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Wnt/ β -catenin Signaling in Osteoarthritis and in Other Forms of Arthritis

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Abstract

Purpose of Review—Arthritis defines a large group of diseases primarily affecting the joint. It is the leading cause of pain and disability in adults. Osteoarthritis (OA) affecting the knee or hip is the most common form among over 100 types of arthritis. Other types of arthritis include erosive hand OA, tempo-romandibular joint (TMJ) OA, facet joint OA, diffuse idiopathic skeletal hyperostosis (DISH), and spondyloarthritis (SpA). However, the specific molecular signals involved in the development and progression of OA and related forms of arthritis remain largely unknown. The canonical wingless/integrated (Wnt)/ β -catenin signaling pathway could play a unique role in the pathogenesis of arthritis. In this review article, we will focus on the molecular mechanisms of Wnt/ β -catenin signaling in the pathogenesis of OA and other types of arthritis.

Recent Findings—Emerging evidence demonstrates that Wnts and Wnt-related molecules are involved in arthritis development and progression in human genetic studies and in vitro studies. Also, mouse models have been generated to determine the role of Wnt/ β -catenin signaling in the pathogenesis of arthritis.

Summary—Wnt/ β -catenin signaling represents a unique signaling pathway regulating arthritis development and progression, and the molecules in this particular pathway may serve as targets for the therapeutic intervention of arthritis. Mediators and downstream effectors of Wnt/ β -catenin signaling are increased in OA as well other forms of arthritis, including DISH and SpA. Through extensive investigations, including pre-clinical studies in transgenic mice and translational and human studies, the Wnt/ β -catenin signaling pathway has been proven to play roles in bone and joint pathology by directly affecting bone, cartilage, and synovial tissue; further, these pathologies can be reduced through targeting this pathway. Continued investigation into the distinct molecular signaling of the Wnt/ β -catenin pathway will provide additional insights toward the therapeutic intervention in arthritis.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

Keywords

Osteoarthritis; Temporomandibular joint (TMJ); Diffuse idiopathic skeletal hyperostosis (DISH); Spondyloarthritis (SpA); Wntless/integrated (Wnt); β -catenin

Introduction

Arthritis is a term used to classify a group of diseases primarily affecting the joint. The main symptoms include pain and stiffness of the joint. Other symptoms in the affected joints can also include redness, warmth, swelling, and decreased the range of motion. Other organs may also be affected by arthritis [1]. People of all ages, sexes, and races can have arthritis. Arthritis is the leading cause of disability and can result in decreased quality of life. Over 100 types of arthritis have been reported. Among them, the most common form is osteoarthritis (OA), usually affecting the knees, hands, and hips [2•]. Other common forms of arthritis include temporomandibular joint (TMJ) OA, spondyloarthritis (SpA), and diffuse idiopathic skeletal hyperostosis (DISH), affecting the axial spine and intervertebral disc. Arthritis is considered to be a multi-factorial disorder, and the risk factors include genetic predisposition, aging, and environmental influences. Investigation of the genetic contributions to the development and progression of arthritis could provide better understanding of the pathological mechanisms and could help to identify potential therapeutic targets.

Several lines of recent evidence suggest that Wnt/ β -catenin signaling may play an important role in regulating the pathogenesis of OA and other forms of arthritis. The canonical Wnt pathway triggers its signaling within cells through regulating intracellular β -catenin levels and subcellular localization of β -catenin. In the absence of Wnt proteins, the β -catenin levels are kept in a steady state. Excess β -catenin proteins are degraded by the enzyme GSK-3 β in a “destruction complex” together with Axin1/Axin2, adenomatous polyposis coli (APC), disheveled (Dvl), and Casein kinase I (CK1) in a phosphorylation-dependent manner [3, 4]. This protein complex phosphorylates specific amino acid residues at the carboxyl terminus of β -catenin protein, encoded by exon3 of the β -catenin gene [5, 6•]. When Wnt ligands bind to its receptors, including Frizzled and low-density lipoprotein receptor-related protein (LRP) 5 or 6, the intracellular portion of the Frizzled and LRP5 and 6 receptors interact with Dvl, APC, and Axin1/Axin2 proteins, leading to the release of β -catenin from the destruction complex and allowing β -catenin to translocate into the nucleus. The β -catenin protein binds to TCF/LEF transcription factors and activates expression of Wnt target genes in the nucleus [4]. Sclerostin and DKK1 are Wnt antagonists, and they competitively bind to Wnt co-receptors, LRP5 and 6, to inhibit Wnt signal transduction [7, 8].

