WISP-1 induces IL-6 production in human synovial fibroblasts through PI3K, AKT and NF-κB pathways

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ABSTRACT INTRODUCTION:
Osteoarthritis (OA) is a chronic joint disorder characterized by slow progressive degeneration of articular cartilage, subchondral bone alteration, and variable secondary synovial inflammation. The exact etiology of OA is not well understood. Wnt-induced secreted protein 1/WISP-1 (CCN4), from the CCN gene family, which is involved in many cellular activities such as growth, differentiation, cell motility, adhesion and division. However, the effect of WISP-1 on OA is mostly unknown. Our preliminary data showed that WISP-1 increased IL-6 production in human synovial fibroblast cells. Whether WISP-1/integrin interaction mediated WISP-1-induced IL-6 production will be examined in this research plan. The analysis of cell signaling for WISP-1 in human synovial fibroblast cells is crucial for the development of novel approaches for treatment of OA. Our preliminary data also showed that WISP-1 induced the phosphoinositide-3-kinase (PI3K) and Akt phosphorylation. In addition, we used Reporter gene assay to measure the NF-κB activity. Our preliminary data also showed that WISP-1 induced the NF-κB activity. Whether PI3K/AKT and NF-κB signaling pathway is involved in WISP-1-mediated IL-6 production in human synovial fibroblast cells will be examined in this plan. These results will help us to understand the complicated processes of OA, and be beneficial for the development of effective anti-OA drugs.

METHODS:
Cell cultures
Human synovial fibroblasts were isolated using collagenase treatment from synovial tissues obtained from knee replacement surgeries of 10 patients with OA after approval by the local ethics committee. Fresh synovial tissues were minced and digested in a solution of collagenase and DNase. Isolated fibroblasts were filtered through 70-µm nylon filters. The cells were grown on plastic cell culture dishes in 95% air–5% CO2 with RPMI 1640 (Life Technologies, Grand Island, NY) that was supplemented with 20mM HEPES and 10% heat-inactivated FBS, 2mM glutamine, 100 U/ml penicillin, and 100 µg/ml streptomycin (pH adjusted to 7.6). Fibroblasts from passages four to nine were used for the experiments. The cells from different patients were treated with WISP-1 separately. All studies carried out on cells from least four patients. Results are expressed of four independent experiments (n=4).

Measurements of IL-6 production
Human synovial fibroblasts were cultured in 24-well culture plates. After reaching confluence, cells were treated with WISP-1, and then incubated in a humidified incubator at 37°C for 24 h. For examination of the downstream signaling pathways involved in WISP-1 treatment, cells were pretreated with various inhibitors for 30 min before WISP-1 (30 ng/ml) administration. After incubation, the medium was removed and stored at -80°C until assay. IL-6 in the medium was assayed using the IL-6 enzyme immunoassay kits, according to the procedure described by the manufacturer. qPCR; Flow cytometric analysis; Western blot analysis; Transfection and reporter gene assay

RESULTS SECTION:
WISP-1 has been involved in pathology of arthritis. The typical pathology of OA includes chronic inflammation of the synovium, which is characterized by infiltrations of inflammatory cells and synovial hyperplasia, especially fibroblast-like synoviocytes. Therefore, we decided to use human synovial fibroblasts to investigate the signaling pathways of WISP-1 in the production of IL-6, an inflammatory response gene. Treatment of OASF with WISP-1 (1-30 ng/mL) for 24 h induced mRNA expression of IL-6 in a concentration-dependent manner (Fig. 1A). In addition, stimulation of cells with WISP-1 also increased protein expression of IL-6 in a concentration-dependent manner (Fig. 1B).

PI3K/Akt signaling pathway can be activated by a variety of growth factors, such as insulin and nerve growth factors. Treatment of cells with WISP-1 led to a significant increase of phosphorylation of p85 subunit of PI3K (Fig. 2A). To explore whether PI3K is involved in WISP-1-induced IL-6 production, PI3K inhibitors Ly294002 and wortmannin were used. As shown in Fig 2B&C, pretreatment of cells with Ly294002 or wortmannin inhibited WISP-1-induced IL-6 production of OASF. We then directly measured the Akt phosphorylation in response to WISP-1 stimulation. Figure 3A shows that WISP-1 increased Akt phosphorylation in a time-dependent manner. Furthermore, Akt inhibitor also antagonized WISP-1-induced IL-6 production (Fig. 3B&C). Taken together, these results indicate that the PI3K and Akt pathway are involved in WISP-1-induced IL-6 production of OASF.

As previously mentioned, NF-κB activation is necessary for the IL-6 production of OASF. To examine whether NF-κB activation is involved in WISP-1-induced IL-6 production, we further examined the upstream molecules involved in WISP-1-induced NF-κB activation. Stimulation of cells with WISP-1 induced IKKα/β phosphorylation in a time-dependent manner (Fig. 4A). Treatment of cells with WISP-1 also caused IkBα phosphorylation in a time-dependent manner (Fig. 4A). Previous studies showed that p65 Ser536 phosphorylation increases NF-κB transactivation. Therefore, the antibody specific against phosphorylated p65 Ser536 was employed to examine p65 phosphorylation. Treatment of cells with WISP-1 for various time intervals resulted in p65 Ser536 phosphorylation (Fig. 4A). To directly determine whether NF-κB is activated after WISP-1 treatment, OASF were transiently transfected with κB-luciferase as an indicator of NF-κB activation. As shown in Fig 4B-C, WISP-1 treatment of cells for 24 hr caused increase in κB-luciferase activity. In addition, P85, AKT, IKKα and IKKβ mutant reduced WISP-1-mediated NF-κB activity. Taken together, these data suggest that activation of PI3K and AKT pathway is required for WISP-1-induced NF-κB activation in OASF.

DISCUSSION:
We explored the signaling pathway involved in WISP-1-induced IL-6 production in human synovial fibroblasts. We found that WISP-1 increases IL-6 production by activating PI3K and Akt which enhances binding of p65 to the NF-κB site and results in the transactivation of IL-6 production. Therefore, this pathway is the common pathway in WISP-1 mediated OA pathogenesis. Furthermore, the discovery of WISP-1 mediated signaling pathway helps us understand the mechanism of OA pathogenesis and may lead us to develop effective therapy in the future.