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Wnt/ β -catenin signaling plays a key role in the development of spondyloarthritis

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Abstract

Spondyloarthritis (SpA) is a group of diseases consisting of psoriatic arthritis (PsA), reactive arthritis, arthritis related to inflammatory bowel disease (a subgroup of juvenile idiopathic arthritis), and ankylosing spondylitis (the prototype of SpA). Axial bone formation and the combination of concurrent erosion and new bone formation are specific characteristics of SpA disease. The use of anti-proinflammatory cytokines, such as inhibitors of tumor necrosis factor α (TNF- α), appears to be the greatest advance in the treatment of SpA over the past 20 years. However, TNF- α blockers do not halt new bone formation. Recent clinical observations and animal studies demonstrate that Wnt signaling proteins and natural Wnt inhibitors, such as DKK1 and sclerostin, are likely to play important roles in the process of ankylosis in SpA, and could potentially serve as therapeutic targets for the treatment of SpA.

Keywords

spondyloarthritis; Wnt signaling pathway; DKK1; sclerostin; bone formation

Spondyloarthritis

In 1974, Moll and colleagues¹ defined a group of rheumatic diseases as spondyloarthritis (SpA), and separated SpA from other rheumatic diseases based on clinical features and a common genetic predisposition. SpA encompasses axial and peripheral spinal arthritis.^{2,3} However, for clinical purposes, SpA is normally divided into five major subtypes, including ankylosing spondylitis (AS), psoriatic arthritis (PsA), reactive spondylitis (ReSpA), SpA associated with inflammatory bowel disease (IBD), and undifferentiated SpA (uSpA).⁴ The most important clinical features of SpA are inflammatory back pain, asymmetric peripheral oligoarthritis, predominantly of the lower limbs, enthesitis, and specific organ involvement, most frequently manifesting as anterior uveitis, psoriasis, and IBD.⁵ The clinical symptoms of SpA diseases are characteristically similar and evidence shows that there are genetic similarities; however, there is also heterogeneity among the different SpA diseases. As

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Conflicts of interest

The authors declare no conflicts of interest

reported in 1973, the major histocompatibility complex (MHC) class I molecule, human leukocyte antigen (HLA)-B27, is known to be the strongest contributing factor to AS. However, a recent study suggests that other genetic factors, such as endoplasmic reticulum amino-peptidase 1 (ERAP-1) and interleukin (IL)-23R, also play an important role in the pathogenesis of SpA.⁴ Interestingly, polymorphisms of the IL-23R gene (*IL23R*) have also been identified in psoriasis and IBD.^{6, 7}

The pathological process of SpA can be divided into three stages:⁸ stage 1, inflammation, in which TNF- α is the principal cytokine involved;⁹ stage 2, erosion; and stage 3, abnormal bony outgrowth (syndesmophytes). Syndesmophytes are the hallmarks of axial SpA. Although the key molecular mechanism is largely unknown and the relationship between inflammation and ankylosis remains controversial, recent findings suggest that Wnt signaling and natural Wnt inhibitors, such as DKK-1 and sclerostin, are involved in stage 3 SpA. Treatment of SpA is primarily based on non-pharmacological and pharmacological treatments, including the use of TNF- α blockers in conjunction with nonsteroidal anti-inflammatory agents. Currently, four anti-TNF- α agents are available and approved for the treatment of AS: infliximab, etanercept, adalimumab and golimumab.¹⁰ However, therapeutic options for patients suffering from the more severe forms of SpA have not been fully developed due to the lack of clear understanding of the cause and progression of the disease.¹⁰⁻¹² New evidence suggests that treatment with anti-TNF- α agents may relieve pain, but might not alter the progression of radiographic bony proliferation and fusion in patients with AS.^{13, 14} Other evidence shows that ankylosis may progress, even with treatment with anti-TNF- α agents.¹⁵⁻¹⁷ While the mechanism leading to bony outgrowth in SpA needs to be further defined, several studies have demonstrated that the Wnt signaling pathway may contribute to the process of SpA. A further understanding of the relationship between Wnt signaling and new bone formation (syndesmophytes or ankylosis) in SpA could shed light on the therapeutics of these diseases.

