REVIEW

WNT Signaling and Cartilage: Of Mice and Men

Bin Ma · Ellie B. M. Landman · Razvan L. Miclea ·
Jan M. Wit · Els C. Robanus-Maandag ·
Janine N. Post · Marcel Karperien

Abstract In adult articular cartilage, the extracellular matrix is maintained by a balance between the degradation and the synthesis of matrix components. Chondrocytes that sparsely reside in the matrix and rarely proliferate are the key cellular mediators for cartilage homeostasis. There are indications for the involvement of the WNT signaling pathway in maintaining articular cartilage. Various WNTs are involved in the subsequent stages of chondrocyte differentiation during development, and deregulation of WNT signaling was observed in cartilage degeneration. Even though gene expression and protein synthesis can be activated upon injury, articular cartilage has a limited ability of self-repair and efforts to regenerate articular cartilage have so far not been successful. Because WNT signaling was found to be involved in the development and maintenance of cartilage as well as in the degeneration of cartilage, interfering with this pathway might contribute to improving cartilage regeneration. However, most of the studies on elucidating the role of WNT signaling in these processes were conducted using in vitro or in vivo animal models. Discrepancies have been found in the role of WNT signaling between chondrocytes of mouse and human origin, and extrapolation of results from mouse models to the human situation remains a challenge. Elucidation of detailed WNT signaling functions will provide knowledge to improve cartilage regeneration.

Keywords Cartilage · Degeneration · Development · WNT

The first three authors contributed equally, and all should be considered first author.

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B. Ma · E. B. M. Landman · J. N. Post · M. Karperien (✉)
Department of Developmental BioEngineering, University of Twente, Drienerlolaan 5, 7522NB Enschede, The Netherlands
e-mail: h.b.j.karperien@utwente.nl

R. L. Miclea · J. M. Wit
Department of Pediatrics, Leiden University Medical Center, Leiden, The Netherlands

E. C. Robanus-Maandag
Department of Human Genetics, Leiden University Medical Center, Leiden, The Netherlands

Introduction

Adult articular cartilage is an avascular tissue composed of a dense extracellular matrix (ECM) predominantly composed of collagens, proteoglycans and glycosaminoglycans, which provide compressive and tensile strength to the cartilage tissue. The chondrocytes residing in this ECM maintain it by remodeling. Because the metabolic activity of chondrocytes is relatively low and cartilage is scarcely populated with cells, cartilage remodeling is a slow process. Articular cartilage is composed of four distinct regions, the superficial zone which faces the synovial cavity, the middle zone, the deep zone and the calcified cartilage zone adjacent to the subchondral bone plate. These zones differ in cell density and matrix composition. Also the chondrocyte morphology differs between zones, as chondrocytes in the superficial zone are small and flattened, middle zone chondrocytes are more rounded and in the deep zone chondrocytes are grouped in columns or clusters. Because chondrocytes rarely divide and are separated from each other by the matrix, it is unclear how they are regulated to maintain the ECM under homeostatic conditions. It is known, however, that gene expression and protein synthesis can be activated upon injury.

Aging and joint disease disrupt the equilibrium within the cartilage tissue and the synthesis of new matrix components is exceeded by the loss of collagens and proteoglycans [1]. This disbalance between anabolic and catabolic processes results in progressive cartilage degeneration. Even though the exact etiology and pathophysiology of cartilage degenerative diseases is largely unknown and there are probably multiple conditions that may trigger its onset, cartilage destruction is considered to be the result of increased expression and activity of proteolytic enzymes, for example as a response to abnormal mechanical loading, genetic predisposition, trauma or inflammation. Matrix metalloproteinases (MMPs) and aggrecanases are the major proteinases that degrade collagens and proteoglycans in joint disease.

Articular cartilage has a limited ability for self-repair. In spite of extensive research efforts to develop treatment options for cartilage degeneration by regeneration of articular cartilage, so far no curative treatment is available to regenerate or restore native articular cartilage.

In order to reverse degeneration and induce regeneration of articular cartilage, it is crucial to have a detailed understanding of chondrogenic differentiation during skeletal development and the maintenance of articular cartilage in adulthood. Using this knowledge, it may be possible to induce chondrogenic differentiation and shifting the balance from catabolism to anabolism. In this review, we describe the processes and molecular components that are involved in skeletal development with emphasis on articular cartilage formation and homeostasis. Because the WNT pathway was found to play an important role in embryonic development and indications of deregulated WNT signaling have been found in degenerative cartilage disease, we focus on the role of the WNT signaling pathway and its components in skeletal development and the maintenance of cartilage homeostasis. We also touch upon the role of WNT signaling in cartilage degeneration. The different components of the WNT signaling pathway that are involved in cartilage development and disease are listed in Table 1.

WNT Signaling

The WNT family of secreted glycoproteins, which are characterized by several conserved cysteine residues, consists of 19 members, of which several encode distinct isoforms arising from differential splicing [2]. The WNT signaling pathway is composed of several components and plays a fundamental role in controlling cell proliferation, cell fate determination, and differentiation by inducing changes in gene expression during embryonic development and in adult cartilage [2, 3]. At least three distinct intracellular signaling pathways are known that are activated by distinct sets of WNTs and Frizzled (FZD) receptors and that lead to unique cellular responses [4]. The canonical WNT/β-catenin pathway is the best described pathway for WNT signal transduction (Fig. 1). In an inactive state, in the absence of a WNT ligand, β-catenin (CTNNB1) is phosphorylated at the NH_2 terminus by glycogen synthase kinase 3β (GSK3β) and casein kinase I (CKI) in a destruction complex, which is brought together by the two scaffolding proteins axin and adenomatous polyposis coli (APC). This phosphorylation targets β-catenin for subsequent ubiquitylation and proteasomal degradation. When WNTs bind to the seven transmembrane frizzled receptor in combination with a coreceptor of the LDL related proteins (LRP) 5 or 6, disheveled (DSH) is activated, resulting in suppression of GSK3β activity. As a result, β-catenin will not undergo phosphorylation and is stabilized in the cytoplasm. Upon reaching a certain level, β-catenin translocates to the nucleus, where it interacts with transcription factors of the T-cell-specific transcription factor/lymphoid enhancer-binding factor (TCF/LEF) family to initiate the transcription of target genes [5, 6].