Wnt/ β -catenin Signaling in OA

OA is a degenerative joint disease often accompanied by low-grade inflammation. Based on radiographic findings, it has been reported that 80% of the population have OA by age 65, although only 60% of those will be symptomatic [9•]. The pathology of OA is characterized by progressive articular cartilage degradation, subchondral bone changes, osteophyte formation at the edges of the joint, inflammation and hyperplasia of the synovial tissue,

degeneration of ligaments and menisci (in the knee), and joint capsule hypertrophy [2••]. With disease progression, arthritis can eventually lead to joint deformity, joint stiffness, severe pain, and limitation of joint movement. Numerous risk factors, including genetic influences, have been proposed to contribute to OA development. Among them, Wnt/ β -catenin signaling may play a unique role in joint tissues through controlling chondrocyte, osteoblast, and synovial cell functions.

In human genetic studies, alterations in genes associated with Wnt/ β -catenin signaling have been suggested as susceptibility factors for OA development. sFRP3 is an antagonist of Wnt signaling and encoded by the gene *sFrp3*. Single-nucleotide polymorphism (SNP) in *sFrp3* results in an Arg324Gly substitution at the carboxyl terminus and is associated with an increased risk for OA in weight-bearing regions of the joint [10, 11]. Based on the findings of expression of Wnt-related genes in articular cartilage, bone, and synovial tissues derived from OA patients, Wnt7b is the most closely linked to OA progression [12]. The upregulation of WISP-1 (Wnt1-inducible-signaling pathway protein 1) was detected in both murine and human OA tissues [13]. Furthermore, the expression of Wnt antagonists is altered in OA patients. For example, DKK1 levels in plasma and synovial fluid are significantly lower in patients with knee OA, and DKK1 levels in these compartments are inversely correlated to the radiographic grading of knee OA [14].

Another potent Wnt signaling antagonist is sclerostin, which is increased in mice with surgically induced OA, in sheep knee OA cartilage, and in human knee OA cartilage. Treatment of sheep cartilage explants with sclerostin results in inhibition of Wnt/ β -catenin signaling and predominately an anti-catabolic effect by reducing *Mmp*, *Adamts*, *Acan*, and *Col2a1* gene expression, as well as IL-1 α -mediated aggrecanolytic activity. In contrast, sclerostin is decreased in regions of bone sclerosis in sheep OA [15]. Sclerostin expression in osteophytes is well characterized as a regulatory mechanism by inhibition of Wnt-mediated endochondral bone formation [16]. Genetic ablation of sclerostin using sclerostin (SOST) KO mice results in a phenotypic increase in bone volume; however, loss of SOST results in increased cartilage damage compared to WT mice in the destabilization of medial meniscus (DMM) OA model [17]. In contrast, conflicting experiments have suggested that sclerostin is not increased in human OA compared to normal cartilage, and pharmacologic inhibition of sclerostin with an antibody does not reduce cartilage degeneration in the rat medial meniscus tear (MMT) posttraumatic OA model. The authors suggest in the setting of sclerostin inhibition, another compensatory molecule, such as a separate Wnt signaling inhibitor, may be reducing the potential benefits of sclerostin inhibition at the cartilage in OA [18].

Several different OA mouse models are currently used in OA research, including spontaneous, surgically or chemically induced, and genetic OA mouse models [19••]. In spontaneous OA mice (STR/Ort mice) and collagenase-induced OA mice, the levels of expression of Wnt and Wnt-related genes are changed in both the articular cartilage and synovium. Among these genes, *Wisp1* expression is significantly increased. *Wisp1* regulates the expression of MMPs and aggrecanases in chondrocytes and macrophages and can induce articular cartilage degradation [13]. *sFrp3*-null mice are prone to surgically or chemically induced OA [20, 21]. In addition, *sFrp3* plays a role in the regulation of chondrocyte

maturation and long bone development [22]. These findings suggest the significance of β -catenin signaling activation during the initiation and progression of OA.