Wnt/ β -catenin signaling

The Wnt family consists of a number of small, cysteine-rich, secreted glycoproteins involved in regulation of a variety of cellular activities with critical roles during development.¹⁸⁻²⁰ Wnt proteins trigger signaling pathways within cells that proceed through several protein complexes including β -catenin. The canonical Wnt signaling pathway regulates β -catenin expression and its subcellular localization. In the absence of Wnts, β -catenin levels are kept in steady state. Free β -catenin proteins are ubiquitinated and degraded by the 26S proteasome.²¹ A multiprotein complex containing the kinases GSK-3 β and casein kinase-1 (CK1) along with the scaffolding proteins Axis inhibition protein 1 (Axin1), Axin2, adenomatous polyposis coli (APC) and disheveled mediates the degradation of excess β -catenin. This complex phosphorylates specific amino acid residues on β -catenin, creating docking sites for the F-box protein/E3 ligase complexes.^{20,22,23} Therefore, inhibition of β -catenin phosphorylation prevents its degradation and increases its cytoplasmic level and facilitates its nuclear translocation. Signaling from Wnt proteins releases β -catenin from its binding proteins, allowing it to move to the nucleus, where it interacts with the TCF/LEF transcription factors to activate expression of target genes.^{19, 20,24,25}

At the cell surface, Wnts interact with two classes of protein: Frizzled receptors and low-density-lipoprotein (LDL)-receptor related protein 5 or 6 (LRP5/6). There are many genes encoding frizzled proteins, including 10 in the human genome. Individual frizzled proteins likely have different affinities for various types of Wnt proteins. Wnt proteins can form a complex with both the cysteine-rich domain (CRD) of frizzled and with LRP5/6 simultaneously, leading to the formation of a dual-receptor complex.^{26,27} The intracellular portion of the receptors communicates this binding information and turn on the pathways that act on β -catenin inside the cell. Following Wnt binding, the intracellular tail of LRP5/6 binds Axin1 (or Axin2) and causes dissociation of β -catenin from its protein complex, activating β -catenin signaling.²⁸ Sclerostin and DKK1 bind Wnt co-receptors LRP5/6 to inhibit Wnt binding and signaling. Sclerostin and DKK1 bind the first β -propeller of LRP5 and LRP6 to inhibit Wnt1 class signaling.^{29,30} DKK1 also binds the third β -propeller to inhibit Wnt3a class signaling.³¹ DKK1 and sclerostin also utilize co-receptors to enhance their inhibitory activity. DKK1 forms a ternary complex with LRP5 or LRP6 and Kremen receptors 1 or 2, which results in internalization of the complex.^{31,32}

Wnt signaling in AS

AS is the major subtype of SpA.¹² AS and other forms of SpA are characterized by two major processes: chronic inflammation of the spine and enthesitis, and progressive ankyloses.³³ An enthesitis is an anatomical zone in which the fibers of tendons, ligaments, and capsules insert into the bone through a fibrocartilaginous connection. The primary disease location in SpA is hypothesized to be the enthesitis.³⁴ Excessive bone formation in AS leads to the formation of bone spurs, such as syndesmophytes and enthesiophytes, which contribute significantly to the permanent disability of patients.³⁵ The Wnt proteins are critically important in normal bone homeostasis, in particular in osteoblastic new bone formation. Therefore, Wnt proteins may also play a role in the process of new bone formation in AS.³⁶⁻⁴⁰ Various components of the Wnt signaling molecules were recently found to be involved in maintaining bone mass. The most studied secreted Wnt inhibitors are the dickkopfs (DKKs), sclerostin, and secreted frizzled related proteins, all of which likely play important roles in bone turnover during the disease progression.⁴¹

DKK1

DKK is a family of cysteine-rich proteins comprising at least four members: DKK1, DKK2, DKK3 and DKK4. Among these, the one best studied is DKK1, which functions as a natural inhibitor of Wnt signaling.^{42,43} DKK1 was recently recognized as a key player in the process of new bone formation in AS,⁴⁴⁻⁴⁷ and DKK1 blockade was shown to lead to the fusion of sacroiliac joints in an animal model of arthritis.⁴⁵ Activation of Wnt signaling by blocking its natural inhibitor DKK1 leads to the formation of osteophytes in peripheral joints.⁴⁴ Heterozygous *Dkk1*-deficient mice (*Dkk1*^{+/-}) showed increased bone formation and bone mass.⁴⁶ *Dkk1* transgenic mice exhibited increased expression of DKK1 and an osteopenic phenotype.⁴⁷ Several recent studies focused on the role of DKK1 in patients with AS; differing results were reported because of the various methodologies utilized in the studies. One study that assessed serum levels of DKK1 using an enzyme-linked immunosorbent assay measuring functional serum DKK1 levels bound to the LRP-6