Another intracellular WNT pathway was first identified in *Drosophila*. This pathway is involved in regulating planar cell polarity by inducing cytoskeletal organization relative to the plane of the tissue in which the cells reside [4, 7]. However, although FZD and DSH were shown to play a role in this pathway, no involvement of LRP, β-catenin or TCF was found and current evidence suggests that no WNT ligands are involved in the regulation of planar cell polarity [4]. FZD is also involved in the WNT/Ca^{2+} pathway, which is another pathway activated by subsets of WNT ligands [4, 6, 8]. In this pathway, binding of a WNT ligand to FZD induces activation of a heterotrimeric G protein, resulting in an increase in intracellular levels of Ca^{2+}. This activates Ca^{2+}-dependent effector molecules such as the transcription factor Nuclear Factor Associated with T cells (NFAT) [4].

The specificity of activation downstream of WNT is determined by selective receptor activation, receptor-mediated endocytosis and the presence of cofactors such as heparin and sulfate proteoglycans [9]. Accumulating evidence has suggested that WNT may activate multiple pathways depending on the engagement of distinct receptors [4]. For example, WNT-5A, which is most often associated with noncanonical pathway, also activates the canonical β-catenin signaling [10]; the canonical WNT-3A also activates the noncanonical Ca^{2+} pathway [11].

WNT signals can be modulated extracellularly by secreted proteins, including members of the secreted Frizzled-related protein (sFRP) family, WNT-inhibitory factor (WIF) 1, Cerberus, SOST and Dickkopf 1 (DKK1). sFRPs, WIF-1 and Cerberus can bind to WNTs directly, thereby

Table 1 Involvement of players of the WNT signaling pathway in cartilage development and disease

| Factor	Development		Disease	
	Expression	Action	Expression	Action
WNT-1	No endogenous expression [34]	Inhibition of cartilage formation [34, 35]	Expressed in RA synovial tissues [79]	Enhances fibronectin and MMP-3 expression in fibroblast-like synoviocytes [79]
WNT-3A	Expressed in early stages of chondrogenesis, decreased when chondrogenic differentiation proceeds [38]	Increases self-renewal and decreases apoptosis of MSCs [36, 37]		Promotes chondrocyte proliferation [11] and induces dedifferentiation [38]
		Blocks *Collagen II* expression and proteoglycan deposition [38]		
WNT-4	Expressed in periphery of joint interzone and hypertrophic chondrocytes [39, 40]	Accelerates chondrocyte maturation [39–41]		
WNT-5A	Expression in perichondrium surrounding condensations [39, 40]	Recruitment of mesenchymal cells [42]	Expressed at high level in chondrocytes [72]; overexpressed in RA synovial tissues [79]	Up-regulates MMP expression [90] and induces chondrocyte dedifferentiation [73] in rabbit.
		Delays chondrocyte differentiation [39–41]		
WNT-5B	Expressed in prehypertrophic chondrocytes and in the perichondrium [38]	Promotes initial steps of chondrogenesis in micromass cultures and delays terminal differentiation in vivo [39]		
WNT-7A	Expressed in dorsal ectoderm in developing limb bud [44]	Inhibition of chondrogenic differentiation in vitro and in vivo [34]	Expressed in rabbit chondrocytes and increased in response to IL-1 [71]	Induces rabbit chondrocyte dedifferentiation [71]
WNT-7B			Up-regulated in OA cartilage and RA synovium [80]; expressed in human chondrocytes and increased in response to IL-1 [72]	Down-regulates MMP expression in human chondrocytes [72]
WNT-14	Expressed in developing joint interzone [46]	Arrests or reverses chondrogenic differentiation [40]		
β-CAT	Expressed at low levels in chondrogenic mesenchymal condensations [25]	Induces expression of Sox9 and promotes chondrogenic differentiation at low levels [25, 26]	Expression elevated in osteoarthritic cartilage [32, 33] and dedifferentiated chondrocytes [69]	Induces OA phenotype in mouse model expressing constitutively active β-catenin [32, 33]; inhibits NF-κB activity and MMP expression in human chondrocytes [72]
FRZB	Expressed in prechondrogenic mesenchymal condensations and in epiphyseal prearticular chondrocytes [46, 48]	Blocks chondrocyte maturation and prevents endochondral ossification in vivo [47, 48]	Multiple variants are expressed in human [74–77]	Risk factor for OA [74–77]; Frzb knockout mice are more sensitive to OA-inducing factors [83, 84]
		Promotes glycosaminoglycan synthesis and expression of Sox9 and *Collagen II* in vitro [49]		
DKK-1	Expressed at sites of programmed cell death in apical ectodermal ridge [93]; expressed at higher level in articular cartilage than growth plate cartilage [50]	Promotes glycosaminoglycan synthesis and expression of Sox9 and *Collagen II* in vitro [93]; inhibits chondrocyte hypertrophy [50]	Expression is increased in RA and decreased in OA [94]	Overexpression ameliorates cartilage destruction in animal models [95, 96]
WIF-1	Expressed in mesenchyme surrounding cartilage elements and articular cartilage [51]	Promotes chondrogenic differentiation [51]		
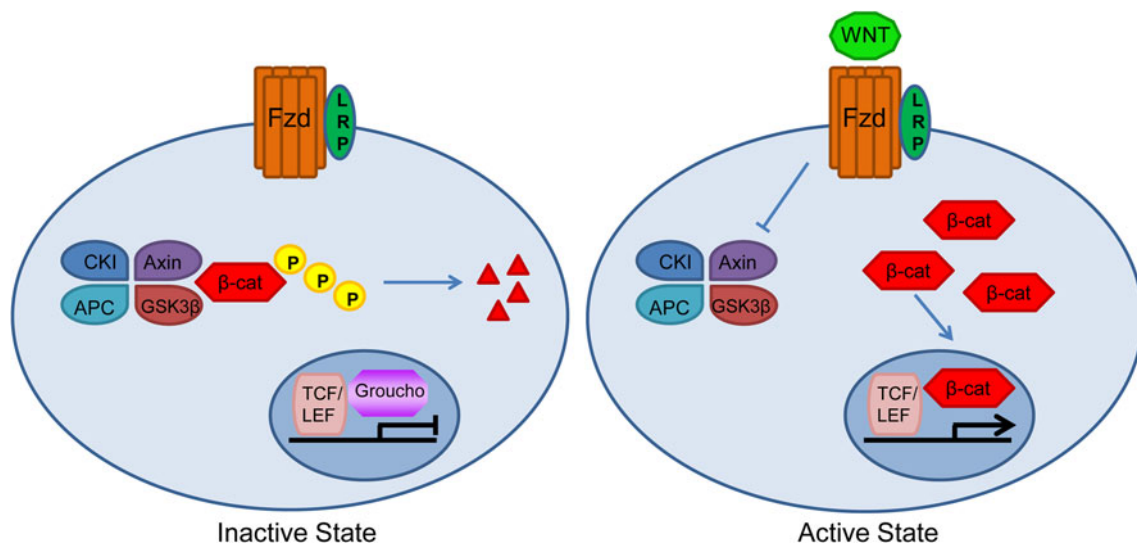
Fig. 1 WNT/β-catenin signaling pathway In the inactive state, β-catenin is phosphorylated by a degradation complex consisting of GSK3β, APC, AXIN and CKI, targeting β-catenin for ubiquitylation and subsequent proteasomal degradation. When a WNT ligand binds

