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REVIEW ARTICLE

Malini Sen · Dennis A. Carson

Wnt signaling in rheumatoid synoviocyte activation

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Abstract Rheumatoid arthritis (RA) is a joint-specific disease with complex pathogenesis. It is characterized by synovial inflammation, cartilage loss, and joint destruction. The reasons why joint damage recurs when therapy is discontinued are not clearly understood. Several lines of evidence suggest that cartilage damage is promoted by the transformed and invasive fibroblast-like synoviocytes (FLS) of the rheumatoid joint. It has been demonstrated in several systems that aberrant wnt-mediated signaling causes blockade of cartilage differentiation and malformation of joints. In this review, we have discussed the importance of wnt–frizzled-mediated signaling in the autonomous activation of FLS in patients with RA. Anti-wnt/anti-frizzled antibodies, frizzled receptor antagonists, or small molecule inhibitors of wnt–frizzled signaling might be useful for therapeutic interventions in RA.

Key words Arthritis · Frizzled · Synoviocyte · Wnt

Introduction

Rheumatoid arthritis (RA) is a fairly common disease in both developed and underdeveloped countries, with an average prevalence of 1%. It is a heterogeneous disease that is the outcome of an interplay between environmental insults and random modulations of the musculoskeletal and immune systems. The unique anatomic and physiological features of diarthroidal joints render them targets for this disease.

The pathology of the rheumatoid arthritic joint lesions has been studied mainly with synovium obtained during joint replacement surgery or arthroscopic surgical procedures. The constituents of a normal synovium are bone marrow – derived macrophages and mesenchymal fibroblast – like synoviocytes (FLS). In long-standing RA, the FLS in the synovial lining and underlying connective tissue transform into a pannus, which then destroys articular cartilage.[1–4] Proinflammatory stimuli such as tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1), which are potent activators of FLS, induce them to produce chemokines, cytokines, matrix-degrading metalloproteinases, and other inflammatory mediators.[5–8] Nevertheless, rheumatoid FLS activation sometimes continues despite antiinflammatory therapy.[3] Furthermore, FLS can be propagated in vitro in the activated state without exposure to proinflammatory cytokines.[3–8] It has been suggested that, in advanced stages of RA, channels or cartilage canals might allow migration into the synovium of bone marrow stem cells with altered properties.[9] The replacement of mature fibroblasts with more primitive cells could alter the regulation of synoviocyte activation. It is important, therefore, to know whether the FLS from long-standing RA express growth modulators characteristic of bone marrow or immature mesenchyme and to determine whether such factors could play a role in synovial fibroblast activation and pannus formation.

We have considered the possibility that various members of the Wnt and Frizzled (Fz) families, which control limb bud formation, connective tissue homeostasis, and hematopoiesis,[10–12] may contribute to autonomous FLS activation. At least 18 Wnt genes, and 12 Fz genes, have been identified to date. The Wnt proteins are secreted glycoproteins that bind to either the cell surface or extracellular matrix and probably act in an autocrine or paracrine fashion.[13,14] The serpentine frizzled proteins, which appear to be G protein-coupled receptors (GPCRs), function as Wnt receptors.[15] In this review, we present a synopsis of current research on the

M. Sen (✉)
Department of Medicine, University of California at San Diego, La Jolla, CA 92093, USA
Tel. +1-858-822-3338; Fax +1-858-534-5399
e-mail: msen@ucsd.edu

D.A. Carson
Department of Medicine and Sam & Rose Stein Institute for Research on Aging, UCSD, La Jolla, CA, USA

importance of Wnt-mediated signaling in the progression and persistence of RA.

