts and a Wnt antagonist—Frzb/Frp3/Sfrp3 (frizzled-related protein/secreted frizzled-related protein 3)—in chick developing limb cartilage.[29–33] Wnt 4, 5a, 5b, 11, and 14 are differentially expressed in the cartilage, perichondrium, and surrounding tissues: Wnt 4 and Wnt14 are dominantly found in the joint regions between two skeletal elements; Wnt 5a is broadly expressed in cartilage from epiphyseal end to metaphysis; Wnt 5b is present in prehypertrophic zone; and Wnt 11 is expressed in the perichondrium during embryonic limb development. Expression patterns of Wnts, frizzleds, Lrp5/6, and extracellular Wnt antagonists, such as Frps/Sfrps and Dickkopfs (DKKs) have been also reported by several studies in chick or mouse developing limb cartilage.[34,35]

The actions of Wnts and Frzb/Frp3/Sfrp3 on chondrogenesis were uncovered using chick embryo or chick limb-bud culture system with retrovirus-driven gene expression (summarized in Table 1). Overexpression of Wnt 5a, 5b, or Frzb/Frp3/Sfrp3 in the developing limbs or limb-bud cultures represents stimulation of chondrogenesis, differentiation of mesenchymal cells to chondrocytes, while overexpression of Wnt 1, Wnt 4, or Wnt 8A inhibits it.[29–32,36–38] In addition to the actions of individual Wnts on chondrogenesis, studies have demonstrated that overexpression of the constitutive active mutant of β-catenin stimulates chondrocyte hypertrophy and endochondral ossification in chick developing limbs.[30,39] In contrast, overexpression of Frzb/Frp3/Sfrp3 and Wnt 5a inhibit chondrocyte hypertrophy, matrix mineralization, and endochondral ossification.[30,37] Specificity of Wnts and Wnt regulators to Wnt signaling pathways (either canonical or non-canonical pathways) are not absolutely restricted and may vary among cell types depending on presence of other regulatory molecules. However, Wnt 1, Wnt 4, and Wnt 8a generally stimulate Wnt/β-catenin signaling while Frzb/Frp3/Sfrp3 inhibits it. Thus, the findings above implicate that β-catenin-mediated canonical Wnt signaling exhibits at least two actions: inhibition of chondrogenesis and stimulation of chondrocyte hypertrophy.

The Roles of Wnt/β-Catenin Signaling in Regulation of Growth Plate Function in Mice

The actions and roles of Wnts and Wnt/β-catenin signaling have been extensively investigated in the transgenic mouse system (Table 1). Mouse embryos that express a constitutive active form of β-catenin mutant in the developing cartilage under the control of *collagen 2* promoter/enhancer exhibit severe dwarfism and skeletal deformity. The cartilage elements strongly reduce synthesis of cartilage matrix molecules and fail to organize growth plate structure.[14,39,40] When β-catenin signaling is activated at a later time point, stimulation of perichondrial bone formation and angiogenesis are observed,[41] and growth plate closure is induced postnatally.[42] These findings indicate that Wnt/β-catenin signaling should be restrictedly controlled for normal cartilage development, and excessive activation of this signaling can lead skeletal disorders.

Inactivation of β-catenin signaling also induces severe skeletal deformity in mice. The conditional ablation of β-catenin in the skeletal cells in the limb using *Prx1/Prrx1* or *Dermo* promoter strongly impairs long bone formation with inhibition of chondrocyte hypertrophy and osteoblast differentiation.[43–45] Ablation of β-catenin using *collagen 2* promoter/enhancer also reveals a similar phenotype in both chondrocytes and osteoblasts in embryos: appearance of the hypertrophic zone expressing *collagen 10* and cartilage matrix calcification are disturbed or delayed; and bone formation is suppressed and characterized by a decrease in gene or protein expression of osteoblast markers, Runx2, Osterix, collagen 1, and alkaline phosphatase.[44,46] The results from these β-catenin conditional knockout mice provide clear evidence that β-catenin mediates an essential pathway to control cartilage and bone development. β-Catenin is a multifunctional molecule, therefore, we should be careful to consider whether the mouse phenotype represents the role of β-catenin-dependent Wnt signaling or reflects other β-catenin function. The conditional double knockout mice for *Lrp5* and *Lrp6* showed similar phenotype to that of β-catenin conditional mutant mice,[47] suggesting that skeletal precursor cells and chondrocytes redundantly utilize Lrp5/6 as a co-receptor of Wnts mediating Wnt/β-catenin signaling. On the contrary, Zhong *et al*[48] have reported that ablation of *Lrp5* and *Lrp6* under the control of *collage 2* promoter did not cause skeletal abnormality, while *Wls* (*Wntless*, an essential protein for Wnt secretion, also called *GRP177* or *Evi*) ablation by the same *collagen 2* promoter induces sever impairment of skeletal formation, and suggest less contribution of β-catenin-dependent Wnt signaling to early cartilage and skeletal development.[48] Thus, the mechanism underlying skeletal defects in the β-catenin mutants needs to be further elucidated. The β-catenin signaling activity in the developing cartilage has been monitored by immunohistochemical staining of β-catenin or target molecules such as Lef1, Tcf1, and Axin2, and analysis of reporter activity in the Wnt reporter mice.[37,44,45,49,50] Summarizing the results, β-catenin signaling is active in the entire growth plate at the early phase of development and becomes limited to hypertrophic zone. However, further evaluation is required for verification of the results since sensitivity of the antibodies used may vary among the experiments, and the Wnt reporter mice generally have limitation to show the signaling activity at postnatal and adult stages.

