The Role of WNT in Rheumatoid Arthritis and its Therapeutic Implication

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Abstract: Rheumatoid arthritis (RA) is a systematic inflammatory and intractable disease, which progressively affects multiple joints. Recent findings strongly suggest a key role of WNT signaling in the disease initiation and progression. In this review, we discuss the role and possibility of treatment by targeting WNT signaling.

Key Words: WNT, rheumatoid arthritis, FRP, β-catenin, synovium, matrix metalloproteinase.

1. OVERVIEW OF RA PATHOLOGY

Rheumatoid arthritis (RA) is a chronic and systematic inflammatory disease that primarily affects small diarthroidial joints in hands and feet, and both genetic and environmental factors contribute to disease susceptibility and progression. Worldwide, the disease affects 0.5-1.0% of the population with a female/male ratio of ~4/1. RA is characterized by the pathological changes of synovium, which include initial vasculitis of the joint, followed by edema, infiltration of inflammatory cells into the synovium, hyperplasia of the synovial lining, and development of the pannus that invades cartilage and subchondral bone. Although exact mechanism of RA pathogenesis is not known, local autoimmune complex in the synovium is considered to be the primary cause of the disease. Aggregation of inflammatory cells in the synovium establishes an enormous cytokine network and triggers a variety of pathological reactions. A number of cytokines including tumor necrosis factor α (TNF-α), interleukin (IL)-1, IL-6, IL-8, IL-12, IL-15, IL-18, chemokine C-X-C motif ligand 12 (CXCL12) and B lymphocyte stimulator (BLyS) are enriched in the RA synovium and synovial fluid. High incidence of the disease in female suggests an involvement of hormonal balance. In fact, estrogens at a physiological level stimulate immune responses but androgens suppressed those responses. The estrogen/androgen ratio in synovial fluid is elevated in both male and female patients suffering from RA, and estrogen in serum is increased in male RA patients [1-3]. Estrogens directly stimulate production of proteins, which are involved in the pathology of RA [4-6].

2. ROLE OF FLS IN DISEASE PROGRESSION

With the initiation and progression of RA, the intimal lining of synovium increases cellularity and becomes hyperplastic with marked increase of macrophage-like synoviocytes and synovial fibroblast-like cells (FLS). Along with disease progression, RA synovium accumulates inflammatory cell infiltration and forms lymphoid aggregates with germinal centers in the sublining region, which consists of $CD4^+$ T cells, B cells and macrophages [7]. In response to pro-inflammatory and inflammatory cytokines, stimulated FLS activate transcription factors such as c-myc and NF-κB and produce a number of growth factors [7, 8]. FLS form the erosive pannus and take a central role in progression of the disease. As they proliferate, they promote angiogenesis and inflammatory reactions, and secrete extracellular matrix (ECM)-degrading proteinases. FLS produce aggrecanase-1 (ADAMTS-4) and matrix metalloproteinases (MMPs), including MMP-1 (interstitial collagenase), MMP-3 (stromelysin 1), MMP-9 (gelatinase B) and MMP-10 (stromelysin 2). Aggrecanase-1 and MMP-1 can digest aggrecan and type II collagen fibrils, respectively [9-11], and are predicted as central proteinases to degrade cartilage by the RA synovium [12]. These aspects of FLS distinguish it from other arthritic conditions such as osteoarthritis (OA) [7, 13, 14].

3. WNT PATHWAY AND TARGET GENES

3.1. WNT Signaling Pathway

WNT is expressed in a variety of pathological conditions [15-17]. Ligation of the WNT molecule to cell surface receptors, frizzled (Fz) and low-density lipoprotein receptor-related protein (LRP)-5 or -6, transmit signaling mediated through β -catenin or other molecules [15, 16, 18] (Fig. **1**). Among WNT-inducible signaling pathways, the ß-catenin-mediated pathway has been characterized extensively. In this canonical pathway, WNT (e.g., WNT1, WNT3, WNT8, and WNT10b)-receptor binding inhibits the kinase activity of glycogen synthase kinase-3β (GSK-3β), liberating β-catenin from degradation and increasing the cytoplasmic free ß-catenin pool. Excess β-catenin translocates into the nucleus and regulates target gene expression through binding with transcription factors (LEF1 or TCF). In the absence of WNT, GSK-3β phosphorylates β-catenin, resulting in its degradation through the ubiquitin-proteasome pathway. On the other hand, WNT5a class (e.g., WNT4, WNT5a, and WNT11) activates the non-canonical WNT pathway. These WNTs bind to receptor tyrosine kinase-like orphan receptor (ROR) and its downstream signal can repress the canonical pathway. WNT pathways are also regulated by the binding of WNT inhibitors; secreted frizzled-related proteins (FRPs), dickkopfs (DKKs), WNT inhibitory factors (WIFs) and scle-