Wnt and frizzled expression profiles in normal and diseased tissues

The Wnt and frizzled proteins are embryonic growth factors that are highly expressed during fetal development. However, the expression profiles of all the different Wnt and frizzled proteins in normal and diseased tissues from adults have not been very well characterized. It has been found that wnt1, wnt7a, and wnt10b have altered expression in glioma and in cancers of the lung and breast, respectively.[16–18] wnt5a overexpression has been found in cells of primary breast tumors that are mostly non-invasive.[19] It is, however, not clearly known whether altered wnt expression in human tissues is a direct cause or effect of disease. Of the different frizzled proteins, expression patterns of only a few (e.g., fz1, fz2, and fz7) have been investigated in normal adult tissues.[20] fz1 RNA has been detected in heart, placenta, lung, kidney, pancreas, prostate, and ovary. fz2 and fz7, on the other hand, are much more differentially expressed in the adult. fz2 is expressed only in the heart, and fz7 is expressed in heart, brain, skeletal muscle and kidney. fz2 is, however, overexpressed in rat artherosclerotic lesions.[21] All the three above-mentioned frizzleds are highly expressed in fetal tissues and many cancer cells.[20]

Work conducted in our laboratory has shown that wnt1, wnt5a, and fz5 are differentially expressed in RA tissue surgical specimens compared to osteoarthritis (OA) tissue surgical specimens and normal adult tissues. Furthermore, we have found that synovial FLS isolated from RA patients express higher levels of wnt1 and wnt5a than synovial FLS.[22,23]

Wnt signal transduction pathways

Of the different potential Wnt signal transduction pathways known from studies of *Drosophila* and *Xenopus*, only the wnt1 signaling pathway has been well characterized in mammalian cells.[24] Several lines of evidence suggest that wnt1 binds to the fz1/fz2 receptors at the cell surface. The wnt1 signal transduction pathway is depicted in Fig. 1. Briefly, activation of wnt1 signal causes phosphorylation of the cytoplasmic protein dishevelled, which then binds and inactivates the Ser/Thr kinase GSK3-β. Inactivation of GSK3-β blocks phosphorylation of cytosolic beta-catenin and inhibits its degradation by the proteasome. The excess beta-catenin then translocates to the nucleus, complexes with transcriptional factors such as Lef/Tcf, and promotes the transcriptional activation of target genes. In colon cancer cells (epithelial cells), nuclear translocation of beta-catenin is responsible for the transcriptional upregulation of myc, cyclin D1, and other genes that promote cell proliferation.[25] In certain cell types (e.g., PC12 neuronal cells), wnt1 signaling can also activate NFκB.[26] Both wnt5a and wnt10b have been reported to activate pathways that do not depend exclusively on beta-catenin.[24]