Wnt/β-catenin signaling is still required for postnatal endochondral ossification. The results from the mice expressing the β-catenin-interacting protein (ICAT) under control of *collagen 2* promoter/enhancer indicate that a decrease in Wnt/β-catenin signaling reduces height of the hypertrophic zone, decreases cell proliferation, and increases cell apoptosis.[49] Similar inhibition of hypertrophic differentiation of chondrocytes is also observed in the β-catenin conditional knockout mouse that induces ablation of β-catenin postnatally.[51] In addition, the growth plate shows derangement of cell alignment with loss of slow-cell cycle cells in the reservoir zone of growth plate.[51] Inhibition of β-catenin signaling delays formation of secondary ossification center characterized by the delay of cavity formation and angiogenesis and decreases in the expression of Vegf and Mmp13.[49] Furthermore, β-catenin deficiency in cartilage induces ectopic cartilaginous masses in perichondrium and periosteum that resemble osteochondromas.[52]

The phenotype of β-catenin mutants also indicates that cartilage and bone development may be controlled in an indispensable manner. Indeed, β-catenin signaling is critical for specification of skeletogenic cell lineage to osteoblasts since β-catenin deficiency stimulates chondrogenic differentiation of skeletal precursor cells and induces ectopic cartilage tissue in the skull and periosteum in long bones at embryonic stages.[43,45] Recent studies demonstrate that β-catenin-deficient chondrocytes decrease osteoprotegerin (Opg) expression or increase RANKL (receptor activator of nuclear factor kappa-B ligand) expression/activity, resulting in an increase in osteoclast formation and decreases in trabecular bone formation and remodeling.[53,54] In addition to the interaction between chondrocytes and other skeletal cells, we should consider an additional control point of Wnt/β-catenin signaling in interplay between cartilage and bone. Several independent research groups have demonstrated that the cells marked by promoter activities of genes (*collagen 2*, *collagen 10*, *aggrecan*, and *Sox9*) associated with chondrocytes/chondrogenic precursors not only reside in the cartilage but also in metaphysis and diaphysis as osteoprogenitors, osteoblasts and osteocytes over time.[55–58] The findings suggest a challenging dogma against the classical consensus that chondrocytes and osteoblasts are derived from distinct lineages. It is very important and interesting to examine whether Wnt/β-catenin signaling regulates such lineage transition, if it is.

In contrast to canonical Wnt/β-catenin signaling pathway, studies on non-canonical pathway in cartilage are limited at this moment although Wnts involved in non-canonical pathway, Wnt 5a and Wnt 11, are expressed in developing cartilage, and Wnt 5a is one of the most dominant Wnts in cartilage.[59] General knockout of *Wnt 5a* and *collagen 2* promoter-directed overexpression of Wnt 5a exhibits shortened limbs with severe skeletal deformity and delay of chondrocyte hypertrophy and endochondral bone formation,[60] suggesting that control of Wnt 5a expression/activity is crucial for cartilage and growth plate development. Wnt 5a is a major Wnt that regulates planar cellular polarity (PCP), which originally refers to the polarity of epithelial cells in a plane during development and organization.[61] This concept can adapt to elongation and cell alignment in other organs. Thus, limb elongation, longitudinal growth of long bones, and columnar structuring of growth plate chondrocytes may be studied as targets of the PCP pathway.[2] Gao *et al*[62] have demonstrated genetic and physical interaction of Wnt 5a with Ror2 (Wnt-binding receptor tyrosine kinase (RTK)) and Vangl2 (Van Gogh (Vang)-like 2) in limb development: *Wnt 5a* null, *Ror2* null, and *Vangl2* mutant or null mice display similar shortening of limbs and cartilage elements; digit chondrocytes in *Ror2* null and *Wnt 5a* null do not show asymmetric localization of Vangl2; and Wnt 5a enhances complex formation of Ror2 and Vangl2 and phosphorylation of Vangl2. These findings indicate that Wnt 5a with Vangl2 and Ror2 regulate limb elongation and chondrocyte arrangement via PCP pathway. Similar to Ror2, Ryk functions as a regulator of PCP pathway in mouse limb development.[63] Involvement of Wnts, likely Wnt 5a in columnar arrangement of growth plate chondrocytes has been indicated by analysis of conditional knockout of *Wls* (*Wntless/GPR177/Evi*).[59] Studies with Wls mutants have provided evidence that Wnts secreted from chondrogenic and osteogenic cells supports skeletal development via canonical and non-canonical Wtn signaling pathways.[48,59]

Wnt Signaling in Genetic Skeletal Diseases

In contrast to ample evidence from animal studies, there are limited number of reports or cases showing that Wnt signaling regulates and is required for cartilage development and growth in human. Robinow syndrome, autosomal dominant (MIM180700), and autosomal recessive (MIM268210) are associated with *WNT5A* and *ROR2* mutations, respectively. They are rare skeletal dysplasia syndromes, characterized by dysmorphic features resembling a fetal face, mesomelic limb shortening, hypoplastic external genitalia in males, and renal and vertebral anomalies.[64] *ROR2* mutation is also linked to brachydactyly, type B1 (MIM113000) that exhibits malformation of the distal phalanges.[65] These genetic features provide evidence that Wnt-mediated PCP pathway is important in the formation and morphogenesis of cartilage skeletal elements in humans.