to the FZD receptor and coreceptors LRP5/6, the degradation complex is disrupted, resulting in stabilization of β-catenin in the cytoplasm. Subsequently β-catenin is translocated to the nucleus and binds to transcription factors TCF/LEF to initiate gene transcription

preventing their interaction with FZD receptors, whereas sFRPs can also bind FZD receptors to form nonfunctional complexes [2, 12]. Both SOST and DKK-1 antagonize WNT signaling by binding to LRP5/6 coreceptors, albeit to distinct regions, and thereby preventing complex formation with the FZD/WNTs [13]. Intracellularly, WNT/β-catenin signaling can be modulated by Inhibitor of β-catenin and T-cell factor (ICAT), which disturbs the interaction between β-catenin and transcription factors TCF/LEF, thereby inhibiting β-catenin-mediated transcription [14, 15].

Skeletal Development

The formation of most of the vertebral skeleton occurs via endochondral bone formation, a process starting with the aggregation, proliferation and condensation of mesenchymal stromal cells (MSCs) at specific locations within the embryo. These MSCs originate from the neural crest (forming craniofacial bones), the sclerotome of the paraxial mesoderm (forming the axial skeleton), or the lateral plate mesoderm (forming the appendicular skeleton) [16]. MSCs commit to the skeletal lineage once they have differentiated into skeletal precursor cells (SPCs), from which chondrocytes and osteoblasts can be derived. Cellular condensations form as the result of altered mitotic activity, and failure of cells to move away from a center or aggregation of cells toward a center, as occurs in limb formation. This process leads to increased mesenchymal cell density, without an increase in cell proliferation. Consequently, cellular condensation is associated with an increase in cell–cell contacts through

cell–cell adhesion molecules and gap junctions that facilitate intercellular communication [17]. Before condensation, MSCs secrete an ECM rich in hyaluronan and collagen type I, preventing intimate cell–cell interaction. As condensation begins, an increase in hyaluronidase activity is observed, so the ECM hyaluronan content decreases, allowing cell migration. The increased cellular interaction as observed during condensation is thought to be involved in triggering signal transduction pathways that initiate chondrogenic differentiation. Adhesion molecules like neural cadherin (N-cadherin) and neural cell adhesion molecule (N-CAM) are expressed in condensing mesenchyme, while they disappear in differentiating chondrocytes. Furthermore, cell–matrix interactions involving fibronectin play an important role in mesenchymal condensation. Fibronectin expression was found to be up-regulated in cellular condensation, and its expression decreases when chondrogenic differentiation continues. At the periphery of these condensations, SPCs form a perichondrial layer, while in the core they differentiate into chondrocytes that produce cartilage specific extracellular matrix (ECM) proteins and continue to proliferate [17].