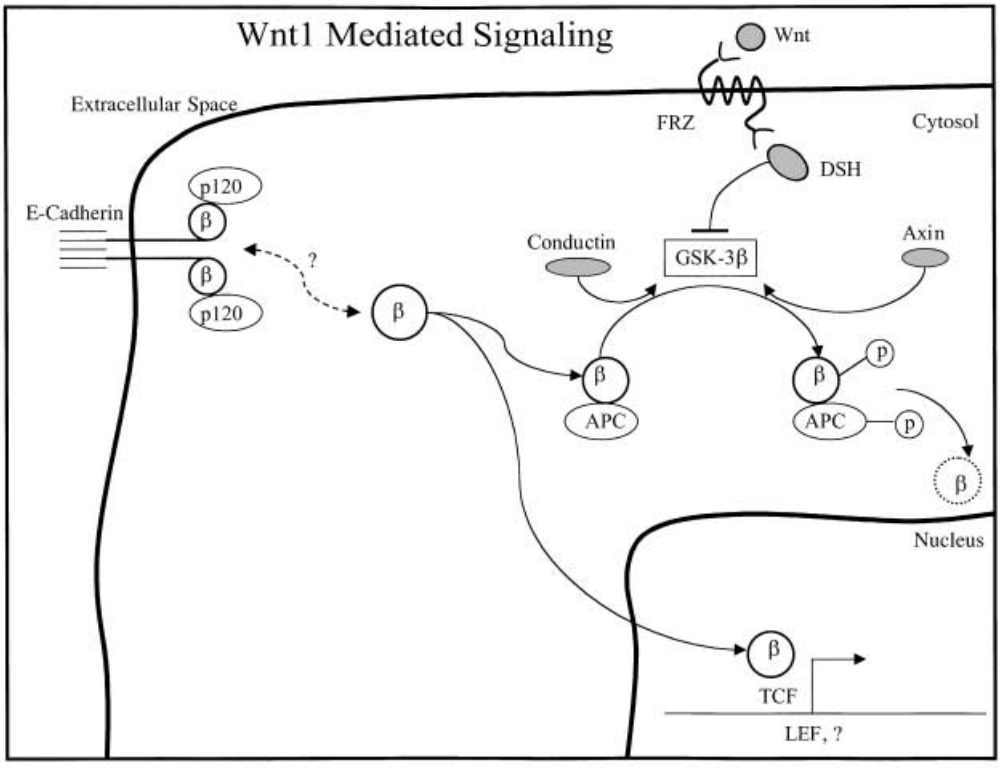

Fig. 1. Schematic diagram of transcriptional activation by Wnt/Fz signaling pathway. Wnt1 binds to its receptor fz. The wnt1/fz complex then activates dishevelled (DSH), which in turn inactivates glycogen synthase kinase-3β (GSK3-β). GSK3-β, which normally phosphorylates β-catenin (β) for degradation, does not phosphorylate it on inactivation. The excess β-catenin enters the nucleus and activates transcription of target genes in association with T-cell factor (TCF). The level of cytosolic β-catenin is probably regulated by complex pathways –complex formation with the cell-surface molecule E-cadherin, complex formation with adenomatous polyposis coli (APC)/axin/conductin, and nuclear shuttling

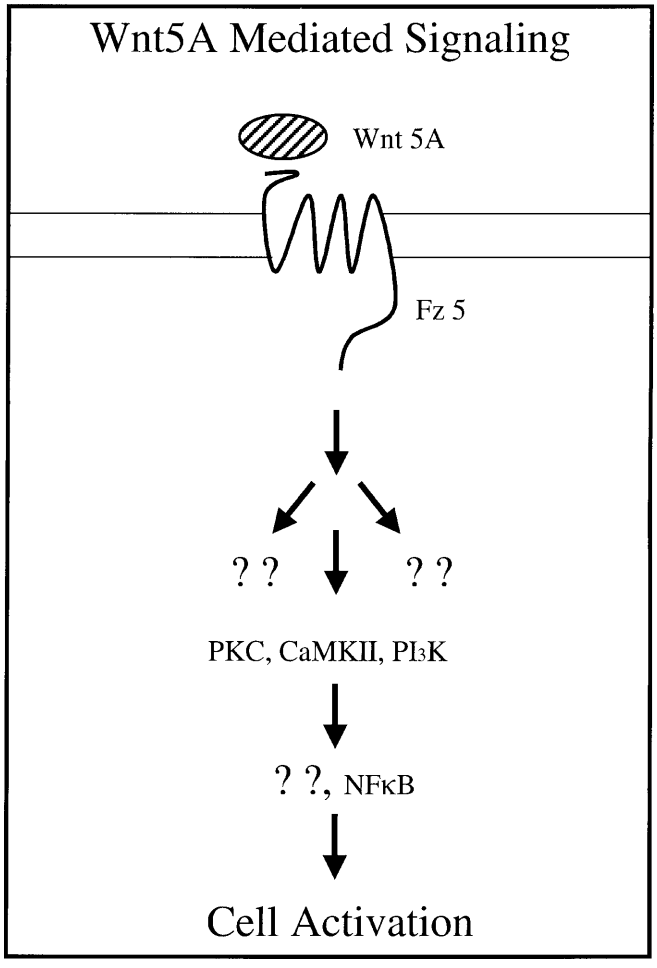

Fig. 2. Schematic presentation of Wnt5a signaling. Wnt5a/Fz5 complex activates a series of components that ultimately activate different kinases such as protein kinase C (PKC), calmodulin kinase II (CaMK11), and PI_3 kinase. These kinases activate NFκB and possibly other transcription factors that in turn activate specific genes such as IL-6, IL-8, IL-15, and RANKL