Genetic mutations in the genes involving Wnt/β-catenin signaling have been reported to be associated with bone disorders. These genes include *LRP5* and *sclerostin* (SOST, an inhibitor of Wnt/β-catenin signaling). *LRP5* mutations are found in osteoporosis-pseudoglioma syndrome (OPPG, MIM259770) characterized by reduction in bone mass, impairment with bone fragility, or osteoporosis by young adulthood. *LRP5* mutations are also associated with other bone disorders such as osteosclerosis (MIM144750), van Buchem disease, type 2 (MIM:607636), and endosteal hyperostosis (MIM:144750). *Sclerostin* mutations are identified in craniodiaphyseal dysplasia (MIM122860), sclerosteosis 1 (MIM269500), and van Buchem disease (hyperostosis corticalis generalisata, MIM239100). *In vitro* culture studies and transgenic mouse studies reveal that the bone phenotype in the patients illustrates function of LRP5 and SOST in bone homeostasis: loss-of-function mutations of *LRP5* is associated with low bone mass and gain-of-function mutations with high bone mass, and *SOST* mutations behave in an opposite way. The phenotype of these bone disorders is generally very evident in skulls or jaws and can be found in cortical bones, however, not associated with growth retardation. Thus, it is unlikely that these gene mutations strongly and directly give impact on cartilage formation and growth plate function. Besides, LRP5 and SOST are not highly expressed in cartilage. However, the mouse experiments reveal an intimate linkage between the cartilage and bone,[55–58] we should keep the possibility in our mind that these mutations might impact cartilage-derived cells, resulting in bone phenotype. Further, we should consider significance of this pathway in cross-interaction with other signaling pathways such as FGF, TGFβ/BMP, and Hedgehog pathways. Mutations of many genes involving these pathways have been found in genetic skeletal diseases in humans.

Wnt SIGNALING IN OA

Expression of Wnts and Wnt-Related Genes in OA

Alterations of expression of Wnts and Wnt-related molecules are found in OA in humans although the entire profile still remains to be clarified and the findings may require further validation (Figure 3b). Nakamura *et al*[66] have examined gene expression of Wnt-related genes/proteins in articular cartilage, bone, and synovial tissue taken from the OA and RA patients and have found that *Wnt 7b* expression is most closely linked to both OA and RA: the expression is high in articular cartilage in OA as well as synovium in RA. In addition, upregulation of *WISP-1* (*Wnt 1-inducible-signaling pathway protein 1*) is detected in human OA cartilage and synovium and in mouse OA cartilage.[67] The bone samples were collected from the patients with hip fracture or hip/knee OA and subjected to expression analysis of Wnt signaling genes. Out of 86 genes, 7 (BCL9, FZD5, DVL2, EP300, FRZB/FRP3/SFRP3, LRP5, and TCF7L1) were differentially expressed in the knee or hip OA compared to the fracture samples, suggesting an alteration of Wnt signaling in bone, possibly subchondral bone in OA.[68] A comprehensive analysis of expression of Wnt signaling molecules using an *ex vivo* injury model of human cartilage have shown upregulation of *Wnt 16* and Wnt target genes (such as *c-MYC*, *CYCLIN D1*, and *AXIN 2*), downregulation of *FRZB/FRP3/SFRP3* and nuclear localization of β-catenin,[69] suggesting that injury induces activation of Wnt/β-catenin signaling in human articular cartilage. Nuclear localization of β-catenin has been reported in articular chondrocytes,[70] osteoblasts, and osteoclasts[71] in human OA samples. OA also alters expression of Wnt antagonists. An increase in gene expression of *DKK1*, an antagonist of Wnt/β-catenin signaling, is detected in affected articular cartilage and synovium in OA patients. DKK1 proteins are detected in both plasma and synovial fluid derived from patients with primary knee OA, and that the DKK1 amount is inversely related to radiographic grading of knee OA.[72] SOST, a potent inhibitor of canonical Wnt signaling pathway by binding to LRP5/6, is reported to be expressed in a late stage of human OA. The protective role of SOST has been demonstrated in sheep and mouse surgically induced OA models.[73] Imai *et al*[74] showed increased expression of *FRP1*, *FRP2*, and *FRP4* mRNA in synovial tissues of OA patients.

Studies have been conducted to examine expression of Wnts and Wnt-related genes in animal models. Spontaneous OA mouse model (STR/Ort mice) and collagenase-induced OA mouse model are used to examine expression of Wnt signaling genes by qPCR.[67] In articular cartilage, Wnt 9A and Wnt 16 are upregulated at the late stage of OA in the *STR/Ort* mouse, but not in the collagenase-induced OA model. In synovium, Wnt expression also changes in a differential manner between these two models: Wnt 2B, Wnt 5B, Wnt 6, and Wnt 16 increase at early phases in both models; and other Wnts including Wnt 2B, Wnt 3, Wnt 7B, and Wnt 9B are also upregulated in STR/Ort mice. WISP-1 expression is strongly increased in both synovium and cartilage in these OA models.[67] Expression of DKK1 is upregulated in both articular cartilage and synovium in the surgery or virus-induced OA mouse model.[75]

In OA, the Wnts and Wnt-related proteins expressed in articular cartilage and synovium may trigger many types of cellular responses directly or indirectly, resulting in cartilage destruction. Canonical and non-canonical Wnt signaling pathways would stimulate expression of matrix proteases including matrix metalloproteinases (Mmp 2, 3, 9, 13, and 14) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs)-4 and 5 in chondrocytes as previously reported[39,76,77] and induce cartilage matrix degradation. Further, both pathways are involved in many aspects of pathological and physiological angiogenesis,[78,79] and Wnt-7b-transfected synovial cells increase expression of tumor necrosis factor-alpha, interleukin-1β and interleukin-6.[66]