The differentiation of chondroprogenitor cells is characterized by the deposition of a cartilaginous extracellular matrix containing collagen types II, IX and XI and aggrecan. One of the earliest markers expressed in cells undergoing condensation is *SOX9*, which is required for the expression of the collagen type II α1 (*COL2A1*) gene and other cartilage specific extracellular matrix proteins [18, 19]. Continuous proliferation of chondrocytes and secretion of ECM contribute to the elongation of the
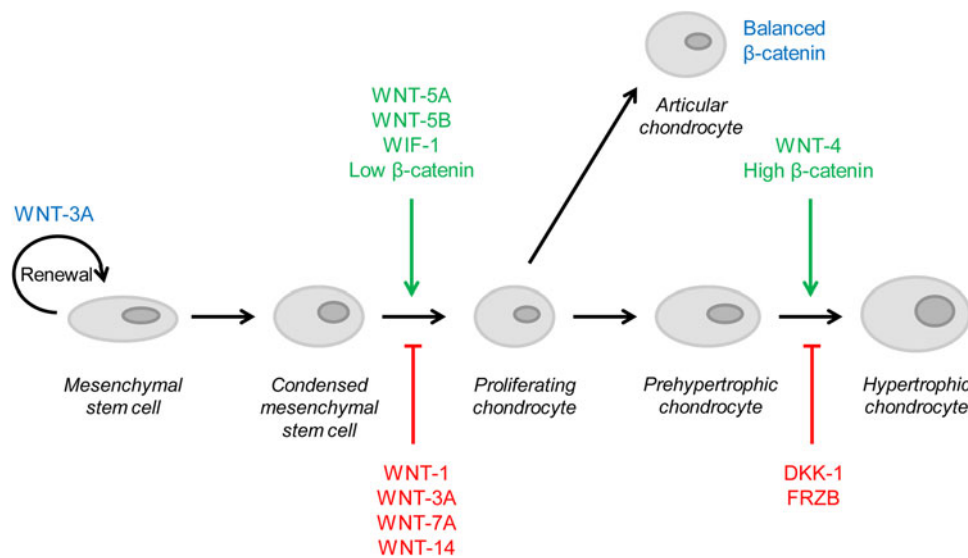
Fig. 2 Involvement of WNT signaling in consecutive stages of chondrogenic differentiation WNT-3A induces proliferation and self-renewal of MSCs, which form mesenchymal condensations at the initial stage of differentiation. Subsequently, chondrocyte differentiation is induced by low levels of β-catenin, WNT-5A, WNT-5B and WIF-1, whereas WNT-1, WNT-3A, WNT-7A and WNT-14 block chondrocyte differentiation. In adult articular cartilage, a fine balance of β-catenin levels is involved in maintaining the chondrocyte phenotype. Hypertrophic differentiation of chondrocytes is induced by WNT-4 and high levels of β-catenin and blocked by WNT antagonists DKK-1 and FRZB

cartilage template, which prefigures the shape of the future bone.

After formation of the cartilaginous template, the core chondrocytes mature and become hypertrophic, secreting a progressively calcified ECM. Simultaneously, perichondrial SPCs differentiate into osteoblasts, forming the future periosteum, which modulates the final shape and size of the cartilage template. After mineralization of the cartilage ECM, vascular invasion and apoptosis of terminal hypertrophic chondrocytes initiate the formation of the primary ossification center. This complex differentiation program radiates centrifugally, leading to the development of trabecular bone [16, 20–24].

Involvement of WNT Signaling in Skeletal Development

In skeletal development, the fate of MSCs to differentiate into either chondrocytes or osteoblasts depends on the expression of the transcription factors SOX9 or RUNX2, respectively. Skeletal precursor cells express both transcription factors, and the intracellular expression levels of β-catenin determine the fate of these cells. High levels of β-catenin inhibit SOX9 expression and activity, while potentiating RUNX2, which results in osteoblast differentiation. In contrast, low β-catenin levels induce SOX9 expression and thereby chondrocyte differentiation [25, 26].

An increasing amount of evidence indicates the important role of WNT/β-catenin signaling in essentially all aspects of skeletal development and maintenance. The role of canonical WNT/β-catenin signaling at subsequent stages of skeletogenesis has been suggested on the basis of the expression patterns of many WNT pathway members, as well as WNT-reporter expression in mice [25–31]. Involvement of WNT signaling in chondrogenic differentiation is depicted in Fig. 2.

Because β-catenin is a key molecule in the canonical WNT signaling pathway, it is the most studied molecule involved in this pathway. In vitro and in vivo data suggest that β-catenin plays an essential role in cell fate determination in skeletal development, as it acts as a molecular switch between chondrocyte and osteoblast differentiation in SPCs. Up-regulation of β-catenin in mesenchymal condensations was found before expression of the osteoblast-specific transcription factors RUNX2 and OSX was detected, indicating that high levels of β-catenin precede osteoblast differentiation. In contrast, β-catenin expression was down-regulated in chondrogenic mesenchymal condensations [25]. Because conditional deletion of β-catenin is prenatally lethal, an essential role for β-catenin in early skeletal development was indicated [25]. Deletion of β-catenin in early embryonic development results in the arrest of osteoblast differentiation at the level of osteochondrogenic progenitor cells, which instead differentiate into chondrocytes [26]. Furthermore, inactivation of β-catenin in micromass cultures enhances chondrocyte differentiation in vitro [25]. Mice in which ablation of β-catenin is induced in cartilage after birth, develop an osteoarthritis-like phenotype. These studies

indicate that β-catenin might be involved in maintaining cartilage in adult indicating that a fine balance of β-catenin is required both for normal chondrogenic differentiation, as well as for the maintenance of cartilage tissue after formation [32, 33].

