Differential Expression Patterns of Secreted Frizzled Related Protein Genes in Synovial Cells from Patients with Arthritis

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ABSTRACT. ***Objective.*** To detect expression of the secreted frizzled related protein (sFRP) gene in synovial cells from patients with arthritis.

Methods. Expression of sFRP-1, 2, 3, 4, and 5 genes was detected in synovial cells from patients with rheumatoid arthritis (RA) and osteoarthritis (OA) by semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis. To identify synovial cell populations expressing sFRP-1, 3, and 4 genes, expression was compared in macrophage-rich populations and fibroblast-like cell-rich populations by RT-PCR. Levels of expression of these genes were also studied in activated peripheral blood mononuclear cells (PBMC) and activated skin fibroblasts.

Results. Expression of the sFRP-1, 3, and 4 genes was observed in both RA and OA synovial cells. sFRP-1 and 4 genes were expressed predominantly in fibroblast-like cell-rich populations, and the sFRP-3 gene was expressed predominantly in macrophage-rich populations. Levels of expression of sFRP-3 and 4 genes were elevated in activated PBMC and activated skin fibroblasts.

Conclusion. Our findings suggest that sFRP-1, 3, and 4 may play different roles in the pathogenesis of synovitis. (J Rheumatol 2002;29:2266–70)

Key Indexing Terms:
SYNOVIAL CELLS
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OSTEOARTHRITIS

Wingless (Wnt) proteins are secreted signaling factors that make up a large family of structurally related proteins, and frizzled (Fz) families are membrane-integrated proteins that function as receptors in transducing the Wnt signal to cytoplasm[1,2]. Wnt/Fz signaling activates Dishevelled, which promotes inactivation of glycogen-synthase-kinase 3 (GSK3) by a mechanism involving protein-kinase C. GSK3 suppresses the activity of ß-catenin by promoting its degradation, and inactivation of GSK3 in response to Wnt/Fz signaling leads to the accumulation of ß-catenin, in turn facilitating interactions with HMG-box transcription factors[2,3]. It has been reported that Wnt/Fz proteins play roles in embryonic development, hematopoiesis, synaptogenesis, and mammary development[3-9]. Recently, several Wnt and frizzled proteins were reported to be expressed in synovial cells in patients with rheumatoid arthritis (RA)[10]. In particular, Wnt5a and Fz5 were overexpressed in RA synovial cells[11]. Additionally, some studies found that Wnt5a/Fz5 proteins were involved in interleukin 6 (IL-6) and IL-15 production by RA synovial fibroblasts[10,11].

Secreted frizzled related proteins (sFRP) have been reported to modulate these Wnt/Fz interactions[12,13]. These proteins consist of roughly 300 amino acids, including a cysteine-rich domain that is typically ~30–50% identical to the cysteine-rich domain of Fz family members[14-18]. Five secreted frizzled related proteins have been reported (sFRP-1, 2, 3, 4, and 5)[3-9]. There are a few reports[3-9] of expressions of sFRP in human tissues and cells. Interestingly, James, *et al*[19] reported expression of sFRP-4 genes in osteoarthritis (OA). However, little is known concerning the expression of sFRP genes in synovial cells from patients with arthritis.

We investigated expression of sFRP in synovial cells from patients with RA and OA to determine the relevance of sFRP to the pathogenesis of these conditions.

MATERIALS AND METHODS

Synovial membrane. Synovial membrane samples were obtained during total knee arthroplasty of 10 RA and 10 OA patients. All patients with RA

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met the American College of Rheumatology 1987 criteria[20]. Patients with OA were diagnosed based on clinical symptoms and typical changes on knee radiographs.

Preparation of synovial cells. Synovial membranes were digested with collagenase type V (Sigma, St. Louis, MO, USA), and synovial mononuclear cells (SMC) were obtained from digested synovial membranes by Ficoll-Hypaque density gradient centrifugation as described[21]. SMC adhered to plates, and adherent cells were used as synovial cells. In some experiments, adherent SMC were reacted with anti-CD163 monoclonal antibody prepared in our laboratory, and then with sheep anti-mouse IgG coated immunomagnetic beads (Dynabeads; Dynal AS, Oslo, Norway), and separated into macrophage-rich populations and fibroblast-like cell-rich populations[21]. The macrophage-rich population was > 80% CD163-positive SMC, and the fibroblast-like cell-rich population was < 15% CD163-positive SMC.