Wnt Signaling in OA Animal Models

Frzb (*Frp3*/*Sfrp3*) null mice display severe cartilage loss compared to wild-type control mice in several OA models (joint instability surgery and intra-articular injection of enzymes).[80,81] Immunohistochemical analysis revealed that *Frzb/Frp3/Sfrp3* null articular cartilage contains more cells exhibiting nuclear translocation of β-catenin after OA induction.[81] In addition, *Frzb/Frp3/Sfrp3* null mice show upregulation of expression of *Mmp3*, but not *Mmp9*, *Mmp13*, *Adamts4*, or *Adamts5* in the cartilage.[81] Recombinant Frzb/Frp3/Sfrp3 inhibits recombinant Mmp3 activity in the *in vitro* assay, indicating a direct inhibition of interaction between the two proteins.[81] However, Frzb/Frp3/Sfrp3 counteracts the action of Wnts on hypertrophic differentiation of chondrocytes in the culture, suggesting that Frzb/Frp3/Sfrp3 would affect the Wnt action at a cellular level as well.[37]

Interestingly, both gain- and loss-of function of β-catenin in cartilage induce osteoarthritic changes in mice. Changes in articular cartilage with the gain-of-function include cell clustering, surface fibrillation, vertical clefts, and chondrophyte/osteophyte formation,[70] whereas the loss-of-function induces a significant increase in number of apoptotic cells[82] and loss of superficial zone and *proteoglycan-4* (*Prg4*) expression.[83] The findings suggest Wnt/β-catenin signaling is a pro-factor for OA in articular cartilage, while a certain level of this signaling is required for maintenance of a healthy status of articular cartilage. The latter idea is supported by the results obtained from the *Lrp6* mutant mice that display apoptosis in articular cartilage and osteoarthritic changes.[84]

Dkk1 likely has multiple roles in OA. Systemic administration of Dkk1 antisense oligonucleotides ameliorated osteoarthritic changes in articular cartilage and subchondral bone in rats.[85] In contrast, forced expression of Dkk1 under the control of *collagen 2a1*[75] or *collagen 1a1*[86] promoter inhibited destruction of articular cartilage in mice. These findings are contradictory regarding the action of Dkk1 in OA. This may be due to a difference in target tissues: systemic administration affects other tissues in addition to cartilage and bone, while the transgenic mouse experiments only targeted cartilage or bone. One of other target tissues may be synovium since Dkk1 enhances angiogenic and inflammatory response and matrix degradation activity in synovial fibroblasts.[72]

Human Genetic Studies in OA

OA is generally considered to be a multifactorial disorder including age and environmental risk factors—obesity, repetitive joint injury, joint malalignment, and genetic risk variants. Loughlin *et al*[87,88] have reported an association between loci 2q31.1 and hip OA, and revealed that the Wnt antagonist, *FRZB/FRP3/SFRP3*, has emerged as gene associated with an increased risk for OA. They have demonstrated that a single-nucleotide polymorphism (SNP) in *FRZB/FRP3/SFRP3* resulting Arg324Gly substitution at carboxyl terminus is associated with hip OA in Caucasian females (and that this variant shows reduced Wnt inhibitory activity.[88] Association of this SNP in *FRZB/FRP3/SFRP3* with OA has been reported in different populations.[89–91] However, large-scale meta-analysis of individual-level data fail to show statistical significance in *FRZB/FRP3/SFRP3* polymorphisms and haplotypes.[92] Further, the two population-based cohort studies (the Rotterdam Study and the Chingford Study) have not detected association among FRZB/FRP3/SFRP3, LRP5, and LRP6 variants with radiographic OA.[93] Additional studies would be required to reach the final conclusion since definition of OA phenotype, quality of scoring, number of cases, and number of control vary among studies. Using genome-wide association studies in OA, Castano Betancourt *et al*[94] demonstrate the correlation between radiographic joint-space widths and the SNP on the gene DOT1-like histone H3 methyltransferase (DOT1L). DOT1L has been demonstrated to exert epigenetic modulation of the β-catenin/TCF4 (TCF7L2) target-gene transcription complex using the *Drosophila* model,[95,96] suggesting that DOT1L may regulate articular cartilage function via Wnt/β-catenin signaling pathway. Association between the same SNP and the risk of OA was reported in a Chinese Han population.[97] Relation of DOT1L with Wnt/β-catenin signaling needs to be clarified in mammalian cells.

CONCLUDING REMARKS

Extensive studies on Wnt signaling using animal models such as *in ovo* avian embryo and transgenic mouse systems reveal multi-function of Wnt signaling in regulation of cartilage and skeletal development. Specifically, the findings emphasize the importance of β-catenin-dependent canonical Wnt signaling in control of many aspects, ranging from the initial step of cartilage formation to the last process of endochondral ossification. It is not still unclear which mechanism(s) underlies the spatiotemporal regulation of this signaling activity. It must be exerted by combination of Wnts, Wnt receptors, and modulators. Further, the Wnt pathway interacts with other signaling pathways including BMP/TGFβ,[98] Hedgehog,[46] retinoid,[99] and epidermal fibroblast growth factor receptor (EGFR)[100] pathways in chondrocytes. These pathways are also center players in regulating cartilage and skeletal development.[8–10] Previous studies generally evaluate the role of Wnt signaling pathway by performing loss-of- and gain-of-function experiments, and have concluded that the inductive changes are responsible for activation or inactivation of Wnt signaling. However, we should revisit the results and re-evaluate the role of Wnt signaling as one of network pathways that regulate cartilage development.