The signal transduction pathway for wnt10b has not been well characterized. A diagram for the wnt5a signaling pathway is shown in Fig. 2. Several reports have suggested that wnt5a-mediated signaling activates calmodulin kinase II and PI_3 kinase in *Xenopus* and protein kinase C (PKC) in other cell types.[24] Some studies have also suggested that wnt5a-mediated signaling antagonizes the wnt1-mediated signal for cell proliferation and acts as a tumor suppressor rather than as a growth promoter.[24] However, the final outcome of wnt signaling, whether wnt1- or wnt5a mediated, in a certain cell type, is dictated to a great extent by the relative density of the different frizzled receptors present on the cell surface. Because there is considerable homology between different members of the wnt and frizzled families, a specific wnt–fz pair is not likely to be the only ligand–receptor combination in a certain cell type. Rather, signaling probably depends on the cumulative action of multiple wnt–fz interactions.

Expression of Wnt and frizzled genes in RA tissue and FLS

Several different wnt and frizzled homologues have been detected at the message level in surgical tissue specimens isolated from the knee of RA patients.[22] In particular, the wnt5a–fz5 ligand–receptor pair is expressed at moderately higher levels in RA tissues compared to normal adult tissues. The expression of wnt1, although less than that of wnt5a and fz5, is also higher in RA tissues than in OA tissues and normal adult tissues. The overexpression is not attributable only to infiltration of the RA tissues with blood leukocytes, because FLS isolated from RA patients also express higher levels of both wnt1 and wnt5a messages than FLS isolated from normal people[22] (and unpublished observation). The high expression of wnt1 in RA FLS was also reproduced by us at the protein level (unpublished observation).

Wnt5A-mediated signaling in RA FLS

Work conducted in our laboratory has indicated that wnt5a overexpression in RA FLS correlates very well with the production of the interleukins IL-6, IL-8, and IL-15. Furthermore, we have shown that transfection of normal synovial FLS with a wnt5a expression vector upregulates the expression of the interleukins both at the message and at the protein levels.[22,23] These three interleukins, IL-6, IL-8, and IL-15, are characteristic of chronic RA.[5–8] IL-6 promotes both proliferation and maturation of B lymphocytes, IL-8 promotes chemotaxis of neutrophils, and IL-15 promotes TNF-α production by monocytes and osteoclastogenesis.

Because RA FLS have been found to express considerable levels of fz5 and fz2, both of which have been found to act as receptors for wnt5a,[27,28] we have hypothesized that wnt5a binds to the fz5/fz2 receptors on the surface of the RA FLS via autocrine/paracrine mechanism(s) to initiate downstream signals that result in interleukin gene induction and protein production. To confirm that wnt5a-mediated signals upregulate interleukin production, we further tested IL-6 and IL-15 production at both the message and protein levels in RA FLS, either transfected with a dominant negative wnt5a expression vector or treated with anti-fz5 antiserum. The dominant negative (dn)wnt5a supposedly functions as an antagonist by sequestering the fz5 receptors, thus inhibiting wnt5a–fz5 binding, and the fz5 antiserum most likely blocks wnt5a binding to fz5. As expected, both dn wnt5a transfection and anti-fz5 antiserum treatment blocked expression of the interleukins in question at both the message and protein level. Antiserum administration also resulted in diminished expression of RANKL at both the message and protein level in RA FLS.[23] It has been reported that RANK–RANKL-mediated signaling promotes osteoclastogenesis, which facilitates bone erosion in rheumatoid arthritis.[29] Because it has been shown that

RANKL expressed by RA FLS contributes to osteoclastogenesis mediated by FLS,[29] it is quite likely that blockade of the wnt5a signaling pathway through anti-fz5 antiserum treatment contributes to blockade of FLS-mediated osteoclastogenesis.