To investigate the mechanisms of Wnt/ β -catenin signaling in OA development, we generated a constitutive β -catenin activation mouse model. Since chondrocyte-specific β -catenin gene deletion or activation leads to embryonic or immediate postnatal lethality [23], we generated β -catenin(*ex3*)^{Col2CreER} mice, which selectively targets β -catenin in chondrocytes in an inducible manner. Amino acids encoded by exon 3 of β -catenin contain three amino acid residues, which can be phosphorylated by the kinase GSK-3 β . Deletion of exon 3 of β -catenin results in the production of a stabilized fusion protein, which is resistant to the GSK-3 β phosphorylation and subsequent ubiquitination and proteasome degradation [6••]. Prevention of β -catenin degradation can lead to the increase of β -catenin protein levels in the nucleus in articular chondrocytes and result in an OA-like phenotype, including progressive loss of articular cartilage and osteophytes formation. A similar phenotype was also confirmed in β -catenin(*ex3*)^{Agc1CreER} mice (unpublished data). Also, we observed upregulation of β -catenin expression in knee joint samples of patients with OA [6••]. Inhibitor of β -catenin and TCF (ICAT) is an intracellular protein that inhibits the interaction of β -catenin and TCF through binding to the armadillo repeats of β -catenin. We generated *Col2-ICAT* transgenic mice in which β -catenin signaling is specifically inhibited in chondrocytes. Delay of growth plate chondrocyte maturation and destruction of articular cartilage were observed in these mice [24, 25•]. These findings suggest that Wnt/ β -catenin signaling plays a critical role in OA development, while the proper level of β -catenin is required for the maintenance of cartilage homeostasis.

Wnt/ β -catenin Signaling in TMJ OA

The temporomandibular joint (TMJ) is a hinge and gliding joint that connects the condyle of the mandibular bone with the temporal articular surface. The TMJ is composed of a concave articular fossa and a convex eminence. Changes in mechanical loading due to excessive or abnormal jaw movement can affect the morphology and composition of the condylar cartilage [26]. It is known that about 40–70% adults have signs of TMJ disorders in US and at least 33% of them have symptoms including pain, limited mandibular motion, or TMJ sounds [27]. TMJ OA is the most common TMJ disorder and is a highly prevalent degenerative disease affecting articular cartilage as well as other TMJ tissues. Common TMJ OA characteristics include progressive cartilage degradation, abnormal subchondral bone remodeling, synovitis, and chronic pain [28•].

The pathologic mechanisms of TMJ OA remain largely unknown. We have investigated the TMJ phenotype in mice with β -catenin overexpression in the condylar cartilage using a β -catenin(*ex3*)^{Col2CreER} mouse model [29••]. These mice developed progressive TMJ defects at 1, 3, and 6 months of age, with decreased condylar cartilage thickness and matrix degradation and increased cell hypertrophy and apoptosis. These findings were also confirmed by joint space narrowing on μ CT analysis. Changes in OA-like defects in TMJ tissues were further demonstrated by analyzing β -catenin(*ex3*)^{Agc1CreER} transgenic mice. *Agc1-CreER*^{T2} targeting efficiency and specificity were examined in *Agc1-CreER*; *ROSA^{mT/mG}* mice, and we showed that *Agc1-CreER*^{T2} mice could efficiently target the

entire condylar cartilage. The most apparent pathological change in β -catenin(*ex3*)^{Agc1CreER} mice was increased hypertrophic chondrocytes expanded in the condylar cartilage layers, as well as disc and cartilage layers of the mandibular fossa, which may be undergoing cell apoptosis [30].

One of the research areas in TMJ disorders is focused on inflammation and remodeling within the subchondral bone. These studies also suggest that Wnt signaling is involved in the pathogenesis of TMJOA. Wnt5a/Ror2 signaling in bone marrow stromal cells of the subchondral bone was enhanced in a surgically induced TMJ OA mouse model. *Cxcl12* and *Rankl* gene expression induced by JNK and Ca²⁺/NFAT signaling pathways was increased, leading to activation of osteoclast differentiation and enhanced subchondral bone turnover [31].