Peripheral blood mononuclear cells (PBMC) were obtained from healthy donors by Ficoll-Hypaque density gradient centrifugation. To obtain a monocyte-rich population (PMB), PBMC suspended in Iscove's modified Dulbecco's medium containing 10% human AB serum were incubated in plastic dishes at 37°C for 1 h, and nonadherent cells were removed by washing twice with phosphate buffered saline. These steps were repeated until > 80% of the resulting adherent cells were positive for nonspecific esterase. Activation of PMB was achieved by incubating PMB for 1 day with 500 units/ml of recombinant interferon-γ (IFN-γ) or 10 ng/ml of lipopolysaccharide (LPS) or 10^{-7} M vitamin D_3. Dermal fibroblasts were prepared from healthy donors in the same manner as for preparation of synovial cells. After 4 passages these cells were stimulated with 1 ng/ml of transforming growth factor-ß (TGF-ß) and 100 units/ml tumor necrosis factor-α (TNF-α).

Semiquantitative RT-PCR analysis. Total RNA was extracted from cells using acid guanidinium thiocyanate-phenol chloroform methods[22]. Different pairs of gene-specific primers based on sequences of cloned human isoforms of sFRP-1 (SARP-2), sFRP-2 (SARP-1), sFRP-3, sFRP-4, and sFRP-5 were designed and used for reverse transcriptase-polymerase chain reaction (RT-PCR) analysis. Five micrograms of RNA were used from each specimen, and 28 cycles of PCR were performed. Expression of GAPDH was used to normalize expressions of the different sFRP isoforms. The following list summarizes the primer pairs: for sFRP-1, 5′-AACATTTCCTTTGAACTTGATTG (forward) and GGGGAAGAAATTAATATGCATTT-3′ (reverse); for sFRP-2, 5′-GGTTAAGTCCAAGCTGGCTCATT (forward) and GATGGTCTCGTCTAGGTCATCGA-3′ (reverse); for sFRP-3, 5′-ACATGACTAAGATGCCCAACCAC (forward) and GAGTCGATCCTTCCACTTCTCAG (reverse); for sFRP-4, 5′-AACATCACGCGGATGCCCAACCA (forward) and GATTACTACGACTGGTGCGCCCG-3′ (reverse); for sFRP-5, 5′-GAGGAGTACGACTACTATGGCTG (forward) and CCTTATTCTTCTTGTCCCAGCGG-3′ (reverse).

Amplified PCR products were ligated into the PCR2.1 vector (Invitrogen, Carlsbad, CA, USA) and these sequences were identified as sFRP. In preliminary experiments, since PCR amplification of sFRP reached plateau by 31 cycles, we used 28 cycles to determine the semiquantatitive amounts of sFRP.

RESULTS

Differential expression of sFRP genes in RA and OA synovial cells. Expression of the sFRP-1 gene was observed in all RA and OA samples. Expression of sFRP-3 gene was observed in 7 of 10 RA samples and 6 of 10 OA samples. There were no differences in level of expression among these samples. Expression of sFRP-4 gene was observed in 8 of 10 RA samples and 6 of 10 OA samples (Figure 1). The sFRP-2 and 5 genes were not detected in any RA or OA sample (data not shown).

Predominant expression of sFRP-3 gene in macrophage-rich populations, and of sFRP-1 and 4 genes in fibroblast-like cell-rich populations from RA synovial cells. To examine which populations of RA synovial cells predominantly expressed sFRP-1, 3, and 4 genes, expression of these genes was compared in macrophage-rich and fibroblast-like cell-rich populations. The sFRP-1 and 4 genes were expressed predominantly in fibroblast-like cell-rich populations. Conversely, the sFRP-3 gene was expressed predominantly in macrophage-rich populations (Figure 2).

Elevated expression of sFRP-3 gene in activated macrophages and sFRP-4 gene in activated skin fibroblasts. To determine the levels of expression of sFRP-1, 3, and 4 genes in cell activation, PBMC and skin fibroblasts were stimulated with LPS or IFN-γ or vitamin D_3 and with both TGF-ß and TNF-α, respectively. Strong expression of the sFRP-3 gene was observed in activated PBMC compared to inactivated PBMC (Figure 3). Similarly, levels of expression of the sFRP-4 gene were elevated in activated skin fibroblasts compared to inactivated skin fibroblasts, and by contrast levels of expression of GAPDH gene were not elevated by similar stimulation.