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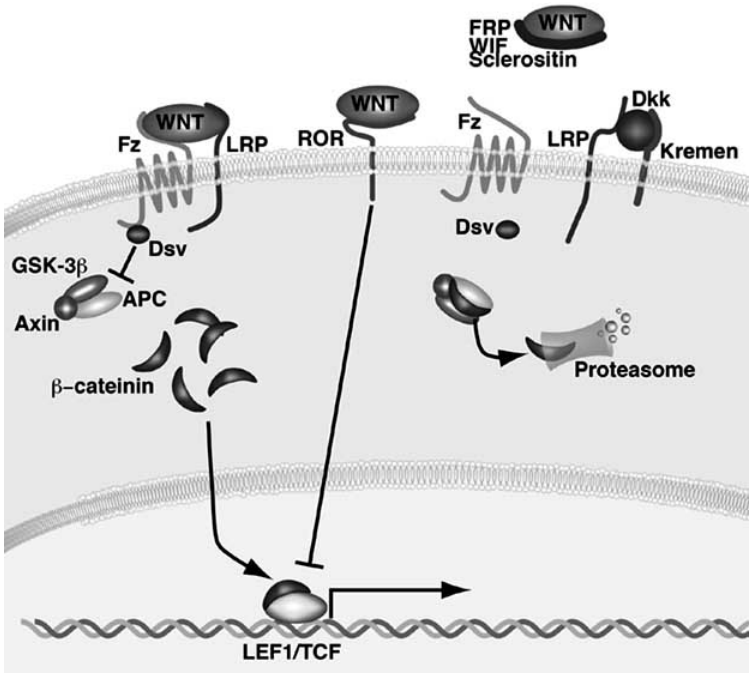

Fig. (1). Schematic illustration of the WNT signaling pathway. Upon the binding of WNT to cell surface receptors; frizzled (Fz) and LRP, disheveled (Dsv) bond on cytoplasmic domain of Fz suppresses the kinase activity of GSK-3β-APC-Axin complex, resulting in the increase of free β-catenin pool. β-catenin translocates into the nucleus, binds to transcription factors (LEF1/TCF and others), and regulates target gene expression. This pathway is referred as the canonical WNT pathway initiated by the WNT1 class. The WNT5a class binds to ROR and its downstream signal suppresses LEF1/TCF activity (the non-canonical WNT pathway). In the absence of WNT or the presence of WNT inhibitors; secreted frizzled-related protein (FRP), WNT inhibitory factors (WIF), sclerostin and dickkopfs (Dkk), GSK-3β-APC-Axin complex degrades β-catenin through ubiquitin-proteasome system.

rostin, by which GSK-3β forces β-catenin to degrade and decreases the free β-catenin pools [15].

3.2. WNT Target Genes

Biologically, WNT signaling stimulates cell proliferation and remodeling of the ECM molecules, and inhibits apoptotic death (www.stanford.edu/~rnusse/wntwindow.html). A number of WNT target genes, including c-Myc [19], MMP-3 [20], uPA [21], CD44 [22], VEGF [23], and fibronectin [24], are upregulated in the synovium of RA patients and play a role in progression of the disease. Therefore, it is straightforward to speculate that the canonical WNT pathway is involved in multi-facets of RA synovial cell phenotypes and in regulating pathology of RA.