Wnt/ β -catenin Signaling in Facet Joint OA

The facet joints are synovial joints at the spine that are located as pairs behind and between two adjacent vertebrae. The articular surfaces of facet joints are lined with hyaline cartilage. The function of the facet joints is to allow flexion and extension and to limit rotation at the spinal motion segment, which consists of two adjacent articulating vertebrae and the connective tissue between them. Facet joint OA can result in significant pain and immobility at the joints affected. The development of facet joint OA can occur in the absence of intervertebral disc (IVD) degeneration; however, IVD degeneration often precedes and precipitates facet joint OA by altering biomechanics at the spinal motion segment [32, 33].

We have demonstrated that conditional activation of β -catenin in cartilage-producing cells, using β -catenin(*ex3*)^{Col2CreER} and β -catenin(*ex3*)^{Agc1CreER} transgenic mice, directly precipitates facet joint OA. These mice were treated with tamoxifen at 2 weeks of age, and facet joints were assessed at 3, 6, and 9 months of age. At each time point, there was significant cartilage degeneration at lumbar facet joints. In these mice, we found that facet joint OA and IVD degeneration occurred simultaneously, as reflected by histologic analysis (unpublished data). We have demonstrated that facet joint OA and IVD degeneration can occur through pathways associated with Wnt/ β -catenin signaling [34••] (unpublished data). We determined that joint degeneration and subsequent pain linked to conditional activation of β -catenin in cartilage producing cells could be inhibited using double mutant β -catenin(*ex3*)/*Mmp13*^{Col2CreER} mice that have β -catenin over-expression and deletion of *Mmp13* [34••]. These results provide additional support for the role of Wnt/ β -catenin signaling in joint degeneration and associated pain and suggest that MMP13 is the critical effector in the degenerative process.

Wnt/ β -catenin Signaling in DISH

Diffuse idiopathic skeletal hyperostosis (DISH) is also called senile ankylosis and ankylosing hyperostosis. It was first described in the 1950s, and the etiology of DISH remains largely unknown. It is not easy to diagnose DISH because standardized diagnostic criteria are not defined [35, 36]. The most common characteristic of DISH is flowing ossification in a “candle wax” appearance along the anterior longitudinal ligament (ALL) on

the anterolateral surface of vertebrae, eventually leading to fusion of the spine. The clinical symptoms of DISH are not limited to the spine. Ossification can also occur in the tendon, ligament, and capsule insertions (enthesis), and non-spinal manifestations have also been observed in the calcanea, patellae, ulnae, and ossa coxae [37].

Changes in metabolic conditions have been linked to DISH [37]; however, the genetic and environmental contributions to this disease remain unclear. Wnt/ β -catenin signaling is activated and localized in the annulus fibrosus, nucleus pulposus, and adjacent cartilage endplates during intervertebral disc development at both embryonic and postnatal stages. Activation of β -catenin signaling results in a severe deterioration of the growth plate cartilage, endplate cartilage, and annulus fibrosus. Overexpression of β -catenin accelerates bone formation at the growth plate and endplate cartilage [34••, 38•]. These findings suggest that β -catenin signaling is required for the development of the intervertebral disc.

Our study also found upregulation of β -catenin protein levels in disc samples—especially annulus fibrosus tissues—derived from patients with disc degeneration as compared to the healthy control subjects [34••]. The serum levels of DKK1, a natural inhibitor of Wnt/ β -catenin, were lower in patients with DISH [39]. We have analyzed the disc phenotype in β -catenin(*ex3*)*Col2CreER* mice [34••] and β -catenin(*ex3*)*Agc1CreER* mice. Overexpression of β -catenin in endplate cartilage cells of disc tissue leads to severe defects in the disc, reduced spine length, as well as extensive osteophyte formation and fusion of adjacent vertebra in the entire spine at 6 months of age, which is similar to the defects observed in DISH disease [34••]. Other deformities include the severe loss of chondrocytes in growth plate and endplate cartilage and disorganization of annulus fibrosus and nucleus pulposus tissues. Mice with β -catenin overexpression driven by the *Col11* promoter have similar defects in the growth plate of lumbar intervertebral discs [38•]. In addition to spine degeneration, similar defects were also noticed in the facet joint and sacroiliac joint, which also sometimes occur in patients with DISH. Furthermore, these defects cause severe pain in mice with β -catenin overexpression (unpublished data).