Interestingly, levels of expression of the sFRP-4 gene were higher in cultured RA synovial fibroblasts without stimulation than in activated skin fibroblasts (Figure 4). On the other hand, levels of expression of sFRP-1 gene were not elevated in activated skin fibroblasts (Figure 4).

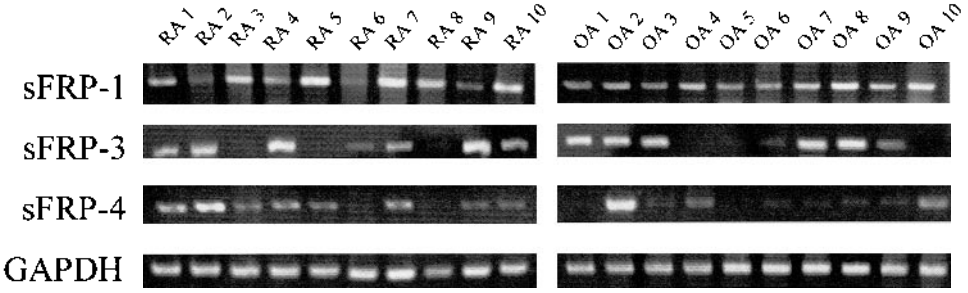

Figure 1. Differential expression of sFRP-1, 3, and 4 and GAPDH genes was detected by semiquantitative RT-PCR analysis in 10 RA and 10 OA synovial cell samples.

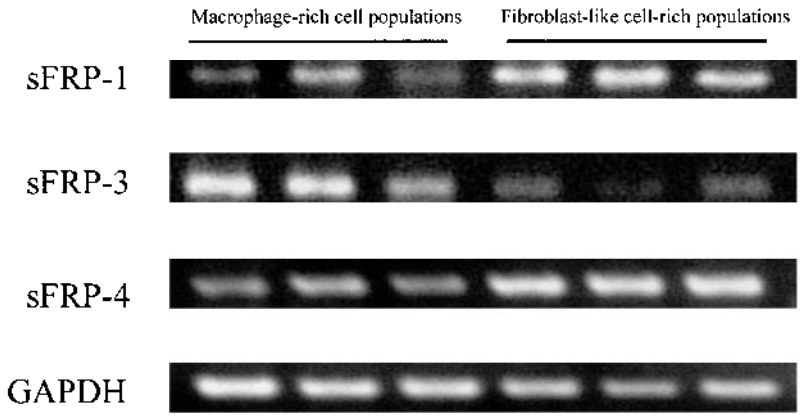

Figure 2. Predominant expression of sFRP-1 and 4 genes in fibroblast-like cell-rich and sFRP-3 genes in macrophage-rich populations of RA synovial cells was detected by semiquantitative RT-PCR analysis.

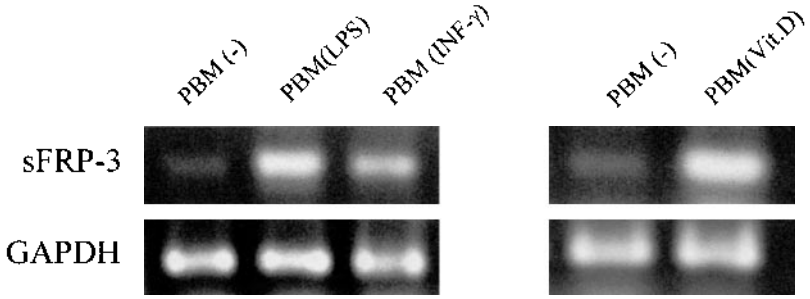

Figure 3. Elevated levels of expression of the sFRP-3 gene in activated monocytes were detected by semiquantitative RT-PCR analysis in PBMC stimulated with LPS, IFN-γ, or vitamin D_3, as described in Materials and Methods.

