Adiponectin enhances IL-6 production in human synovial fibroblast via an adipoR1 receptor, AMPK, p38 and NF-κB pathway

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Introduction: Adipose tissue is a ubiquitous tissue, which can be found as a structural component of many organs of the human body. Adipocyte has the ability to synthesize and release proinflammatory molecules, complement factors, signaling molecules, growth factors, and adhesion molecules [1], suggesting an integrated function of adipocytes in tissue inflammation. Among these molecules are IL-6, macrophage migration inhibitory factor, leptin and adiponectin. Adiponectin was originally described as an adipocytokine exclusively expressed by adipose tissue. However, the signaling pathway for adiponectin on IL-6 production in synovial fibroblasts is mostly unknown. In the present study, we explored the intracellular signaling pathway involved in adiponectin-induced IL-6 production in synovial fibroblast cells.

Materials and Methods: Cell cultures: Human synovial fibroblasts were isolated by collagenase treatment from synovial tissue obtained from knee replacement surgeries of ten patients with rheumatoid arthritis synovial fibroblasts (RA) and osteoarthritis (OA).

Measurements of IL-6 production: Human synovial fibroblasts were cultured in 24-well culture plates. After reaching confluence, cells were treated with various stimulators, and then incubated in a humidified incubator at 37°C for 24 h. IL-6 in the medium was assayed using the IL-6 enzyme immunoassay kits, according to the procedure described by the manufacturer.

mRNA analysis by reverse transcriptase-polymerase chain reaction (RT-PCR).
Western Blot Analysis.
Transfection and reporter gene assay.
AMPK in vitro kinase assay.
DNA affinity protein-binding assay (DAPA).
Chromatin immunoprecipitation assay.

Results: Adiponectin is significantly higher in synovial fluid of patients with osteoarthritis and rheumatoid arthritis [1]. Treatment of rheumatoid arthritis synovial fibroblast (RASF) or osteoarthritis synovial fibroblast (OASF) with adiponectin for 24 hr induced IL-6 production in a concentration-dependent manner (Fig. 1A), this induction occurred in a time-dependent manner (Fig. 1B). After adiponectin treatment for 24 hr, the amount of IL-6 released had increased in both RASF and OASF cells (Fig. 1B). To further confirm this stimulation-specific mediation by leptin without LPS contamination, polymyxin B, an LPS inhibitor, was used. We found that polymyxin B completely inhibited LPS-induced IL-6 release. However, it had no effect on adiponectin-induced IL-6 release in both RASF and OASF (Fig. 1C&D). To investigate the role of adipoR1 and R2 subtype receptors in adiponectin-mediated increase of IL-6 production, we assessed the distribution of these adiponectin receptor subtype receptors in human synovial fibroblasts by RT-PCR analysis. The mRNAs of adipoR1 and adipoR2 subtype receptors could be detected in RASF and OASF (Fig. 2). Upon adiponectin treatment for 12 hr, the mRNA levels of IL-6 and adipoR1 subtype receptor were evidently increased, whereas other subtypes adipoR2 receptor mRNA remained unchanged (Fig. 2). Adiponectin has been shown to increase fatty acid oxidation via activation of AMPK. Fig. 3A&B show that adiponectin enhanced AMPK phosphorylation at the Thr172 and activity in a time-dependent manner. Pretreatment of cells for 30 min with AMPK inhibitors [araA or compound C ] or transfection with adipoR1 siRNA markedly attenuated the adiponectin-induced AMPK kinase activity (Fig. 3C).

Discussion: It has been reported that adiponectin is significantly higher in synovial fluid of patients with osteoarthritis and rheumatoid arthritis [1]. Here we further identify IL-6 as a target protein for the adiponectin signaling pathway that regulates cell inflammatory response in both RASF and OASF. Using pharmacological and genetic inhibitors show that these inhibitors all attenuated adiponectin-induced IL-6 release, indicating adiponectin through the same signaling pathway to induce IL-6 production in RASF and OASF. We also show that potentiation of IL-6 by adiponectin requires an activation of the adipoR1 receptor, AMPK, p38, IKK and NF-κB signaling pathway. These findings suggest that adiponectin acts as an inducer of inflammatory cytokines such as IL-6 and as an enhancer of the inflammatory response in RA and OA.


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