Wnt signaling in cartilage development and degeneration

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The Wnt signaling network, which is composed of Wnt ligands, receptors, antagonists, and intracellular signaling molecules, has emerged as a powerful regulator of cell fate, proliferation, and function in multicellular organisms. Over the past two decades, the critical role of Wnt signaling in embryonic cartilage and bone development has been well established, and much has been learnt regarding the role of Wnt signaling in chondrogenesis and cartilage development. However, relatively little is known about the role of Wnt signaling in adult articular cartilage and degenerative cartilage tissue. This review will briefly summarize recent advances in Wnt regulation of chondrogenesis and hypertrophic maturation of chondrocytes, and review data concerning the role of Wnt signaling in the maintenance and degeneration of articular chondrocytes and cartilage. [BMB reports 2008; 41(7): 485-494]

Wnt signaling components and pathways

The Wnt signaling components are a family of secreted glycoproteins, and 19 human Wnt genes have been identified to date (see Wnt homepage at http://www.stanford.edu/~rnusse/wntwindow.html). Wnt regulates a variety of biological processes including embryonic development, body patterning, tissue morphogenesis, and tumorigenesis (1). Wnt family members can be classified into two groups depending on their role in axis induction of Xenopus embryos (2) and in transformation of mammary epithelial cells (3): the Wnt-1 class (for example, Wnt-1, -3a, -7a, and -8), which activates the canonical Wnt pathway; and the Wnt-5a class (for example, Wnt-4, -5a, and -11), which activates the noncanonical Wnt pathway. Wnt ligands bind the seven transmembrane receptor frizzled (Fzd) and the co-receptor lipoprotein-related proteins 5 and 6 (LRP-5 and LRP-6) in the canonical Wnt pathway, whereas noncanonical pathways do not require LRP-5 and LRP-6 (1). Wnt signaling is antagonized by two families of antagonists: the Dickkopf (Dkk) family binds to LRP-5/6 and antagonize canonical pathway, whereas the secreted frizzled-related protein (sFRP) family binds directly to Wnt ligands and inhibits both canonical and noncanonical Wnt pathways (4) (Fig. 1).

In the canonical Wnt pathway, Wnt binding to Fzd and LRP receptors induces phosphorylation of Dishevelled (Dvl) by casein kinases, which in turn causes inhibition of glycogen synthase kinase (GSK)-3β. In the absence of Wnt signaling, GSK-3β phosphorylates β-catenin, resulting in its ubiquitination and proteasomal degradation. In the presence of Wnt signal, GSK-3β is inhibited and the unphosphorylated β-catenin is stable in the cytosol and travels into the nucleus where it acts as a co-activator with Tcf/Lef transcription factors (1). β-Catenin-Tcf/Lef transcriptional activity regulates expression of a number of target genes such as cyclin D1, c-Jun, c-Myc, E-cadherin, and matrix metalloproteinases (MMP), including MMP-7 and MMP-26 (5). Noncanonical Wnt signaling acts via a β-catenin-independent mechanism that increases intracellular Ca2+ concentrations or controls cell polarity by activation of Rho GTPases (6). The planar cell polarity (PCP) pathway regulates the orthogonal polarity of cells within an epithelium that activates the small GTPase RhoA and c-Jun amino (N)-terminal kinase (JNK). Activation of Rho GTPases induces changes in the cytoskeleton and microtubules. The Wnt/Ca2+ pathway regulates cell movements in the early stage of development. Fzd appears to activate phospholipase C and phosphodiesterase to increase free intracellular Ca2+ and to decrease intracellular cyclic guanosine monophosphate (cGMP) concentrations. However, the noncanonical pathways in animals are poorly understood (Fig. 1).

It seems that both canonical and noncanonical pathways regulate embryonic skeletal development including chondrogenesis, hypertrophic maturation of chondrocytes, endochondral ossification for long bone development, and joint development. Prior to reviewing the role of Wnt signaling in cartilage development and degeneration, we will provide a brief overview of our current knowledge of cartilage development and destruction.