Human genetics and animal experiments provide evidence that canonical Wnt signaling participates in the pathogenesis of OA. The target tissues of this signaling in OA likely are not only articular cartilage but also synovium and subchondral bone. It is likely that excessive activation of Wnt/β-catenin signaling enhances articular cartilage destruction and possibly promotes subchondral bone remodeling. Articular cartilage has very limited reparative capacity. It is very difficult and may not be realistic to attempt to regenerate articular cartilage once severely destructed. Thus, we should make efforts to develop a method that delays osteoarthritic degenerative changes and enhances regenerative potential of articular chondrocytes or progenitors at an early phase of OA. Complete inhibition of Wnt/β-catenin signaling induced cell apoptosis[82] and gave a negative impact on articular progenitors,[83] while transient activation of this signaling thickened a surface layer of articular cartilage.[42] From this point of view, mild and transient activation of Wnt/β-catenin signaling may be effective to enhance cell survival and regenerative potential.

Although animal experiments are essential to elucidate the cellular and molecular mechanisms by which Wnt signaling regulates cartilage biology and pathology, we should be careful to apply the findings and knowledge to humans. Diversity and complexity in regard to human biology must be considered to be much larger compared to other organisms since humans are the apex of evolution of biological complexity. Thus, we should not overestimate the results of animal experiments and use them to obtain overall trends of Wnt signaling roles in cartilage during skeletal development and in skeletal disorders. A large population of aged people is affected by OA sooner or later. Thus, etiology of OA may vary. Wnt signaling must be involved in a certain number of OA patients, but such subpopulation still contains variability in extent, quality, and the target tissue of Wnt signaling. Precision medicine will be required to define whether, and which specific types of Wnt signaling is involved in individual conditions.

ACKNOWLEDGMENTS

This work is partially supported by the National Institutes of Health RO1 Grants: AR046000, AR062908, and AR056837 and Arthritis Foundation Innovative Research award (#6389).

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

1. Capdevila J, Izpisua Belmonte JC. Patterning mechanisms controlling vertebrate limb development. Annu Rev Cell Dev Biol 2001;17: 87–132.
2. Yang Y. Wnt signaling in development and disease. Cell Biosci 2012;2: 14.
3. Baron R, Kneissel M. WNT signaling in bone homeostasis and disease: from human mutations to treatments. Nat Med 2013;19:179–192.
4. Lories RJ, Corr M, Lane NE. To Wnt or not to Wnt: the bone and joint health dilemma. Nat Rev Rheumatol 2013;9:328–339.
5. Moss ML, Moss-Salentijn L. Vertebrate cartilage. In: Hall BK (ed). *Cartilage*. Academic Press Inc: New York, 1983, pp 1–24.
6. Nishimura R, Hata K, Ono K *et al.* Regulation of endochondral ossification by transcription factors. Front Biosci (Landmark Ed) 2012;17:2657–2666.
7. Yeung Tsang K, Wa Tsang S, Chan D *et al.* The chondrocytic journey in endochondral bone growth and skeletal dysplasia. Birth Defects Res C Embryo Today 2014;102:52–73.
8. Long F, Ornitz DM. Development of the endochondral skeleton. Cold Spring Harb Perspect Biol 2013;5:a008334.
9. Ballock RT, O'Keefe RJ. Physiology and pathophysiology of the growth plate. Birth Defects Res C Embryo Today 2003;69:123–143.
10. Adams SL, Cohen AJ, Lassova L. Integration of signaling pathways regulating chondrocyte differentiation during endochondral bone formation. J Cell Physiol 2007;213:635–641.
11. Krakow D. Skeletal dysplasias. Clin Perinatol 2015;42:301–319.
12. Teven CM, Farina EM, Rivas J *et al.* Fibroblast growth factor (FGF) signaling in development and skeletal diseases. Genes Dis 2014;1: 199–213.
13. Akiyama H, Chaboissier MC, Martin JF *et al.* The transcription factor Sox9 has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of Sox5 and Sox6. Genes Dev 2002;16:2813–2828.
14. Akiyama H, Lyons JP, Mori-Akiyama Y *et al.* Interactions between Sox9 and beta-catenin control chondrocyte differentiation. Genes Dev 2004;18:1072–1087.
15. Smits P, Dy P, Mitra S *et al.* Sox5 and Sox6 are needed to develop and maintain source, columnar, and hypertrophic chondrocytes in the cartilage growth plate. J Cell Biol 2004;164:747–758.
16. Dy P, Wang W, Bhattaram P *et al.* Sox9 directs hypertrophic maturation and blocks osteoblast differentiation of growth plate chondrocytes. Dev Cell 2012;22:597–609.
17. Komori T, Yagi H, Nomura S *et al.* Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell 1997;89:755–764.
18. Ueta C, Iwamoto M, Kanatani N *et al.* Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes. J Cell Biol 2001;153:87–100.
19. Pacifici M, Koyama E, Shibukawa Y *et al.* Cellular and molecular mechanisms of synovial joint and articular cartilage formation. Ann NY Acad Sci 2006;1068:74–86.
20. Archer CW, Dowthwaite GP, Francis-West P. Development of synovial joints. Birth Defects Res C Embryo Today 2003;69:144–155.
21. Khan IM, Redman SN, Williams R *et al.* The development of synovial joints. Curr Top Dev Biol 2007;79:1–36.
22. Las Heras F, Gahunia HK, Pritzker KP. Articular cartilage development: a molecular perspective. Orthop Clin North Am 2012;43:155–171.
23. Becerra J, Andrades JA, Guerado E *et al.* Articular cartilage: structure and regeneration. Tissue Eng Part B Rev 2010;16:617–627.
24. Poole AR. What type of cartilage repair are we attempting to attain? J Bone Joint Surg Am 2003;85-A(Suppl 2):40–44.
25. Goldring MB, Goldring SR. Osteoarthritis. J Cell Physiol 2007;213: 626–634.