4. WNT ACTIVATION IN FLS

Understanding the molecular mechanism(s) contributing to destructive features of RA synovial cells may allow for the development of novel diagnostic and therapeutic strategies in RA patients. Previous reports described that WNT1, WNT5a, and WNT7a were preferentially expressed in RA synoium [24-26]. In our previous study, we showed that WNT5a and WNT10b are preferential expressed in RA-FLS, and that WNT10b expression in synovial lining cells, fibroblasts and endothelial cells is increased in parallel with synovial inflammation, fibrosis and angiogenesis in RA synovial tissues. In these WNT10b-expressing cells, ß-catenin was also positively stained, implicating the activation of canonical WNT pathway [27]. The data above from our and other laboratories strongly suggest that the activation of WNTs expression during disease progression is likely given the heterogeneous nature of the disease. However, it is beyond dispute that multiple WNT members are expressed in RA synovial tissues. In great contrast to the WNT family, FRP1, 2 and 3 are expressed in all of OA synovial fibroblasts but are negligible in RA-FLS, and FRP1 was localized in synovial lining cells, fibroblasts and endothelial cells in OA synovial tissues which were same cell types as WNT expression in RA tissues. Expression of WNT10b and β-catenin were negligible in OA synovium [27]. These findings are supported by a recent report on DKK1 expression in RA [28]. DKK1 is a WNT antagonist that cross-links LRP with Kremen and prevents WNT binding to its receptor. In RA synovium, DKK1 is expressed along with inflammations but serum levels of DKK1 is decreased with the progression of the disease. Blockage of DKK1 by the antibody increases nuclear accumulation of β-catenin in FLS. Overall, RA syovium activates WNT signaling pathway through WNTs expression and negligible expression of WNT inhibitory proteins, and also may be involved in the pathology of RA (Fig. **2**).

5. WNT AND BONE

Genetic mutation and deletion of WNTs and their related molecules result in severe bone and osteoblast anomalies. The canonical WNT pathway is prerequisite to osteoblast differentiation from its precursor cells [29], and stimulates osteoblasts to express osteoclast-stimulating factors (RANKL

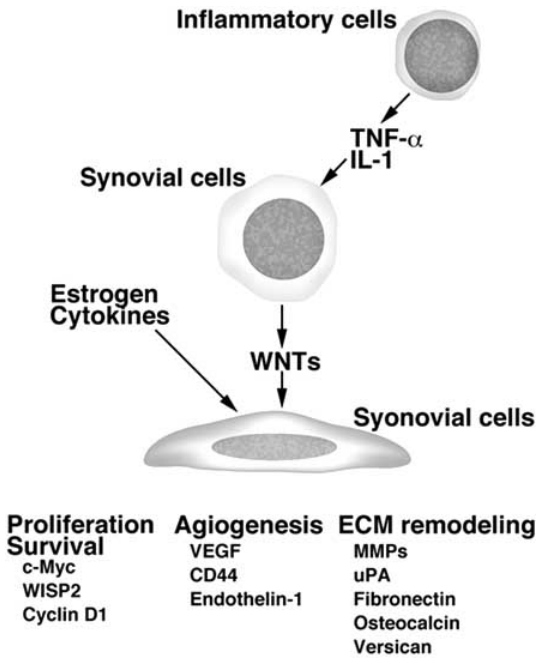

Fig. (2). Involvement of WNTs in the pathology of RA. Pro-inflammatory cytokines (TNF-α and IL-1) secreted from inflammatory cells in the synovium stimulate expression of WNTs from synovial cells. Secreted WNTs act in autocrine and/or juxtacrine fashions, and induce target gene expression in combination with other signaling pathways and estrogen. WNT-stimulated cells initiate phenotypic alterations of cells and disease progression through enhancement of cellular proliferation and survival, angiogenesis, and extracellular matrix (ECM) remodeling.

and M-CSF), but suppresses expression of osteoprotegerin, which inactivates osteoclasts [30, 31]. WNT inhibitors, FRP2, FRP3, WIF and DKK1, are strongly expressed at the late stage of osteoblast differentiation. Inhibition of DKK1 by the antibody upregulates osteoprotegerin expression and blocks bone erosion by reducing osteoclast numbers in a TNF-α transgenic RA mouse model [28]. However, deletion of FRP1 or DKK1 genes increase the bone mass [32, 33]. Therefore, the role of WNT is different largely between the RA synovium and bone. In regard to chondrocytes, expression of WNTs and activation of WNT pathways are still unknown, although one report described a very slight increase of WNT10b mRNA in RA chondrocytes [26]. WNT signals play a critical role in the cartilage development and integrity, but its action is largely affected by chondrocyte differentiation status [34, 35]. It is a fact that RA chondrocytes express many WNT target genes, which are involved in the pathology of RA. Future studies are required to define the roles of the WNT pathway.