Cartilage development

Cartilage is comprised primarily of matrix such as collagens and proteoglycans containing sparse populations of chondrocytes, which perform matrix-generation and maintenance functions. Cartilage development is initiated by differentiation of mesenchymal cells to chondrocytes, a process known as chondro-
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Fig. 1. Wnt signaling pathways. The Wnt signaling network is composed of Wnt ligands, receptors, antagonists, and intracellular signaling molecules. Wnt ligands bind to the Fzd receptor and the LRP-5/6 co-receptors, which initiate the β-catenin-dependent canonical pathway and β-catenin-independent noncanonical pathway.

Fig. 2. Schematic summary of cartilage development and endochondral ossification. Chondrogenesis is initiated by mesenchymal condensation. Differentiated chondrocytes undergo hypertrophic maturation during endochondral bone formation. Marker extracellular matrix (ECM) molecules and regulatory factors at different stages of development are indicated.

Chondrogenesis (7-9) (Fig. 2). The initial step of chondrogenesis is the recruitment of mesenchymal chondroprogenitor cells to future sites of skeletal development, which is followed by cellular aggregation or condensation and the differentiation of mesenchymal cells to the chondrogenic lineage. Differentiated chondrocytes express specific extracellular matrix (ECM) molecules such as type II collagen and aggrecan to form cartilage tissue. Chondrogenesis is dependent on signals generated by cell-ECM and cell-cell adhesion interactions, which in turn are modified by the cell’s response to growth and differentiation factors. Several factors, including bone morphogenetic proteins, fibroblast growth factors, insulin-like growth factor 1, Indian hedgehog, parathyroid hormone-related peptide, and Wnt, are known to regulate discrete steps in chondrogenesis. These factors exert...
their effects by initiating intracellular signaling pathways, including the protein kinase C (PKC) (10), protein kinase A (11), and mitogen-activated protein kinase (12, 13) pathways. Extracellular signaling molecules and subsequent activation or inhibition of intracellular signaling pathways modulate gene expression profiles to regulate chondrogenic processes. Sox-9 is a master transcription factor that regulates chondrocyte differentiation in cooperation with L-Sox-5 and Sox-6 (14).

Differentiated chondrocytes have two distinct fates (15-17). One is to remain as chondrocytes to form articular cartilage and function in joint development. Articular cartilage plays an important role in withstanding mechanical stress associated with joint movement at the ends of long bones. The other fate of differentiated chondrocytes is maturation into hypertrophic chondrocytes to function as a template for long bone during endochondral ossification (Fig. 2). During this process, the size of chondrocyte cells increases after stop proliferation and the hypertrophic chondrocytes initiate expression of type X collagen, alkaline phosphatase, Runx2, osteopontin, and MMP-13. Hypertrophic chondrocytes also express vascular endothelial growth factor to induce attraction for blood vessels in association with other factors, which is required for replacement of cartilage by bone and for equating osteoblast and osteoclast levels. Hypertrophic chondrocytes are eventually subjected to apoptosis, and the remaining cartilage ECM molecules are replaced by bone matrix produced from osteoblasts. A number of factors regulating hypertrophic maturation of chondrocytes have been identified, including Indian hedgehog, Runx, and Wnt. Thus, hypertrophic chondrocytes play an essential role in coordinating chondrogenesis and osteogenesis, as hypertrophic chondrocytes provide a scaffold for subsequent bone formation.

Cartilage degeneration

Articular cartilage has an avascular structure consisting of a large amount of ECM and a small number of chondrocytes. Homeostasis of cartilage tissue is maintained by the balance of synthesis and degradation of ECM by chondrocytes. Articular cartilage is the major target tissue for destruction in both rheumatoid arthritis (RA) and osteoarthritis (OA), resulting in destruction of ECM homeostasis. This process involves inflammation, degradation of ECM by MMP, loss of differentiated phenotypes, and apoptosis of chondrocytes, as shown in Fig. 3 (18-21).