26. Firestein GS. Evolving concepts of rheumatoid arthritis. Nature 2003;423:356–361.
27. Clevers H, Nusse R. Wnt/beta-catenin signaling and disease. Cell 2012;149:1192–1205.
28. Cadigan KM, Waterman ML. TCF/LEFs and Wnt signaling in the nucleus. Cold Spring Harb Perspect Biol 2012;4:pii: a007906.
29. Rudnicki JA, Brown AM. Inhibition of chondrogenesis by Wnt gene expression in vivo and in vitro. Dev Biol 1997;185:104–118.
30. Hartmann C, Tabin CJ. Dual roles of Wnt signaling during chondrogenesis in the chicken limb. Development 2000;127: 3141–3159.
31. Wada N, Kawakami Y, Ladher R *et al*. Involvement of Frzb-1 in mesenchymal condensation and cartilage differentiation in the chick limb bud. Int J Dev Biol 1999;43:495–500.
32. Duprez D, Leyns L, Bonnin MA *et al*. Expression of Frzb-1 during chick development. Mech Dev 1999;89:179–183.
33. Hartmann C, Tabin CJ. Wnt-14 plays a pivotal role in inducing synovial joint formation in the developing appendicular skeleton. Cell 2001;104:341–352.
34. Witte F, Dokas J, Neuendorf F *et al*. Comprehensive expression analysis of all Wnt genes and their major secreted antagonists during mouse limb development and cartilage differentiation. Gene Expr Patterns 2009;9:215–223.
35. Loganathan PG, Nimmagadda S, Huang R *et al*. Comparative analysis of the expression patterns of Wnts during chick limb development. Histochem Cell Biol 2005;123:195–201.
36. Church V, Nohno T, Linker C *et al*. Wnt regulation of chondrocyte differentiation. J Cell Sci 2002;115:4809–4818.
37. Enomoto-Iwamoto M, Kitagaki J, Koyama E *et al*. The Wnt antagonist Frzb-1 regulates chondrocyte maturation and long bone development during limb skeletogenesis. Dev Biol 2002;251: 142–156.
38. Enomoto-Iwamoto M, Kitagaki J, Koyama E *et al*. Dual roles of the Wnt antagonist, Frzb-1 in cartilage development. In: Shapiro IM, Boyan BD, Anderson HC (eds). *The Growth Plate*. IOS Press: Amsterdam, 2002, pp 235–244.
39. Tamamura Y, Otani T, Kanatani N *et al*. Developmental regulation of Wnt/beta-catenin signals is required for growth plate assembly, cartilage integrity, and endochondral ossification. J Biol Chem 2005;280:19185–19195.
40. Guo X, Day TF, Jiang X *et al*. Wnt/beta-catenin signaling ins sufficient and necessary for synovial joint formation. Genes Dev 2004;18: 2404–2417.
41. Dao DY, Jonason JH, Zhang Y *et al*. Cartilage-specific beta-catenin signaling regulates chondrocyte maturation, generation of ossification centers, and perichondrial bone formation during skeletal development. J Bone Miner Res 2012;27:1680–1694.
42. Yuasa T, Kondo N, Yasuhara R *et al*. Transient activation of Wnt/{beta}-catenin signaling induces abnormal growth plate closure and articular cartilage thickening in postnatal mice. Am J Pathol 2009;175: 1993–2003.
43. Hill TP, Spater D, Taketo MM *et al*. Canonical Wnt/beta-catenin signaling prevents osteoblasts from differentiating into chondrocytes. Dev Cell 2005;8:727–738.
44. Hu H, Hilton MJ, Tu X *et al*. Sequential roles of Hedgehog and Wnt signaling in osteoblast development. Development 2005;132: 49–60.
45. Day TF, Guo X, Garrett-Beal L *et al*. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell 2005;8: 739–750.
46. Mak KK, Chen MH, Day TF *et al*. Wnt/beta-catenin signaling interacts differentially with Ihh signaling in controlling endochondral bone and synovial joint formation. Development 2006;133:3695–3707.
47. Joeng KS, Schumacher CA, Zylstra-Diegel CR *et al*. Lrp5 and Lrp6 redundantly control skeletal development in the mouse embryo. Dev Biol 2011;359:222–229.
48. Zhong ZA, Zahatnansky J, Snider J *et al*. Wntless spatially regulates bone development through beta-catenin-dependent and independent mechanisms. Dev Dyn 2015;244:1347–1355.
49. Chen M, Zhu M, Awad H *et al*. Inhibition of beta-catenin signaling causes defects in postnatal cartilage development. J Cell Sci 2008;121: 1455–1465.