A number of WNT ligands have been implicated in the regulation of various aspects of endochondral ossification. Although WNT-1 is not endogenously expressed during limb development, overexpression of WNT-1 in chick embryos in vivo was found to cause skeletal abnormalities such as truncation or deletion of skeletal elements [34, 35]. Because retroviral expression of WNT-1 in limb bud micromass cultures resulted in severely inhibited cartilage formation, this might be the underlying mechanism for these skeletal abnormalities in vivo [35]. WNT-3A regulates the expansion of the MSC population, through increasing self-renewal and decreasing apoptosis [36, 37]. Expression of WNT-3A is decreased when chondrogenic differentiation progresses. Furthermore, addition of exogenous WNT-3A blocked the collagen type II expression and suppressed the deposition of sulfated proteoglycans, indicating that down-regulation of WNT-3A is required for chondrogenesis [38]. WNT-4 was found to be involved in joint formation and cartilage development, as it is expressed in developing joints, in the periphery of the joint interzone and in a subset of hypertrophic chondrocytes [39, 40]. Different effects of WNT-4 were found during different stages of skeletal development. Misexpression of WNT-4 resulted in the shortening of long bones and histological examination of developing limbs revealed that cartilage elements in these limbs showed an expanded hypertrophic zone and a thicker osteoid layer of the bone collar, compared to the contralateral control limb [41]. These findings indicate that WNT-4 accelerates chondrocyte maturation, which results in an accumulation of terminally differentiated hypertrophic chondrocytes at the expense of immature round chondrocytes at the ends of the cartilage elements [40]. During skeletal development, WNT-5A is initially expressed in the mesenchyme around the developing condensations, indicating that WNT-5A might be involved in the recruitment of mesenchymal cells into the chondrogenic lineage. At later stages, WNT-5A expression was found in the perichondrium, indicating the involvement of WNT-5A in the formation of bone. Expression of WNT-5B was restricted to prehypertrophic chondrocytes, as well as cells in the outer layer of the perichondrium [39, 40, 42]. WNT-5A and WNT-5B both promote the first steps of chondrogenesis in micromass cultures, whereas cartilage elements in which WNT-5A was misexpressed are smaller in size and show a delay in the maturation of hypertrophic chondrocytes histologically and molecularly [39, 41]. Expression of WNT-7A was found in the dorsal ectoderm in the developing limb [43]. Chondrogenesis was blocked by WNT-7A in micromass cultures in vitro as well as after in vivo overexpression in chick embryos [34]. Furthermore, WNT-7A induces a chondroinhibitory effect, which is mediated by MAP kinase and AP1 signaling [44]. WNT-14 expression was, like WNT-4, found in the developing joint interzone [45]. WNT-14 is implicated in the initial steps of joint development and it was found to arrest and even reverse chondrogenic differentiation. Ectopic expression of WNT-14 in the prechondrogenic region prevents prechondrogenic cells from further differentiating into chondrocytes, with down-regulated expression of chondrogenic markers *Collagen II* and *Sox9* [40]. In the mature joint, WNT-14 continues to be expressed in the synoviocytes and the joint capsule.

The regulation and specificity of WNT signaling is not only dependent on the presence of specific ligands and receptors, but also on the action of endogenous antagonists of WNT signaling. FRZB expression was specifically found in mesenchymal prechondrogenic condensations and at later stages in epiphyseal prearticular chondrocytes [46, 47]. Overexpression of *Frzb* in vivo blocks chondrocyte maturation at an early hypertrophic stage and prevents endochondral ossification [47]. In addition, *Frzb* knockout mice, in which a mild activation of WNT/β-catenin signaling was observed, exhibit accelerated hypertrophic chondrocyte maturation [48]. Addition of FRZB to micromass cultures of MSCs promoted glycosaminoglycan synthesis, as well as gene expression and protein expression of SOX9 and collagen type II. DKK-1 was found to have a similar effect, although to a lesser extent [49]. A recent study provided evidence that the WNT antagonists DKK-1 and FRZB in combination with the bone morphogenetic protein (BMP) antagonist GREM1 are significantly higher expressed in articular cartilage compared to growth plate cartilage [50]. The authors provided evidence that these antagonists are natural breaks of hypertrophic differentiation and subsequent endochondral ossification of articular cartilage. WIF-1 expression was found in the mesenchyme surrounding cartilage elements forming in the limb during early skeletal development. In late embryonic and postnatal development, WIF-1 expression was observed in articular cartilage. Moreover, WIF-1 interferes with WNT-3A mediated inhibition of chondrogenesis in micromass cultures [51].

Cartilage Degeneration in Arthritis

Arthritis is a joint disorder that usually involves one or more joints. Osteoarthritis (OA), also known as degenerative arthritis, is the most common form of arthritis. It is a heterogeneous disease characterized by progressive degradation of joint cartilage, typical bone changes and signs of mild synovitis particularly in more advanced stages of

the disease. In contrast, rheumatoid arthritis (RA) is a chronic, systemic inflammatory disorder that may affect many tissues and organs, but principally involves synovial joints. In both OA and RA, articular cartilage is the primary target for damage as a result of loss of cartilage homeostasis. Cartilage homeostasis in healthy joints is maintained by the balance of synthesis and degradation of extracellular matrix (ECM). Cartilage undergoes destruction when this balance is lost.

In the adult, articular chondrocytes are fully differentiated cells that play a critical role in the pathogenesis of OA by responding to adverse environmental stimuli by promoting matrix degradation and down-regulating processes essential for cartilage repair. Multiple risk factors have been implicated in the initiation and progression of OA, including mechanical injury, genetics and aging [52]. For instance, in response to traumatic injury chondrocytes activate general gene expression, which results in increased expression of inflammatory mediators, cartilage-degrading proteinases, and stress response factors [53, 54].

Synovial inflammation likely contributes to deregulation of chondrocyte function, amongst others by secreting cytokines that impact chondrocyte activity [55]. Chondrocytes can respond to a number of cytokines and chemokines in the joint tissue and joint fluid. These cytokines and chemokines can be produced by other cells such as fibroblast-like synoviocytes (FLS) which play an important role in the pathogenesis of RA [56]. IL-1β and TNF-α are able to induce the synthesis of ECM degrading enzymes such as MMPs as well as the production of other pro-inflammatory mediators such as prostaglandin E2 (PGE2) and nitric oxide (NO) [57, 58]. In addition, the association of the increased levels of catabolic enzymes and inflammatory mediators such as prostaglandins and NO and the levels of cytokines like IL-1β and TNF-α in synovial fluid and joint tissue has been established [59]. Pro-inflammatory cytokines induce loss of the chondrocytic phenotype of chondrocytes in the matrix and can induce chondrocyte apoptosis [60, 61]. These findings indicate that the pro-inflammatory mediators are crucial mediators of cartilage degeneration.