Synovial inflammation is a factor that likely contributes to dysregulation of chondrocyte function in arthritic cartilage (18, 20, 21). The key aspects of inflammation include metabolic signals and degradation that are driven by cytokine cascades and the production of inflammatory mediators, which favor an imbalance between the catabolic and anabolic activities of the chondrocytes. Inflammatory cytokines such as interleukin (IL)-1β and tumor necrosis factor (TNF)-α appear to play important roles in cartilage destruction. These cytokines increase the synthesis of prostaglandin E2 (PGE2) by stimulating the expression or activity of cyclooxygenase (COX)-2 and produce nitric oxide via inducible nitric oxide synthase. The proinflammatory cytokines are essential mediators of cartilage ECM degradation, loss of the differentiated phenotype and apoptosis of chondrocytes.

One of the central pathophysiological features in arthritic cartilage is loss of the cartilage ECM. Catabolism of cartilage ECM is regulated by MMP and tissue inhibitor MMP (TIMP), the activities of which are regulated by various local mediators such as cytokines, growth factors, prostaglandins, matrix breakdown products, complement, oxygen species, and neuropeptides (19, 22, 23). The MMPs can degrade all components of the cartilage ECM, and several MMPs are associated with arthritic cartilage destruction, including MMP-1, -2, -3, -7, -9, -13, and MT1-MMP (24). Classical collagenases (MMP-1, -8, and -13) and MT1-MMP can degrade fibrillar collagens includ-
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Regulation of chondrogenesis and cartilage development by Wnt signaling has been extensively studied and several review papers are available (46). In this review, we will briefly summarize current knowledge of Wnt regulation of cartilage development. The role of Wnt signaling has been elucidated by mis-expression or forced expression of Wnt signaling components in chick embryos, transgenic and knock-out mice, and forced expression in micromass culture system of mesenchymal cells derived from chick or mouse embryos. Using these approaches, it has been demonstrated that a variety of different Wnt signaling components positively or negatively regulate different stages of chondrogenesis and cartilage development (Fig. 4).

Wnt signaling in cartilage development

Cartilage development is initiated by chondrogenesis, which requires mesenchymal condensation and cartilage nodule formation. Various Wnt signaling components regulate chondrogenesis at the stages of mesenchymal condensation and/or cartilage nodule formation. For example, forced expression of Wnt-4 (47) and Fzd-7 (48) inhibits mesenchymal condensation, whereas forced expression of Wnt-7a (49, 50) and Wnt-14 (51) blocks transition of condensation to cartilage nodules. Wnt-1 and -7a also inhibit chondrogenesis without

Fig. 4. Schematic representation of the Wnt action involved in chondrogenesis and hypertrophic maturation of chondrocytes. The actions of individual components of the Wnt signaling network in the regulation of chondrogenesis and chondrocyte maturation are indicated.
significant effects on early condensation (52). Wnt-3a is also known to inhibit chondrogenesis through a β-catenin-dependent pathway. Chondrogenesis was inhibited by Wnt-3a via a β-catenin-dependent mechanism, although it has not been determined whether the inhibitory effects require transcriptional activity of β-catenin (53). In chick micromass culture systems, Wnt-3a or accumulation of β-catenin inhibited chondrogenesis by stabilizing cell-cell adhesion via β-catenin and independently of β-catenin transcriptional activity (38, 54). By contrast, stimulation of canonical pathways by active Lef promoted chondrocyte differentiation in a Sox-9-dependent manner (55). Inappropriate activation of Wnt signaling generally results in truncated skeletal elements in vivo and inhibition of chondrogenesis. However, some components of Wnt signaling pathways promote chondrogenic differentiation of mesenchymal cells. For example, Wnt5-a and Wnt-5b are known to promote early chondrogenesis in vitro by increasing cartilage nodule formation (47) and also to coordinate chondrocyte proliferation and differentiation by differentially regulating cyclin D1 and p130 expression (36).