6. WISP EXPRESSION BY WNT AND ESTROGEN PATHWAYS

In addition to WNT target genes described above, WNT1 inducible signaling pathway proteins (WISPs) are multidisciplinary growth factors expressed in various pathological conditions, and stimulate cell proliferation and migration, angiogenesis, and tissue fibrosis [36]. WISPs belong to the CCN family and consist of three members (WISP1-3). We found that WISP2, but not WISP1 and WISP3, is preferentially expressed in fibroblasts of RA synovial tissues [5]. Its expression was synergistically activated by the canonical WNT pathway and estrogen (Fig. **3**). WISP2 expression was abolished when RA-FLS were treated with an estrogen antagonist (ICI 182,780) or transfected with a dominant-negative form of β-catenin. There are four estrogen-responsive elements and 10 WNT signaling-responsive sites (LEF1 or TCF binding elements) within 1 kb 5'-upstream promoter region of the WISP2 gene. In fact, estrogen receptors and LEF1/TCF directly bind on the promoter region and synergistically upregulate the expression of target genes [37, 38]. Although WISP3 was negligibly detected in our study, genetic mutations of *WISP3* gene are associated with progressive pseudorheumatoid dysplasia and juvenile idiopathic arthritis [39, 40]. Since both WNT and estrogen are stimulated in the RA syovium, synergistic action between WNT and other pathways may regulate target gene expression and contribute to establish the pathological conditions.

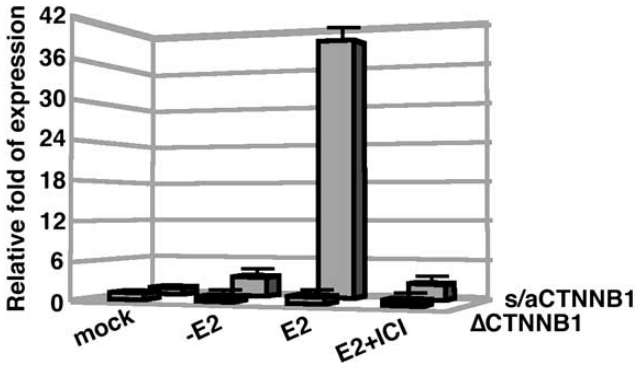

Fig. (3). Synergistic action of WNT signaling on *WISP2* expression. *WISP2* expression in RA synovial fibroblasts expressing constitutive active β-catenin (s/aCTNNB1), dominant-negative β-catenin (ΔCTNNB1) or vector alone (mock) were measured by real-time PCR. Cells were stimulated by 10^{-9} M 17-β-estradiol (E2) for 12 h with or without 1 μM estrogen antagonist (ICI). Expression levels in the absence of E2 were depicted as –E2. Reproduce from Tanaka *et al.* (*Biochem. Biophys. Res. Commun*., **2005**, *334*, 973).

7. BLOCKAGE OF WNT PATHWAY AS A RA THERAPEUTIC STRATEGY

Altogether, above data strongly implicate the possibility that biological modifiers for molecules activated in RA synoviocytes can be candidates for therapeutic reagents to RA patients. Anti-TNF-α antibody (infliximab and adalimumab), TNF-α decoy receptor (etanercept), IL-1 receptor antagonist (anakinra), cytotoxic T-lymphocyte antigen 4 decoy protein (abatacept), and anti-CD20 antibody (rituximab) are clinically approved to use for RA treatment by the FDA. The use of TNF-α inhibitor is expected to suppress activation of cytokine network, synovial hyperplasia, angiogenesis and osteoclast activation [41], and demonstrated to slow cartilage and bone degradation [42]. In addition, a selective estrogen receptor modulator (raloxifene analog LY117018) successfully inhibits the progression of RA in an animal model without side effects of long-term therapy [43]. Combination therapy using biological modifiers and traditional disease-