50. Dao DY, Yang X, Flick LM *et al*. Axin2 regulates chondrocyte maturation and axial skeletal development. J Orthop Res 2010;28: 89–95.
51. Candela ME, Cantley L, Yasuaha R *et al*. Distribution of slow-cycling cells in epiphyseal cartilage and requirement of beta-catenin signaling for their maintenance in growth plate. J Orthop Res 2014;32:661–668.
52. Cantley L, Saunders C, Guttenberg M *et al*. Loss of beta-catenin induces multifocal periosteal chondroma-like masses in mice. Am J Pathol 2012;182:917–927.
53. Wang B, Jin H, Zhu M *et al*. Chondrocyte beta-catenin signaling regulates postnatal bone remodeling through modulation of osteoclast formation in a murine model. Arthritis Rheumatol 2014;66: 107–120.
54. Golovchenko S, Hattori T, Hartmann C *et al*. Deletion of beta catenin in hypertrophic growth plate chondrocytes impairs trabecular bone formation. Bone 2013;55:102–112.
55. Ono N, Ono W, Nagasawa T *et al*. A subset of chondrogenic cells provides early mesenchymal progenitors in growing bones. Nat Cell Biol 2014;16:1157–1167.
56. Park J, Gebhardt M, Golovchenko S *et al*. Dual pathways to endochondral osteoblasts: a novel chondrocyte-derived osteoprogenitor cell identified in hypertrophic cartilage. Biol Open 2015;4: 608–621.
57. Zhou X, von der Mark K, Henry S *et al*. Chondrocytes transdifferentiate into osteoblasts in endochondral bone during development, postnatal growth and fracture healing in mice. PLoS Genet 2014;10: e1004820.
58. Yang L, Tsang KY, Tang HC *et al*. Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. Proc Natl Acad Sci USA 2014;111:12097–12102.
59. Zhu X, Zhu H, Zhang L *et al*. Wls-mediated Wnts differentially regulate distal limb patterning and tissue morphogenesis. Dev Biol 2012;365: 328–338.
60. Yang Y, Topol L, Lee H *et al*. Wnt5a and Wnt5b exhibit distinct activities in coordinating chondrocyte proliferation and differentiation. Development 2003;130:1003–1015.
61. Devenport D. The cell biology of planar cell polarity. J Cell Biol 2014;207:171–179.
62. Gao B, Song H, Bishop K *et al*. Wnt signaling gradients establish planar cell polarity by inducing Vangl2 phosphorylation through Ror2. Dev Cell 2011;20:163–176.
63. Andre P, Wang Q, Wang N *et al*. The Wnt coreceptor Ryk regulates Wnt/planar cell polarity by modulating the degradation of the core planar cell polarity component Vangl2. J Biol Chem 2012;287: 44518–44525.
64. Roifman M, Marcelis CL, Paton T *et al*. De novo WNT5A-associated autosomal dominant Robinow syndrome suggests specificity of genotype and phenotype. Clin Genet 2015;87:34–41.
65. Bacchelli C, Wilson LC, Cook JA *et al*. ROR2 is mutated in hereditary brachydactyly with nail dysplasia, but not in Sorsby syndrome. Clin Genet 2003;64:263–265.
66. Nakamura Y, Nawata M, Wakitani S. Expression profiles and functional analyses of Wnt-related genes in human joint disorders. Am J Pathol 2005;167:97–105.
67. Blom AB, Brockbank SM, van Lent PL *et al*. Involvement of the Wnt signaling pathway in experimental and human osteoarthritis: prominent role of Wnt-induced signaling protein 1. Arthritis Rheum 2009;60:501–512.
68. Velasco J, Zarrabeitia MT, Prieto JR *et al*. Wnt pathway genes in osteoporosis and osteoarthritis: differential expression and genetic association study. Osteoporos Int 2010;21:109–118.
69. Dell'accio F, De Bari C, Eltawil NM *et al*. Identification of the molecular response of articular cartilage to injury, by microarray screening: Wnt-16 expression and signaling after injury and in osteoarthritis. Arthritis Rheum 2008;58:1410–1421.
70. Zhu M, Tang D, Wu Q *et al*. Activation of beta-catenin signaling in articular chondrocytes leads to osteoarthritis-like phenotype in adult beta-catenin conditional activation mice. J Bone Miner Res 2008;24: 12–21.
71. Monaghan H, Bubb VJ, Sirimujalin R *et al*. Adenomatous polyposis coli (APC), beta-catenin, and cadherin are expressed in human bone and cartilage. Histopathology 2001;39:611–619.