Wnt regulation of hypertrophic maturation of chondrocytes
Differentiated chondrocytes permanently maintain chondrocyte properties to form joint articulating cartilage or they undergo hypertrophic maturation during endochondral bone formation. During endochondral ossification, a variety of different Wnt signaling components regulate hypertrophic maturation of chondrocytes (57) (Fig. 4). In addition to the ability to promote early chondrogenesis, forced expression of Wnt-5a (47, 50, 56-58) and -5b (47, 56) inhibits hypertrophic maturation of chondrocytes. Chondrocyte maturation and mineralization were also blocked or delayed by the forced expression of FrzB-1 (59), Fzd-1, or Fzd-7 (50, 60). Knockout of the Wnt antagonist sFRP1 reduced the height of the growth plate and increased calcification of the hypertrophic zone, indicating accelerated endochondral ossification. Knockout of sFRP1 also accelerated differentiation of hypertrophic chondrocytes, as demonstrated by experiments using mouse embryo fibroblasts (61). A loss-of-function study in the mouse system has demonstrated that Wnt-5a regulates outgrowth of the limbs (62).

In contrast to the inhibition of chondrocyte maturation, Wnt-4 accelerates terminal chondrocyte differentiation, whereas it blocks initiation of chondrogenesis in a chick micromass culture system (47). Overexpression of Wnt-4 or the constitutively active form of β-catenin also promoted growth plate chondrocyte terminal differentiation (60, 63), whereas knockout of β-catenin delayed chondrocyte maturation and endochondral bone formation (64). Viral overexpression of Wnt-8c and -9a in chick sternal chondrocytes enhanced hypertrophic maturation by upregulating type X collagen and Runx2 (65, 66).

Wnt signaling in cartilage degeneration
In contrast to the body of knowledge regarding the role of Wnt signaling chondrogenesis and cartilage development, relatively little is known regarding the role of Wnt signaling in the maintenance and destruction of cartilage tissue. However, recent data suggest that abnormal Wnt signaling may contribute to cartilage destruction in RA and OA by regulating synthesis and degradation of cartilage matrix, chondrocyte apoptosis, and inflammation. Preliminary evidence was obtained by the expression of Wnt signaling components in RA and OA cartilage tissue, although expression of Wnt signaling components itself does not prove the active involvement of Wnt signaling in cartilage degeneration.

Correlated expression of Wnt signaling components in RA and OA cartilage
RA is a typical autoimmune disease and a cascade of disease progression is activated by T-cell-mediated immune responses. These include infiltration of the synovial lining by blood leukocytes and production of inflammatory cytokines, prostaglandins, and MMPs (67). In RA cartilage, for example, several Wnt family members such as Wnt-1, -3a, -10b, -11, and -13 and frizzled receptors including Fzd-2, -5, and -7 are expressed in RA synovial tissue (68). Among these, Wnt-5a and Fzd-5 are overexpressed in RA synovial tissue compared with a panel of normal adult tissue. In addition, cultured RA fibroblast-like synoviocytes express higher levels of IL-6, IL-8, and IL-15, compared with normal synovial fibroblasts. Transfection of normal fibroblasts with a Wnt-5a reproduced this pattern of cytokine expression and stimulated IL-15 secretion (68). By contrast, inhibition of Wnt-5a/Fzd-5 signaling blocks RA synoviocyte activation by blocking cytokine production (69). Activation of canonical Wnt signaling also increases expression of degradative enzymes such as MMP-3 in the same cell system (70). Other components such as Wnt-7a (71) and Wnt-10B (72) are also detected in OA synovium.