receptor showed that DKK1 levels decreased in AS patients, in contrast with patients with rheumatoid arthritis (RA), who exhibited increased DKK1 levels.⁴⁴ Another study reported that patients with AS receiving anti-TNF- α treatment had significantly higher serum DKK1 levels than patients with AS who did not receive anti-TNF- α treatment. Despite increased serum levels, the DKK1 in the sera of AS patients was found to be less able to suppress β -catenin translocation to the nucleus than that in the control sera, suggesting that the DKK1 in AS patients might not be fully functional.⁴⁸ The German SpA Inception Cohort (GESPIC) study, with a two-year follow-up of AS patients, demonstrated that patients with lower DKK1 levels were more prone to the development of syndesmophytes.⁴⁹ A similar finding from a study conducted in Korea also suggests that DKK1 levels were lower in AS patients than in control subjects.⁵⁰ The high levels of functional DKK1 were associated with protection from radiographically-detected syndesmophyte formation in the GESPIC study.⁴⁹ Although the assay methods used were different in these studies, the findings suggest that DKK1 levels were decreased in AS patients and that these patients had a higher risk of developing syndesmophytes. In addition, although circulating DKK1 levels had increased with anti-TNF- α treatment in AS patients, these increased DKK1 proteins might not have been fully functional.

Sclerostin

Sclerostin is another Wnt inhibitor that suppresses the Wnt pathway. The levels of sclerostin in osteocytes and in the sera of RA, AS, and osteoarthritis (OA) patients have been investigated; sclerostin levels were found to be significantly lower in AS patients than those in RA and OA patients and healthy controls.⁵¹ Sclerostin also inhibits bone morphogenetic protein (BMP) signaling. Mechanical loading of osteocytes has been reported to stimulate BMP,⁵² while a decrease of DKK1 and sclerostin levels could activate Wnt and BMP signaling.⁵³ These findings may explain why new bone formation selectively involves the entheses, which are subjected to high mechanical loading.

Risk loci

Certain risk loci linked to the Wnt/ β -catenin pathway are related to neo-ossification/ankylosis in AS patients.⁵⁴ Anthrax toxin-receptor 2 (ANTXR2), a membrane-bound molecule, can interact with LRP6⁵⁵ and potentially affect new bone formation. LRP6 is an important cell surface receptor in the Wnt/ β -catenin pathway affecting osteoblast activity. A recent genome-wide association study performed on Han Chinese with AS⁵⁶ detected two risk loci likely related to bone formation, *HAPLN1-EDIL3* at 5q14.3 and *ANO6* at 12q12.1. *HAPLN1* has been shown to be involved in osteophyte formation⁵⁷ in Japanese women with SpA. *EDIL3* has an inhibitory effect on Wnt/ β -catenin signaling.⁵⁸

Wnt signaling in PsA

The findings mentioned above point to the possible use of DKK1 and sclerostin as biomarkers for patients with increased risk of development of progressive ankylosis. It has been suggested that DKK1 is a serum-soluble bone turnover biomarker in PsA.⁵⁹ However, findings on DKK1 levels in PsA patients are very different from the findings in AS patients. Levels of DKK1 have been reported to be elevated in patients with PsA compared to healthy

controls.^{59,60} These findings suggest that, unlike other types of SpA, the new bone formation observed in PsA patients may be regulated by different signaling mechanisms. Therefore, DKK1 may not be useful as a biomarker for PsA. The significance of DKK1 up-regulation in patients with PsA needs further investigation.

