Transforming Growth Factor-β Stimulates Constitutive and IL-1β-induced MCP-1 Expression in Human Synovial Cells Through ERK/AP-1 Pathway

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Introduction:
Arthrofibrosis is one of the most significant complications after trauma and surgery around joints. Our previous study (1) demonstrated that synovial hyperplasia, infiltration of inflammatory cells, and overproduction of TGF-β were observed in the fibrotic sites of human synovial tissues. Excessive TGF-β contributes to pathogenesis of tissue fibrosis, and TGF-β not only plays a critical fibrogenic role, but also has chemotactic properties. Monocytes infiltration became apparent after TGF-β intraarticular injection and TGF-β may play a direct role in arthritic inflammation (2). Monocyte chemotactic protein-1 (MCP-1) is one of the most potent macrophage-recruiting molecules, and MCP-1 has been also implicated in a variety of fibrotic disease (3). Recently, TGF-β has been shown to up-regulate MCP-1 expression in human macrophages (4). The aim of this study was to elucidate whether TGF-β modulates constitutive and IL-1β-induced MCP-1 production in human synovial cells and to in vestigate the signaling pathways involved.

Materials and methods:

Cell culture
Synovial tissues were obtained from non-arthritic patients during ligament reconstruction surgery. The tissues were digested with collagenase, and isolated cells were cultured in DMEM containing 10% FBS. The cultures were subjected to the different stimulation.

MCP-1 ELISA
After a 24 hours-starvation, confluent cells were treated with TGF-β1 and IL-1β. MCP-1 concentrations in the culture supernatant were assayed with ELISA kit for MCP-1 (AN’ALYZA).

Real-time PCR
Total RNA was isolated from confluent cells with TRizol reagent. Quantitative real-time PCRs for MCP-1 mRNA were performed in the LightCyclerTM instrument (Roche Diagnostics) using FRET probes with the QuantiTestTM Probe PCR Kit. The results of EMSA revealed that TGF-β stimulated both NF-κB and AP-1 activity (Fig.2A). In contrast, IL-1β-induced NF-κB activity was inhibited by TGF-β (Fig.2B).

Results

TGF-β stimulates constitutive and IL-1β-induced MCP-1 production

TGF-β induced MCP-1 protein expression in human synovial cells in a dose- and time-dependent manner (Fig.1A). IL-1β has been known to induce MCP-1 in synovial cells, TGF-β significantly provoked constitutive and IL-1β-induced MCP-1 protein production and MCP-1 mRNA expression (Fig.1B).

TGF-β stimulates constitutive and IL-1β-induced MCP-1 promoter activity

Next we examined whether AP-1 or NF-kB is responsible for increased MCP-1 production by TGF-β. TGF-β elicited transcriptional activity of the MCP-1 promoter. And the combination of TGF-β and IL-1β synergistically increased MCP-1 promoter activity. Although TGF-β inhibited IL-1β-induced NF-kB-driven luciferase activity, TGF-β increased the activity of deleted promoter construct either with or without IL-1β, suggesting that the AP-1 binding region affects the activation of MCP-1 transcription induced by TGF-β (Fig.2B).

Discussion
In the present study, we provided evidence that TGF-β up-regulates the expression of MCP-1 in human synovial cells, and concurrent treatment with TGF-β and IL-1β synergistically enhances MCP-1 production through the activation of AP-1. The phosphorylation of ERK might be involved in this process. It suggests that TGF-β generates chemotactic activities via MCP-1 production, and recruited macrophages release greater amounts of profibrotic cytokines, such as TGF-β. This putative activation loop may lead to the perpetuation of inflammatory response and contribute to the pathogenesis of arthrofibrosis.

References

Fig.1. Stimulation of MCP-1 production by TGF-β.

Fig.2. Effects of TGF-β on the promoter activities of MCP-1. Activation of the ERK1/2 -AP-1 pathway might be involved in MCP-1 expression by TGF-β.

Fig.3. Effects of TGF-β on the DNA binding activity of AP-1 and NF-kB.

Fig.4. Effects of TGF-β on IL-1β-induced MAPK activation.