In contrast to the early inflammatory events that precipitate RA, OA is a degenerative joint disease that is preceded by biochemical and biomechanical changes in cartilage and bone. Compared with normal cartilage tissue, OA cartilage shows altered expression patterns of Wnt signaling components. For instance, FrzB-2, a recently identified sFRP, is strongly expressed in human OA chondrocytes but only negligible levels are detected in normal cartilage chondrocytes. The expression level of FrzB-2 is well correlated with the extent of cartilage destruction, and the possible involvement of FrzB-2 in chondrocyte apoptosis has been suggested (73). sFRP3 is found in human OA chondrocytes. A single nucleotide polymorphism variant with an Arg324Gly substitution in sFRP3, which shows reduced antagonistic activity of Wnt signaling without affecting binding to Wnt, is associated with OA of the hip in females (74). Wnt-7a expression is upregulated in OA cartilage (71). Consistent with this, β-catenin has been found to accumulate in OA cartilage (33, 75). More recently, a systematic analysis of the Wnt signaling pathway revealed upregulation of Wnt-16, downregulation of FrzB, upregulation of Wnt target
genes, and nuclear localization of β-catenin in acutely injured cartilage owing to mechanical stress. Expression levels of Wnt-16 and β-catenin were barely detectable in preserved cartilage areas in OA, but were markedly upregulated in areas of the same joint with moderate to severe OA damage (76).

**Modulation of chondrocyte functions by Wnt signaling**

So far, there is no direct evidence that Wnt signaling regulates pathogenic disease of cartilage tissue. However, Wnt signaling may be involved in cartilage degeneration, as indicated by the observation that Wnt signaling regulates chondrocyte functions related to cartilage degeneration (Fig. 5). For example, protein levels of β-catenin, which accumulates in OA chondrocytes, are very low in differentiated articular chondrocytes; however, these low levels were increased by post-translational stabilization during phenotypic loss caused by a serial monolayer culture or exposure to IL-1β. Moreover, ectopic expression or inhibition of β-catenin degradation caused cessation of cartilage-specific ECM molecule synthesis via activation of β-catenin-Tcf/Lef transcriptional activity (38). In addition to the inhibition of ECM molecule synthesis in articular chondrocytes, accumulation of β-catenin and activation of β-catenin-Tcf/Lef transcriptional activity induced expression of COX-2, an important enzyme in prostaglandin synthesis and inflammatory responses (75). The function of β-catenin is regulated by a variety of β-catenin-binding proteins (77). One of the interesting molecules involved in regulation of β-catenin function in chondrocytes is α-catenin (78). It has been shown that α- and β-catenins accumulate in dedifferentiated chondrocytes by avoiding proteasomal degradation. β-catenin degradation was ubiquitination-dependent, whereas α-catenin was proteasomally degraded in an ubiquitination-independent manner. The accumulated α-catenin and β-catenin are present as complexes in the cytosol and nucleus. The α-catenin and β-catenin complex blocked proteasomal degradation of α-catenin and inhibited β-catenin-Tcf/Lef transcriptional activity, suppression of type II collagen expression associated with ectopic expression of β-catenin, inhibition of the proteasome, or Wnt signaling. Together, these results indicate that ubiquitin-independent degradation of α-catenin regulates β-catenin signaling and maintenance of the differentiated phenotype of articular chondrocytes (78).

The inhibitory effects of β-catenin on cartilage-specific ECM molecule synthesis and COX-2 expression suggest that the canonical Wnt pathway causes loss of cartilage-specific ECM molecule synthesis and induction of inflammatory enzymes in articular chondrocytes. Indeed, it has been shown that Wnt-3a (54) and Wnt-7a (33) cause cessation of collagen type II expression in chondrocytes, and that this is due to the stimulation of β-catenin-Tcf/Lef transcriptional activity. In the case of Wnt-3a-stimulated chondrocytes, Wnt-3a caused the expression of c-Jun and its phosphorylation by c-Jun N-terminal kinase (JNK), resulting in activation of AP-1. AP-1 activation suppressed the expression of Sox-9, an important transcription factor regulating type II collagen expression, indicating that Wnt-3a causes dedifferentiation of chondrocytes by activation of the β-catenin-Tcf/Lef transcriptional complex and the c-Jun/AP-1 pathway (54). Similar to Wnt-3a action, Wnt-7a caused expression of COX-2 via the canonical pathway in chondrocytes (Hwang et al., unpublished data). Wnt-7a also inhibited NO-induced apoptosis of chondrocytes by activating cell survival signaling, such as phosphatidylinositol 3-kinase.