Wnt/ β -catenin Signaling in SpA

Spondyloarthritis (SpA) includes a group of inflammatory rheumatic diseases that often affects teenage and young adults, particularly males. SpA can result in axial spinal defects and peripheral joint arthritis. Clinically, SpA can be divided into five major subtypes, including ankylosing spondylitis (AS), peripheral SpA, reactive arthritis (Reiter's syndrome), psoriatic arthritis (PsA), and enteropathic arthritis, the SpA is associated with inflammatory bowel disease (IBD) [1].

Low back pain is the main symptom of SpA disease, and it most often occurs in axial SpA. The pathological process of SpA can be described with three stages: inflammation, erosion, and bony outgrowths (syndesmophytes) [40]. Syndesmophytes are the hallmarks of axial SpA, suggesting that bone turnover, or particularly the bone formation process, is activated during disease progression. The natural inhibitors of Wnt signaling, DKK1 and sclerostin, are involved in syndesmophyte formation in SpA patients. The German SpA Inception Cohort (GESPIC) study assessed AS patients over 2 years and showed that patients with

lower DKK1 levels were more susceptible to the development of syndesmophytes [41]. Sclerostin levels were significantly lower in AS patients [42]. Mouse genetic studies have also suggested the role of β -catenin in SpA disease. Activation of Wnt signaling by blocking DKK1 leads to osteophyte formation in peripheral joints [43]. *Dkk1* heterozygous KO mice showed a high bone mass phenotype [44], and *Dkk1*-overexpressing transgenic mice had a low bone mass phenotype [45]. In addition, severe destruction in spine tissues was observed in 3- and 6-month old β -catenin(*ex3*)^{Col2CreER} mice, including progressive loss of growth plate and endplate cartilage, disorganized annulus fibrosus and nucleus pulposus tissues, and increased osteophyte formation in the disc of the entire spine [34••, 46••]. These studies suggest that the activation of β -catenin signaling may contribute to the development and progression of SpA.

Studies in a rat IBD model, in which the activation of Wnt/ β -catenin signaling was observed, suggested that this activation may lead to the suppression of transplanted mesenchymal stem cells to differentiate into intestinal epithelium [47]. Wnt signaling components have been tested as potential markers for the diagnosis and treatment of IBD [48]. Aminosalicylate mesalazine is the drug used clinically for IBD treatment, and studies suggest that this drug may have an inhibitory effect on Wnt/ β -catenin signaling [49].

Chondrocyte β -catenin Signaling in Cartilage Degeneration and Bone Remodeling

OA in articular joints, TMJ, and spine may share similar pathological mechanisms of disease progression in the cartilage tissues. During the initial stage of OA, articular chondrocytes have low metabolic rates and undergo hypertrophy and apoptosis; the articular chondrocytes demonstrate progressively decreased expression of cartilage specific genes, such as *Col2a1* and *aggrecan*, increased levels of hypertrophic marker genes, such as *Runx2* and *Col10a1*, and increased expression of genes encoding for catabolic enzymes, such as *Mmp13*, *Adamts4*, and *Adamts5*. Decreased synthesis of anabolic components and increased synthesis of catabolic enzymes in cartilage can eventually result in progressive cartilage fibrillation and degeneration. Subchondral bone changes and osteophyte formation occur during OA progression. Transgenic mice with conditional activation of β -catenin signaling in *Col2a1*- or *Agc1*-expressing cells exhibit severe cartilage degeneration, subchondral bone erosion, and osteophyte formation. These data suggest that Wnt and Wnt-related proteins expressed in chondrocytes trigger different types of cellular responses that directly or indirectly precipitate pathologic phenotypes in arthritis.