As was mentioned before, abnormal mechanical loading or synovial inflammation may induce a disequilibrium between the catabolic and anabolic activity of chondrocytes. The relation between increased production of proteinases, including MMPs, MMP-1, MMP-3, MMP-8, MMP-13, and the aggrecanases, particularly ADAMTS-5, with cartilage damage has been documented [62, 63]. FLS in the synovium also produce pro-MMP-3 (precursor form of MMP-3 or stromelysin 1), which in its mature form enhances cartilage degradation [56]. Production and activities of these proteinases are regulated by various mediators such as cytokines, growth factors, prostaglandins, matrix breakdown products, and oxygen species [64, 65]. It has also been shown

that expression of the *COL2A1* gene is suppressed in upper zones of OA cartilage with progressing matrix destruction, whereas global *COL2A1* gene expression is increased in late-stage OA cartilage compared to normal and early degenerative cartilage suggesting a compensatory mechanism [66]. Cessation of cartilage ECM molecule synthesis can be caused by a number of factors such as pro-inflammatory cytokines [60] and NO [61]. Anabolic factors such as BMP-2, activin A and tumor necrosis factor-β (TGF-β) superfamily members might be responsive for the compensatory increase in COL2A1 expression [67, 68]. Importantly, once the cartilage is severely degraded the chondrocyte is unable to reproduce the complex arrangement of collagen laid down during development. Therefore, the imbalance between catabolic and anabolic activities of the chondrocytes is a key contributor to cartilage degeneration.

Phenotypic Modulation of Chondrocyte Function by WNT Signaling

In addition to its function in chondrogenesis and chondrocyte maturation, WNT signaling is also involved in the maintenance of fully differentiated chondrocyte phenotypes and may therefore play a crucial role in cartilage homeostasis throughout adult life. When differentiated chondrocytes are exposed to inflammatory factors such as IL-1 and retinoic acid or when cultured in monolayer, their phenotype is rapidly lost and cells become fibroblast-like. This process is known as dedifferentiation and it is accompanied by increased β-catenin protein expression [69] and differential expression of components of the WNT signaling pathway, such as DKK1, FRZB, WNT-5A, and FZD-4, -8, -9 (unpublished data). Accumulation of β-catenin by ectopic expression or inhibition of its degradation results in a decrease of cartilage-specific ECM molecule synthesis through activation of TCF/LEF transcriptional activity in rabbit chondrocytes [69]. Conditional deletion of the *APC* gene, which results in up-regulation of β-catenin in mature chondrocytes, also results in a complete loss of the chondrocyte phenotype in vivo [70]. In addition, WNT-3A and WNT-7A caused loss of type II collagen synthesis via stimulation of β-catenin–TCF/LEF transcriptional activity [38]. Moreover, WNT-3A induced the expression of c-Jun and its phosphorylation by c-Jun N-terminal kinase (JNK), resulting in activation of AP-1. AP-1 could suppress the expression of SOX9, a major transcription factor regulating COL2A1 expression [38]. In contrast, WNT-3A inhibited chondrogenesis of mesenchymal cells by stabilizing cell-cell adhesion in a manner independent of β-catenin transcriptional activity [38]. It has also been shown that WNT-7A inhibited NO-induced apoptosis by activating cell survival signaling, such as phosphatidylinositol 3-kinase and AKT, independent of β-catenin transcriptional activity [71].

Together, these results suggest that WNT proteins regulate chondrocyte functions via different mechanisms.

However, all these studies were performed in animal chondrocytes, whereas the function of WNT signaling in human chondrocytes has so far not been well studied. A recent study reported that WNT-3A promoted human articular chondrocyte proliferation through the β-catenin-dependent canonical pathway while simultaneously inducing loss of expression of chondrocyte marker genes via a β-catenin-independent noncanonical pathway (Fig. 3) [11]. Dedifferentiation of human chondrocytes in vitro could not be reversed by inhibition of the canonical WNT pathway either by knock-down of β-catenin or by addition of a TCF/β-catenin inhibitor (unpublished data). Remarkably, during human chondrocyte dedifferentiation the noncanonical WNT-5A is strongly up-regulated which coincided with a down-regulation of COL2A1 expression. Knockdown of WNT-5A reversed COL2A1 expression, again suggesting that dedifferentiation in human chondrocytes appears independent of β-catenin (unpublished data). These findings are in contrast with observations in rabbit chondrocytes, in which β-catenin-TCF/LEF transcriptional activity contributed to chondrocyte dedifferentiation [69]. The controversial findings in human and animal chondrocytes suggest that the exact function of the canonical WNT pathway in articular cartilage may be species-dependent. Such species difference was also observed in the regulation of MMP expression in human and animal chondrocytes [72].

In contrast to WNT-3A and WNT-7A, WNT-5A and WNT-11 primarily regulate cartilage-specific ECM
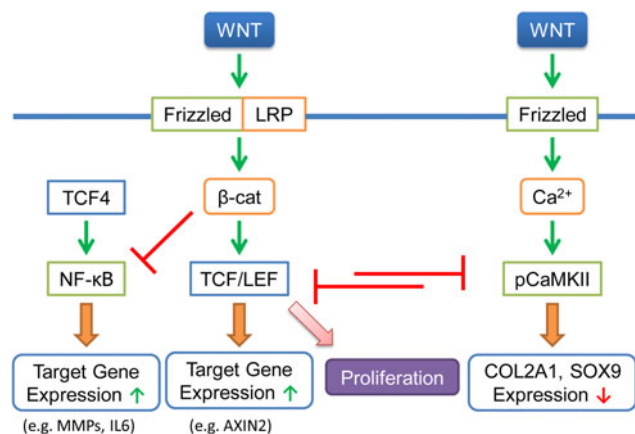