Wnt signaling in SpA associated with IBD

Some studies examined the role of Wnt/ β -catenin pathway in AS related to IBD.⁶¹⁻⁶³ Xing *et al.*⁶¹ recently created a rat IBD model and found that canonical Wnt signaling was activated in rats with IBD, possibly leading to the suppression of mesenchymal stem cell transplants (MSCTs) differentiating into intestinal epithelium. This research suggests that the canonical Wnt signaling pathway is activated to promote proliferation of intestinal stem cells in IBD. Annalucia *et al.*⁶² reported that Wnt pathway components could be used as predictive markers for the diagnosis, prevention and treatment of IBD. Carina *et al.*⁶³ found that the drugs used clinically for IBD treatment, such as aminosalicilate mesalazine (5-ASA), acted by inhibiting the Wnt/ β -catenin pathway, indicating that inhibition of the Wnt/ β -catenin pathway may protect from IBD. Other studies suggest that the Wnt signaling pathway is indirectly associated with IBD; these findings also suggest a role for the Wnt signaling pathway in IBD-associated disease.⁶⁴⁻⁶⁷

Cartilage β -catenin signaling in SpA

We have recently generated chondrocyte-specific β -catenin gene (*Ctnnb1*) conditional activation mice, *Ctnnb1(ex3)^{Col2ER}* mice, by breeding *Ctnnb1(ex3)^{lox/lox}* mice with *Col2-Cre^{ERT2}* transgenic mice.^{68,69} Our findings in *Ctnnb1(ex3)^{Col2ER}* mice include the following. (1) Severe destruction in disc tissues of the spine in 3- and 6-month-old *Ctnnb1(ex3)^{Col2ER}* mice, including severe loss of cartilage tissue in the growth plate and endplate, disorganized annulus fibrosus (AF) and nucleus pulposus (NP) tissues, and large amounts of osteophyte formation in the disc tissue of the entire spine, was observed. These anomalies were associated with the up-regulation of *Mmp13* and *Adamts5* expression in disc tissues of *Ctnnb1(ex3)^{Col2ER}* mice.⁷⁰ (2) Deletion of *Mmp13* or *Adamts5* in *Ctnnb1(ex3)^{Col2ER}* mice significantly reversed the defects observed in disc tissues of *Ctnnb1(ex3)^{Col2ER}* mice.⁷¹ (3) Severe knee joint destruction associated with accelerated maturation of articular chondrocytes was found in 5- and 8-month-old *Ctnnb1(ex3)^{Col2ER}* mice.⁷² (4) Severe cartilage damage in temporomandibular joint (TMJ) tissues was found in 3- and 6-month-old *Ctnnb1(ex3)^{Col2ER}* mice; this phenotype was also significantly reversed by deletion of *Mmp13* or *Adamts5* in the *Ctnnb1(ex3)^{Col2ER}* background.⁷³ (5) Severe damage and loss of proteoglycan at the cartilage surface of the facet joint, demonstrated by Alcian blue and Safranin O staining, were observed in 6- and 9-month-old *Ctnnb1(ex3)^{Col2ER}* mice. Additionally, constant pain was experienced by 4-9 month-old *Ctnnb1(ex3)^{Col2ER}* mice (unpublished data).

As we know, SpA has two major phases, inflammation and bony overgrowth. Spine and joints are the major organs affected in the body during the disease progression. Currently it is unknown if inflammation and bony overgrowth (ankylosis) are linked or separated events. Severe defects in spine, including the degradation of tissues in disc⁷⁰ and in facet joint

(unpublished data), were observed in cartilage-specific β -catenin conditional activation mice. In addition to the defects in spine, β -catenin conditional activation mice also showed OA-like changes in knee joint.⁷² These animals also experience severe pain (unpublished data). Based on these observations, we hypothesize that inflammatory cytokine(s) released during the inflammation phase of the disease causes up-regulation of β -catenin signaling, leading to bony overgrowth during disease progression. The key questions that need to be addressed are: which inflammatory cytokine(s) up-regulates β -catenin signaling during the disease progression; and what is the molecular mechanism involved in the β -catenin up-regulation process.

It has been reported that IL-1 β increased β -catenin protein levels in rabbit articular chondrocytes.⁷⁴ In addition, it has been shown that TNF- α and IL-1 β upregulated β -catenin signaling in other types of cells.^{75,76} Currently we are actively searching which cytokine(s) up-regulates β -catenin signaling in chondrocytes during the progression of SpA disease.

Conclusions

New bone formation is a hallmark of SpA and a major therapeutic challenge. Recent findings suggest that Wnt signaling and the natural Wnt inhibitors DKK1 and sclerostin are likely to play important roles in the process of ankylosis in SpA. Our findings suggest that activation of β -catenin signaling in cartilage tissue may be the key event leading to spine and joint destruction in patients with SpA. Understanding the molecular mechanisms of the Wnt signaling pathway and new bone formation or ankylosis in SpA could shed light into novel therapeutics for these diseases.

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