Table 1. Criteria for the Classification of Rheumatoid Arthritis*

Criterion	Definition
1. Morning stiffness	Morning stiffness in and around the joints, lasting at least 1 h before maximal improvement
2. Arthritis of 3 or more joint areas	At least 3 joint areas simultaneously have had soft tissue swelling or fluid observed by a physicians.
3. Arthritis of hand joints	At least 1 area swollen (as defined above)
4. Symmetric arthritis	Simultaneous involvement of the same joint areas (as defined in 2) on both sides of the body
5. Rheumatoid nodules	Subcutaneous nodules, over bony prominences, or extensor surface, or in juxtaarticular regions, observed by a physician
6. Serum rheumatoid factor	Demonstration of abnormal amounts of serum rheumatoid factor by any method for which the result has been positive in <5% of normal control subjects
7. Radiographic changes	Radiographic changes typical of rheumatoid arthritis on posteroanterior hand and wrist radiographs, which must include erosions or unequivocal bony decalcification localized in or most marked adjacent to the involved joints

* The criteria defined by American College of Rheumatology at 1987 are reproduced from a reference by Arnett, F.C. *et al*., *Arthritis Rheum.,* **1988**, *31*, 315-324,.

For classification purpose, a patient shall be said to have rheumatoid arthritis if he/she has satisfied at least 4 or these 7 criteria. Criteria 1 through 4 must have been present for at least 6 weaks.

modifying antirheumatic drugs (DMARDs) is an attractive paradigm as a next avenue of therapeutic strategies [44].

Recently chemical compounds targeting β-catenin to disrupt its binding with transcription factors have been developed; FH535 [45], ICG-001 [46], CPG049090 and PKF115-584 [47]. Modified non-steroidal anti-inflammatory drug and NO-donating aspirin dissociates the β-catenin-TCF complex [48]. NO_2 moiety of NO-aspirin inhibits the interaction between β-catenin and TCF that could account for this effect are their S-nitrosylation or tyrosine nitration, resulting in downregulation of TCF transcriptional activity, expression of WNT target genes, and cell proliferation. Another compound, FJ9 (a non-electrophilic indole-2-carbinol-based chemical scaffold), inhibits the binding between Fz and disheveled and suppresses nuclear translocation of β-catenin. Its systematic administration inhibits the growth of tumor xenografts in mice [49]. Since WNT signaling plays a significant role in the initiation and progression of various diseases including arthritis, malignant neoplasm, type II diabetes and obesity, development of effective anti-WNT drugs is an attractive strategy to treat patients suffering not only from RA but also other diseases [50]. Although we should pay attention to the risk of toxicity and impairing host defense since WNT pathways may affect to innate and adaptive immunities of patients, unveiling the molecular pathways that play a critical role in the pathology of RA will provide more fruitful information to us.

CONCLUDING REMARKS

RA patients now benefit from basic research. However, the use of biological modifiers also increases the risk of infections and malignancies. One-quarter to one-third of patients only have transient improvement or negligible benefit from the therapy. In addition, direct costs for the therapy using biological modifiers are substantially more than that of traditional DMARDs. Currently, diagnosis of RA is largely dependent on clinical symptoms (Table **1**), which means that the disease is already advanced in almost all of patients when diagnosis has been made and the starting point of treatment is delayed. Biopsy analysis of clinically symptom less joints of patients with early RA highlights the poor correlation of clinical assessment and development of the disease [14, 51]. To make sure patients receive effective therapies, one of the most important thing is early treatment. To this end, biochemical markers which can predict disease in the early stage should be developed along with detecting systems are required to treat the patients effectively. Recently, it has been reported that serous level of antibody to cylic citrullinated peptide (anti-CCP) is significantly elevated in RA patients. However, the diagnostic reliability for early stage patients is a controversial issue [52, 53]. Understanding the molecular mechanisms of initiation and progression of RA and the development of diagnostic strategies at an early stage are prerequisites for the future.

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