**Fig. 5.** Schematic representation of selected Wnt proteins in the regulation of articular chondrocytes. A. In primary culture articular chondrocytes, Wnt-3a and -7a blocks type II collagen expression, whereas Wnt-5a inhibits this expression via the noncanonical pathway. By contrast, Wnt-11 enhances type II collagen expression via the noncanonical pathway. B. Wnt-7a in chondrocytes inhibits type II collagen expression and induces cyclooxygenase (COX-2) expression via the canonical pathway. Wnt-7a also causes accumulation of β-catenin and increased β-catenin concentrations stabilize cell-cell adhesion and inhibit chondrogenesis. Wnt-7a blocks nitric oxide (NO)-induced apoptosis by activation of survival signals.
and Akt, independent of β-catenin-Tcf/Lef transcriptional activity (33). We also observed that Wnt-7a inhibits chondrogenesis of mesenchymal cells induced by micromass culture, and that the inhibitory effects of Wnt-7a are due to its ability to stabilize cell-cell adhesion independent of β-catenin-Tcf/Lef transcriptional activity (Hwang et al., unpublished data). This is consistent with the observation that accumulation of β-catenin inhibits chondrogenesis by stabilizing cell-cell adhesion (38).

In contrast to Wnt-3a and -7a, Wnt-5a and -11 regulate cartilage-specific ECM molecule synthesis through the non-canonical pathway (79). IL-1β in chondrocytes upregulated Wnt-5a and downregulated Wnt-11 expression. Wnt-5a inhibited type II collagen expression, whereas knockdown of Wnt-5a small interfering RNA (siRNA) blocked the inhibitory effects of IL-1β on type II collagen expression. In contrast to the inhibitory effects of Wnt-5a, Wnt-11 stimulated type II collagen expression. Wnt-5a and Wnt-11 did not cause accumulation of β-catenin or activation of the β-catenin-Tcf/Lef transcriptional complex. Instead, Wnt-5a activated JNK, and inhibition of JNK blocked Wnt-5a inhibition of type II collagen expression. By contrast, Wnt-11 activated PKC and inhibition of PKC blocked Wnt-11 stimulation of type II collagen expression, indicating that Wnt-5a and Wnt-11 have opposing effects on type II collagen expression by signaling through distinct noncanonical Wnt pathways (79). It has also been shown that Wnt-5a expression is regulated by histone deacetylase (HDAC) in chondrocytes, in association with acetylation of the Wnt-5a promoter. Inhibition of HDAC in chondrocytes inhibits type II collagen expression. HDAC inhibition also promoted the expression of Wnt-5a, and knockdown of Wnt-5a blocked the ability of HDAC inhibitors to suppress type II collagen expression (80).

**Conclusion**

Over the past two decades, extensive studies on Wnt regulation of chondrogenesis and cartilage development have shown that Wnt signaling has positive and negative regulatory effects on cartilage development. Based on the role of Wnt signaling in cartilage development, it is not surprising that increasing evidence indicates the involvement of Wnt signaling in the regulation of differentiated chondrocyte functions and cartilage disease. Although our current knowledge of the role of Wnt signaling in cartilage tissue is limited, a variety of Wnt signaling components might also regulate the maintenance and destruction of cartilage tissue. For example, in addition to the above-mentioned Wnt signaling components such as β-catenin, Wnt-3a, Wnt-5a, Wnt-7a, and Wnt-11, other signaling molecules such as Fz-1 and Fz-6 are downregulated by IL-1β in chondrocytes, indicating the possible involvement of these molecules in the regulation of cartilage function. Therefore, a challenge for the future is to fully understand the role of the Wnt signaling network in the maintenance of cartilage tissue and pathogenesis of joint disease.

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