Fig. 3 Role of WNT signaling in human chondrocytes WNTs may activate both canonical and noncanonical pathways in a cellular context dependent manner, most likely involving differential expression of FZD receptors at the cell surface. The canonical WNT/β-catenin pathway induces proliferation and expression of target genes such as *AXIN2*. It represses NF-κB signaling through an inhibitory interaction of β-catenin with NF-κB, consequently inhibiting expression of target genes such as MMPs and IL6. In contrast, TCF4 is able to potentiate NF-κB signaling independent of β-catenin. The noncanonical WNT signaling decreases COL2A1 and SOX9 expression through Ca²⁺/CaMKII pathway

molecule synthesis through the noncanonical pathway [73]. Stimulation of rabbit chondrocytes with IL-1β up-regulated *WNT-5A* and down-regulated WNT-11 expression. WNT-5A inhibited COL2A1 expression via the JNK pathway, whereas WNT-11 stimulated COL2A1 expression via the PKC pathway, indicating that WNT-5A and WNT-11 have opposing effects on COL2A1 expression by signaling through distinct noncanonical WNT pathways in rabbit chondrocytes [73]. In human chondrocytes, WNT-5A was also found to block COL2A1 expression, in agreement with its effects in rabbit chondrocytes [72]. Interestingly, WNT-3A is also able to down-regulate *COL2A1* and *SOX9* expression through the noncanonical Ca²⁺/CaMKII pathway (Fig. 3) [11]. All these findings substantiate the role of noncanonical cascade in the deregulation of chondrocyte function. Collectively, a direct role of the β-catenin-dependent canonical pathway is unlikely in human chondrocyte dedifferentiation.

WNT Signaling in Cartilage Degeneration

In light of the involvement of WNT signaling in cartilage development and the maintenance of adult chondrocyte phenotype and cartilage homeostasis, dysfunction of the WNT pathway may lead to cartilage tissue disease. Indeed, differential expression of WNT pathway components has been documented in joint disorders such OA and RA. In several genome-wide association studies, the WNT antagonist *FRZB* has emerged as a candidate gene associated with an increased risk for OA [74–77]. A single-nucleotide polymorphism in *FRZB* resulting in an Arg324Gly substitution at the carboxyl terminus, which shows diminished ability to antagonize WNT signaling in vitro, was associated with hip OA in females [75]. The correlation of elevated circulating levels of DKK-1, another WNT antagonist, with reduced progression of radiographic hip osteoarthritis (RHOA) in elderly women has also been suggested [78]. This is in line with the proposed role of DKK-1 with FRZB and GREM1 as natural brakes of chondrocyte hypertrophy. Derailed hypertrophic differentiation in articular cartilage has been implemented in the pathogenesis of OA, at least in a subset of patients [50]. Likewise, differential expression of various WNTs and their receptors has been reported in human joint disorders [79]. For example, overexpression of WNT-5A and FZD5 has been found in RA synovial tissues in comparison to a panel of normal adult tissues [79], while the canonical WNT-7B is up-regulated in OA cartilage and RA synovium [80]. In addition, increased expression of the WNT target gene *WISP-1* was found in both mouse OA models and in human OA cartilage [81]. A systematic analysis of the WNT signaling pathway revealed up-regulation of WNT-16, down-regulation of FRZB, up-regulation of WNT target

genes, and nuclear localization of β-catenin in injured cartilage [82]. In addition, in OA, WNT-16 and β-catenin were barely detectable in preserved cartilage areas, but were dramatically up-regulated in areas of the same joint with moderate to severe OA damage [82]. These findings were subsequently corroborated by observation of increased nuclear β-catenin staining in human OA cartilage compared to control [32]. Therefore, these studies indicate that increased WNT signaling is associated with cartilage degeneration.

To explore the exact function and underlying mechanism of WNT signaling in joint biology and disease, a variety of studies have been conducted in animal models. Although a noteworthy developmental phenotype is not developed, $Frzb^{-/-}$ mice display greater cartilage loss in comparison to wild type controls when exposed to factors known to induce OA, like enzymatic treatment (papain-induced OA), accelerated instability (collagenase-induced ligament and meniscal damage) or inflammation (mBSA-induced monoarthritis) [83, 84]. The mild phenotype might be explained by partial compensation by other antagonists like DKK-1 and GREM1 [50]. Cartilage degradation in the $Frzb^{-/-}$ mice is associated with up-regulation of β-catenin and MMP-9. Interestingly, it was also shown that cartilage injury results in increased WNT activity and decreased expression of FRZB in vitro [85]. In postnatal mouse models, β-catenin signaling has been selectively activated in articular chondrocytes in 3- and 6-month-old mice using an inducible conditional transgenic approach and tissues were analyzed two months later. In 5-month-old mice reduced articular cartilage area and matrix production is observed [32]. In 8-month-old mice cell cloning, surface fibrillation, vertical clefting, and chondrophyte/osteophyte formation is observed. At this age, complete loss of articular cartilage layers and the formation of new woven bone in the subchondral bone area is also found. These findings indicate that activation of β-catenin signaling in articular chondrocytes in adult mice leads to the development of an OA-like phenotype. At both ages, activation of β-catenin accelerates chondrocyte maturation [32]. Because the phenotype 2 months after the inducible conditional activation of β-catenin in 6-month-old mice is more pronounced than in 3-month-old-mice, β-catenin's role in chondrocytes may be age dependent. This is further underscored by the phenotype of transgenic mouse embryos with conditional activation of β-catenin in collagen type II positive chondrocytes. Constitutive activation of β-catenin in chondrocytes is not compatible with the formation of chondrocytes and the phenotype of these mice resembles the phenotype of embryos lacking Sox9 expression in chondrocytes [86]. A similar phenotype is found in mice with a conditional inactivation of the *Apc* gene in chondrocytes [70]. Thus, in organogenesis, activation of β-catenin in collagen type II positive chondrocytes arrests further chondrogenesis and induces a complete loss of the chondrocyte phenotype reverting in a more undifferentiated cell type [70, 86], while in adult articular cartilage activation of β-catenin accelerates chondrocyte maturation and induces typical signs of OA [32].