It has not yet been investigated if wnt5a-mediated signaling acts through a beta-catenin dependent pathway as does wnt1-mediated signaling in the RA FLS. However, we have found that transfection of synovial fibroblasts by a wnt5a expression vector activates NFκB (unpublished observation); this is not surprising because the three interleukins that are activated by wnt5a signaling have NFκB binding sites in their promoters.[30–32] A gene microarray analysis of both RA FLS and wnt5a transfectants would help in elucidating which genes are activated via wnt5a signaling. Such studies are underway.

Wnt1-mediated signaling in RA FLS

Wnt1 was one of the first wnts to be cloned, characterized, and shown to be involved in limb bud formation and embryogenesis.[10] It is, therefore, quite likely that abnormalities in wnt1-mediated signaling might play a role in the progression of a joint disease such as rheumatoid arthritis.

As mentioned before, we have found considerable expression of wnt1 both in RA tissue and RA FLS. We have also found that increased expression of wnt1 in RA FLS correlates with increased expression of the extracellular matrix protein fibronectin at the protein level (unpublished observation). Increased production of fibronectin has been reported to occur in fibrotic diseases, not only in RA but also in scleroderma and mixed connective tissue disease.[3,33] Fibronectin promotes cell movement, cell adhesion, and cell survival,[34,35] and may contribute to hyperplasia in the rheumatoid joint in the absence of classical proinflammatory factors.

Work conducted in our laboratory has revealed that transfection of synovial fibroblasts with a wnt1 expression vector leads to increased synthesis of the fibronectin protein. The same results have been reproduced by transfecting synovial fibroblasts with beta-catenin, which has been reported to be a downstream modulator wnt1 signaling (unpublished observation). To confirm that the wnt1–beta-catenin pathway contributes to fibronectin synthesis in RA FLS, we investigated if transfection of RA FLS with dominant negative versions of lef and tcf (transcriptional mediators of the wnt1–beta-catenin pathway) abrogates fibronectin synthesis by the RA FLS. As expected, transfection by both dn lef1 and dn tcf4 downregulated fibronectin synthesis by the RA FLS (unpublished observation). Futhermore, transfection with both dn lef1 and dn tcf4 promoted cell death, thus implying that wnt1 might act as a survival factor in RA FLS.

Prospects for the use of Wnt signaling blocking agents for therapeutic intervention

It will be important to investigate if injection of retroviral or adenoviral derivatives of dn wnt5a, dn lef, or dn tcf can block disease progression in animal models of RA. If positive, the experimental results obtained from animal models will pave the way for therapeutic interventions in human RA, with antibodies, receptor antagonists, or small molecule inhibitors of wnt–fz signaling.

Conclusions

The wnt–fz signaling pathways are active in FLS obtained from patients with RA and upregulate the expression of IL-6, IL-8, IL-15, RANKL, and the extracellular matrix protein fibronectin, all of which are characteristic of chronic RA. To date, our studies have focused on wnt5a and wnt1 signaling. It will be important to investigate if other wnt proteins (e.g., wnt13, which is highly expressed in connective tissues, and wnt14, which is required for synovial joint development[36]) are also involved in the progression of RA.

The upregulated synthesis of cytokines, chemokines, and extracellular matrix components induced by wnt1 and wnt5a signaling in the joint FLS has the potential to promote joint inflammation and fibrosis, ultimately leading to joint destruction. However, given the fact that wnt signaling is also operational in wound healing, it is also possible that unregulated wnt signaling is part of an abortive attempt to remodel joints on the verge of destruction. Experiments on animal models of arthritis will suggest if wnt signaling pathways would be attractive targets for the development of new therapeutic agents in rheumatoid arthritis.

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