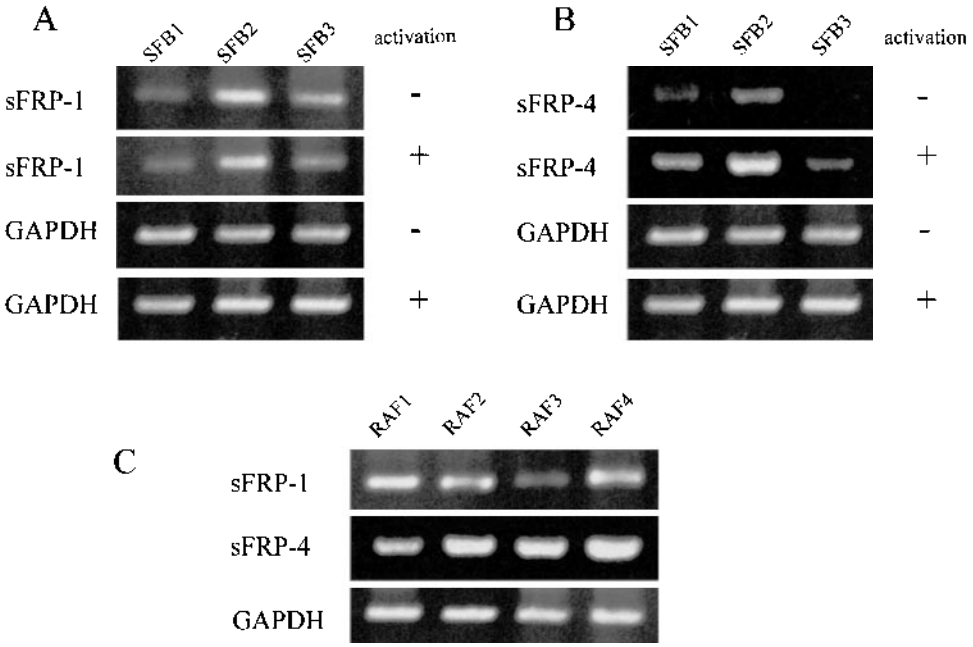

Figure 4. Elevated levels of expression of sFRP-1 and 4 genes were detected by semiquantitative RT-PCR analysis in skin fibroblasts (SFB) from 3 healthy donors stimulated with TGF-ß and TNF-α (A and B), and in cultured RA fibroblasts (RAF) from 4 patients with RA (C).

DISCUSSION

sFRP are described as modulators of Wnt/Fz signal transduction. This sFRP family shares the Wnt binding domain of the frizzled protein, although lacking their 7-transmembrane segment[12-17]. However, the pathophysiological functions of these proteins are still unclear. Additionally, it is difficult to determine corresponding Wnt/Fz ligand-receptor pairs because some Wnt proteins can interact with multiple Fz receptors.

In this study, we first determined expression of the sFRP-1, 3, and 4 genes in RA and OA synovial cells. Unexpectedly, no differences were found in frequency of expression of sFRP-1 or 3 genes between RA and OA synovial cells, although elevated levels of expression of the sFRP-4 gene were found more frequently in RA synovial cells. Predominant expression of sFRP-1 and 4 genes was observed in RA fibroblast-like cell-rich populations, and of sFRP-3 genes in RA macrophage-rich populations.

Sen, *et al* reported that Wnt5a/Fz signaling increased IL–6 and IL-15 production, and that this signaling was partially blocked by recombinant sFRP-1[11]. These findings, together with those for expression of the sFRP-1 gene and elevated expression of the sFRP-3 and 4 genes in cell activation, suggest that sFRP might be involved in the pathogenesis of inflammatory diseases including arthritis[23].

Vitamin D_3 increased expression of sFRP-3 gene in PBMC, suggesting that sFRP-3 may play a role in the differentiation of monocytes, since vitamin D_3 is known as an agent to differentiate monocytes to macrophages. On this point, Berg, *et al* reported expression of Wnt2B and Wnt10B in hematopoietic cells but not in progenitor cells and/or mature cells, and speculated that the Wnt family played a role in function in hematopoietic growth[5].

In cultured RA synovial fibroblasts without stimulation, expression of sFRP-4 was higher than in activated skin fibroblasts. The reason for this may be that cultured RA synovial fibroblasts can be maintained in the activated state for several months in *in vitro* culture[23].

Members of the sFRP family are known to possess a C-terminal netric domain, which is homologous with the C-terminal domains of netins, complement proteins C3, C4, C5, type I procollagen C-proteinase enhancer proteins, and N-terminal domains of tissue inhibitors of metalloproteinases (TIMP)[24]. The N-terminal domain of TIMP has been reported to inhibit and form complexes with active forms of matrix metalloproteinases (MMP)[25]. Thus, in addition to the relevance of sFRP to the Wnt/Fz family, sFRP secreted by synovial cells may play important roles as modulators in cartilage degeneration by interacting with MMP and TIMP. In support of this, it has been suggested that sFRP-4 protein in synovium may be involved in the cartilage degeneration in arthritis by inducing apoptosis of chondrocytes[19].

Our findings suggest that sFRP-1, 3, and 4 may play different roles in the pathogenesis of synovitis.

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