72. Honsawek S, Tanavalee A, Yuktanandana P *et al*. Dickkopf-1 (Dkk-1) in plasma and synovial fluid is inversely correlated with radiographic severity of knee osteoarthritis patients. BMC Musculoskelet Disord 2010;11:257.
73. Chan BY, Fuller ES, Russell AK *et al*. Increased chondrocyte sclerostin may protect against cartilage degradation in osteoarthritis. Osteoarthritis Cartilage 2011;19:874–885.
74. Imai K, Morikawa M, D'Armiento J *et al*. Differential expression of WNTs and FRPs in the synovium of rheumatoid arthritis and osteoarthritis. Biochem Biophys Res Commun 2006;345:1615–1620.
75. Oh H, Chun CH, Chun JS. Dkk-1 expression in chondrocytes inhibits experimental osteoarthritic cartilage destruction in mice. Arthritis Rheum 2012;64:2568–2578.
76. Yuasa T, Otani T, Koike T *et al*. Wnt/beta-catenin signaling stimulates matrix catabolic genes and activity in articular chondrocytes: its possible role in joint degeneration. Lab Invest 2008;88:264–274.
77. Nalesso G, Sherwood J, Bertrand J *et al*. WNT-3A modulates articular chondrocyte phenotype by activating both canonical and noncanonical pathways. J Cell Biol 2011;193:551–564.
78. Maruotti N, Corrado A, Neve A *et al*. Systemic effects of Wnt signaling. J Cell Physiol 2013;228:1428–1432.
79. Reis M, Liebner S. Wnt signaling in the vasculature. Exp Cell Res 2013;319:1317–1323.
80. Thysen S, Luyten FP, Lories RJ. Loss of Frzb and Sfrp1 differentially affects joint homeostasis in instability-induced osteoarthritis. Osteoarthritis Cartilage 2015;23:275–279.
81. Lories RJ, Peeters J, Bakker A *et al*. Articular cartilage and biomechanical properties of the long bones in Frzb-knockout mice. Arthritis Rheum 2007;56:4095–4103.
82. Zhu M, Chen M, Zuscik M *et al*. Inhibition of beta-catenin signaling in articular chondrocytes results in articular cartilage destruction. Arthritis Rheum 2008;58:2053–2064.
83. Yasuhara R, Ohta Y, Yuasa T *et al*. Roles of beta-catenin signaling in phenotypic expression and proliferation of articular cartilage superficial zone cells. Lab Invest 2011;91:1739–1752.
84. Joiner DM, Less KD, Van Wieren EM *et al*. Heterozygosity for an inactivating mutation in low-density lipoprotein-related receptor 6 (Lrp6) increases osteoarthritis severity in mice after ligament and meniscus injury. Osteoarthritis Cartilage 2013;21:1576–1585.
85. Weng LH, Wang CJ, Ko JY *et al*. Control of Dkk-1 ameliorates chondrocyte apoptosis, cartilage destruction, and subchondral bone deterioration in osteoarthritic knees. Arthritis Rheum 2010;62: 1393–1402.
86. Funck-Brentano T, Bouaziz W, Marty C *et al*. Dkk-1-mediated inhibition of Wnt signaling in bone ameliorates osteoarthritis in mice. Arthritis Rheumatol 2014;66:3028–3039.
87. Loughlin J, Mustafa Z, Smith A *et al*. Linkage analysis of chromosome 2q in osteoarthritis. Rheumatology (Oxford) 2000;39:377–381.
88. Loughlin J, Dowling B, Chapman K *et al*. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci USA 2004;101:9757–9762.
89. Valdes AM, Loughlin J, Oene MV *et al*. Sex and ethnic differences in the association of ASPN, CALM1, COL2A1, COMP, and FRZB with genetic susceptibility to osteoarthritis of the knee. Arthritis Rheum 2007;56:137–146.
90. Lane NE, Lian K, Nevitt MC *et al*. Frizzled-related protein variants are risk factors for hip osteoarthritis. Arthritis Rheum 2006;54: 1246–1254.
91. Min JL, Meulenbelt I, Riyazi N *et al*. Association of the Frizzled-related protein gene with symptomatic osteoarthritis at multiple sites. Arthritis Rheum 2005;52:1077–1080.
92. Evangelou E, Chapman K, Meulenbelt I *et al*. Large-scale analysis of association between GDF5 and FRZB variants and osteoarthritis of the hip, knee, and hand. Arthritis Rheum 2009;60:1710–1721.
93. Kerkhof JM, Uitterlinden AG, Valdes AM *et al*. Radiographic osteoarthritis at three joint sites and FRZB, LRP5, and LRP6 polymorphisms in two population-based cohorts. Osteoarthritis Cartilage 2008;16:1141–1149.
94. Castano Betancourt MC, Cailotto F, Kerkhof HJ *et al*. Genome-wide association and functional studies identify the DOT1L gene to be involved in cartilage thickness and hip osteoarthritis. Proc Natl Acad Sci USA 2012;109:8218–8223.
95. Mohan M, Herz HM, Takahashi YH *et al*. Linking H3K79 trimethylation to Wnt signaling through a novel Dot1-containing complex (DotCom). Genes Dev 2010;24:574–589.
96. Mahmoudi T, Boj SF, Hatzis P *et al*. The leukemia-associated Mllt10/Af10-Dot1l are Tcf4/beta-catenin coactivators essential for intestinal homeostasis. PLoS Biol 2010;8:e1000539.
97. Zhou Y, Bi F, Yang G *et al*. Association between single nucleotide polymorphisms of DOT1L gene and risk of knee osteoarthritis in a Chinese Han population. Cell Biochem Biophys 2014;70:1677–1682.
98. Li TF, Chen D, Wu Q *et al*. Transforming growth factor-beta stimulates cyclin D1 expression through activation of beta-catenin signaling in chondrocytes. J Biol Chem 2006;281:21296–21304.
99. Yasuhara R, Yuasa T, Williams JA *et al*. Wnt/beta-catenin and retinoic acid receptor signaling pathways interact to regulate chondrocyte function and matrix turnover. J Biol Chem 2010;285:317–327.
100. Zhang X, Zhu J, Li Y *et al*. Epidermal growth factor receptor (EGFR) signaling regulates epiphyseal cartilage development through beta-catenin-dependent and -independent pathways. J Biol Chem 2013;288:32229–32240.