The most important matrix metalloproteinase involved in cartilage degradation is MMP13. High MMP13 expression has been found in patients with articular cartilage destruction [50]. Mice with *Mmp13* overexpression develop an OA-like phenotype with articular cartilage degradation [51]. To determine the specific role of *Mmp13* in articular cartilage in OA development, we generated *Mmp13*^{Col2CreER} conditional KO mice by deleting *Mmp13* in *Col2*-expressing articular chondrocytes at the postnatal stage [52••]. OA was induced by meniscal-ligamentous injury (MLI) surgery [53, 54]. The progression of OA was ameliorated in mice with *Mmp13* deletion post-surgery. Articular cartilage volume and

cartilage matrix secretion were increased, and chondrocyte apoptosis was reduced in *Mmp13* conditional KO mice. Furthermore, we found that treatment with a specific MMP13 inhibitor attenuated MLI-induced OA severity. Rescue effects were also observed in β -catenin(*ex3*)/*Mmp13*^{Col2CreER} and β -catenin(*ex3*)^{Col2CreER}; *Adamts5*^{-/-} double mutant mice. Deletion of *Mmp13* or *Adamts5* significantly reversed the TMJ OA and intervertebral disc degeneration phenotypes observed in β -catenin(*ex3*)^{Col2CreER} transgenic mice [29•, 34•]. The ADAMTS family of proteases contributes to proteoglycan/aggrecan depletion during arthritis. Deletion of *Adamts5* or double KO of both *Adamts4* and *Adamts5* prevented cartilage degradation in a surgically induced knee OA mouse model [55, 56]. In β -catenin overexpressing mice, β -catenin(*ex3*)^{Col2CreER}, expression of both *Adamts4* and *Adamts5* genes in disc tissues was upregulated [31]. These findings revealed that *Mmp13* and *Adamts4/5* are critical for cartilage erosion during progression, and pharmacologic inhibition of these enzymes may serve as an alternative strategy for osteoarthritis treatment.

The mechanisms governing how chondrocytes interact with adjacent osteoclasts or osteoblasts to regulate bone remodeling have not been fully defined. Cartilage-specific β -catenin signaling is critical for the formation of primary and secondary ossification centers and perichondrial bone formation during joint development [57]. The β -catenin(*ex3*)^{Col2CreER} overexpression mice have increased bone mass, while β -catenin^{Col2CreER} conditional KO mice have reduced bone mass during postnatal bone growth [58, 59]. In addition to subchondral bone erosion and osteophyte formation in OA, β -catenin signaling in chondrocytes may play a crucial role in osteoblast and osteoclast function. β -catenin signaling in chondrocytes can regulate bone remodeling in a manner dependent on RANKL/osteoprotegerin (OPG). OPG serves as a decoy receptor by competing with RANK. It binds to RANKL in stromal/osteoblast precursor cells, but does not transduce the signal to the osteoclast lineage cells, which are supposed to interact with stromal/osteoblast precursor cells and receive RANKL signals. In this way, OPG exerts an inhibitory effect on osteoclast differentiation. RANKL and OPG are expressed in hypertrophic chondrocytes in growth plates and can modulate osteoclast differentiation [60,61]. To investigate the function of endogenous OPG in chondrocytes, we generated chondrocyte-specific *Opg* transgenic mice using the *Col2a1* promoter to drive *Opg* expression in chondrocytes [62•]. *Col2-Opg* transgenic mice displayed reduced numbers of osteoclasts and delayed formation of the secondary ossification center. As a consequence, the bone mass in the proximal metaphysis of the tibiae was increased. These findings suggest that *Opg* expressed in hypertrophic chondrocytes may regulate adjacent osteoclast formation and contribute to local bone remodeling during bone growth.

Summary

Although significant progress has been made in arthritis research in recent years, there is still limited understanding about the molecular mechanism(s) of OA initiation and progression. The canonical Wnt/ β -catenin signaling pathway represents a key signaling pathway involved in regulation of the pathogenesis of arthritis in different tissues containing cartilage. The various types of arthritis commonly share similar pathological features, such as degeneration of cartilage matrix, subchondral bone changes, and the formation of osteophytes, and result in joint stiffness and pain. Activation of canonical β -catenin

signaling may represent a key signaling event leading to cartilage degradation and bony overgrowth. However, how β -catenin signaling is turned on or activated and the upstream factors involved remain unknown. In addition, we also do not know if Wnt/ β -catenin signaling is regulated by inflammatory cytokine(s), which could serve as a link between inflammation and cartilage and bone changes observed in OA-related processes.

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