Activation of WNT/β-catenin signaling in rabbit and mouse chondrocytes stimulates the expression of cartilage matrix degrading MMPs [87, 88]. In a spontaneous guinea pig OA model, development of OA is associated with increased β-catenin expression in cartilage [87]. Interestingly, inhibition of β-catenin signaling in articular chondrocytes also causes OA-like cartilage degradation in a Col2a1-ICAT transgenic mouse model [14]. In 6-month-old or elder Col2a1-ICAT transgenic mice, ICAT expression is associated with significant increase in articular chondrocyte apoptosis and cartilage destruction. Consistent with increased TUNEL staining, Bcl-2 and Bcl-x expression are decreased and caspase 9 and caspase 3/7 activity are increased in chondrocytes, suggesting that increased cell apoptosis may contribute to articular cartilage destruction observed in Col2a1-ICAT transgenic mice. Because ICAT may have other cellular targets than β-catenin, it is unclear whether the OA-like phenotype in these mice can solely be attributed to inhibition of β-catenin. Taken together, these findings have led to the hypothesis that in postnatal cartilage low levels of WNT/β-catenin signaling are required for maintenance of normal cartilage function and that deregulation of this pathway may contribute to the development and progression of cartilage degeneration. This hypothesis is supported by strong experimental evidence in animal models. The support for such a role in human OA, however, is less strong and is predominantly based on circumstantial evidence showing associations between increased β-catenin levels and an OA phenotype in cartilage specimens. Evidence for a causal relationship in human is currently lacking.

In contrast to its pro-catabolic role in animal cartilage by inducing ECM degrading enzymes such as MMPs, WNT activation was found to inhibit MMP expression in a TCF/LEF-independent pathway in human articular chondrocytes [72]. In animal cells, the WNT pathway regulates MMP expression through β-catenin-TCF/LEF transcriptional activity [72, 89]. In human chondrocytes, activation of canonical WNT blocks MMP expression through an inhibitory interaction of β-catenin with NF-κB (Fig. 3) [72]. This species difference in the regulation of pro-catabolic MMP expression gives rise to the question whether canonical WNT signaling is a pathogenic factor in human cartilage degeneration. However, similar to findings in animal models, in fibroblast-like synoviocytes from RA patients, activation of canonical WNT signaling by *WNT-1* transfection increases expression of MMP-3, while interference with WNT signaling using anti-WNT-1 blocking antibody or the WNT antagonist sFRP-1 decreases MMP-3 expression [90]. WNT signaling may exhibit complicated functions in joint disease

by activating multiple cascades and interacting with other pathways, which might also be tissue dependent. In addition, the heterogeneity of cell cultures, age of donors and stages of differentiation of chondrocytes are alternative explanations for the observed differences between human and animal chondrocytes. Furthermore, the cellular context might play a decisive factor in determining how cells respond to β-catenin. Factors that may influence a cell's response to WNT/β-catenin signaling are amongst others the presence and concentration of specific combinations of Frizzled or LRP5/6 receptors at the cell surface [4] and/or intracellular regulators of canonical WNT signaling that may compete for binding to β-catenin. For example, detailed comparison of Frizzled receptors expression between human and animal chondrocytes as a function of age and cellular differentiation state are lacking. In addition, a number of intracellular targets of β-catenin have been identified such as TCF/LEF transcription factor, NF-κB and SOX9 [3, 72, 86]. These factors may compete for binding to β-catenin and consequently the relative abundance of these factors in a cell may influence β-catenin's response. Detailed side-by-side analysis of the expression of these factors in chondrocytes from distinct species are lacking. Therefore, the role of WNT signaling in human cartilage degeneration remains elusive and more studies should be focused on extrapolation of knowledge obtained from animal models to the human situation. Our recent study also reveals that TCF4 induces MMP expression and apoptosis, probably through potentiating NF-κB signaling (Fig. 3) and its expression is up-regulated in OA cartilage compared to normal human cartilage (unpublished data). Thus, TCF4 may serve as a potential therapeutic target for OA.

A role of noncanonical WNT signaling in cartilage degeneration is suggested by the induction of MMP expression by WNT-5A in rabbit chondrocytes [91] and repression of chondrocyte marker gene expression by WNT-5A or the WNT-3A-mediated Ca^{2+} pathway [11]. It has been suggested that canonical and noncanonical pathways reciprocally inhibit each other [11]. Therefore, blocking the canonical/β-catenin pathway will also cause articular chondrocyte dedifferentiation through de-repression of the Ca^{2+} pathways. Stimuli such as IL-1 [92] and biomechanics [93], which change CaMKII, may significantly influence the outcome of WNT signaling by switching the balance between β-catenin and CaMKII. Thus, IL-1-mediated procatabolic activity in cartilage may partially come from its enhancement of the Ca^{2+} cascade activity initiated by WNT. The opposing induction of the β-catenin pathway by IL-1 serves as a negative feedback to counteract its pro-catabolic activity [72]. Because of the complicated properties of WNT in activating multiple pathways and WNT signaling components showing diverse functions in human chondrocytes, more specific targeted therapy should be developed with respect to treating human joint disease by manipulating WNT signaling.

Conclusion

A remarkable effort has unraveled the stage-dependent regulatory role of WNT signaling in chondrogenesis and cartilage development. Consequently, cumulating evidence has suggested the involvement of WNT signaling in cartilage disease. Although extensive animal studies have indicated that excessive WNT signaling may lead to cartilage destruction, the exact function of WNT signaling in human cartilage is still largely unclear. Species differences in WNT function in chondrocytes have been observed. The future challenge for research is to properly extrapolate our knowledge of WNT signaling in animal models to the human situation. Given the fact that WNT signaling is a complex network, accumulation of our understanding of the involvement of specific WNT components in cartilage degeneration will facilitate the development of more effective and specific treatments for joint disease.

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