The Runx2 transcription factor and MMP-13 upregulation in the stromal cell of Giant Cell Tumor of bone

Isabella Mak1,2, Rob Cowan1,2, Nigel Colterjohn1,2, Gurmit Singh1,2, Michelle Ghert1,2
1McMaster University, Hamilton, ON, Canada; 2Juravinski Cancer Centre, Hamilton, ON, Canada

Introduction: Matrix metalloproteases (MMPs) play a key role in the degradation of the extra-cellular matrix. MMP-13 is a type-I collagenase that is induced in joint diseases such as osteoarthritis. Giant Cell Tumor of bone (GCT) is a locally destructive tumor with metastatic potential. The histological appearance of GCT reveals a high number of osteoclast-like multinucleated giant cells together with spindle-shaped, mesenchymal stromal cells (1). Cell culture experiments with GCT specimens in our lab have shown that the mesenchymal stromal cell expresses high levels of MMP-13.

Elevated expression of MMP-13 in the joints of arthritic patients coincides with increased levels of pro-inflammatory cytokines interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) (2). It has been shown that IL-1β induces the phosphorylation of Runx2 in human chondrosarcoma cells via the p38 MAPK kinase pathway (3). Runx2 is a transcription factor that is necessary for osteoblast differentiation with a binding site in the promoter region of MMP-13 (4). We have found Runx2 to be constitutively expressed in GCT stromal cells.

The objectives of this study were: 1. To determine if IL-1β and/or TNF-α upregulate MMP-13 in GCT stromal cells, and 2. To identify the signaling pathways involved in cytokine-induced MMP-13 upregulation with a focus on the Runx2 transcription factor.

Materials and Methods: Immunohistochemistry on GCT tissue samples embedded in paraffin blocks after formalin fixation was used to detect the expression of Runx2, IL-1β and TNF-α. Primary cell lines and cell culture of GCT stromal tumor cells from fresh GCT tissue were obtained from patients following Ethics Board approval and patient consent. The tissue was processed and maintained as described by Ghert et al (1). Following several successive passages, the mesenchymal stromal cells became the homogeneous cell type whereas the multinucleated giant cells were eliminated from the culture. Real-time reverse transcription-polymerase chain reaction (RT-PCR) was used to analyze the expression of Runx2, MMP-2 and -13 from GCT stromal cells treated with cytokines (IL-1β and TNF-α, 1.0 ng/ml). Effects of signaling pathway inhibitors (SB203580 – p38 inhibitor, PD98059 – ERK inhibitor, SP60012530 – JNK inhibitor or DMSO) on cytokine-induced expression of Runx2, MMP-2 and -13 were also evaluated using real-time PCR. All experiments were performed in triplicate.

Results: Immunohistochemical staining of GCT tissue samples confirmed the presence of IL-1β and TNF-α in the GCT microenvironment (Fig. 1A, B). Staining was strongly positive in the cytoplasm of multinucleated giant cells, and faintly in the stromal cells. The expression of Runx2 localized heavily in the nuclei of the stromal cells, and occasionally in some of the nuclei of the multinucleated giant cells (Fig. 1C). Following stimulation with incremental concentrations of cytokines (IL-1β or TNF-α), the level of MMP-13 mRNA expression in the stromal cells increased dramatically over 100-fold (Fig. 2), however, the expression levels of MMP-2 and Runx2 remained stable. After co-stimulation with cytokines and signaling pathway p38, ERK and JNK inhibitors on GCT stromal cell cultures, the expression of Runx2, MMP-2 remained unchanged (data not shown).

However, MMP-13 mRNA expression decreased incrementally with ERK and JNK (but not p38) inhibition (Fig. 3). However, MMP-13 mRNA expression decreased incrementally with ERK and JNK (but not p38) inhibition (Fig. 3).

Discussion: In this study, we have shown that exposure to IL-1β and TNF-α, strongly expressed in the giant cells, induces a significant upregulation of MMP-13 mRNA expression in GCT stromal cells consistent with findings in chondrosarcoma (3). We have also demonstrated that ERK and JNK pathway inhibition differentially down-regulates cytokine-induced MMP-13 expression in GCT stromal cells while sparing Runx2 expression. Given the fact that Runx2 is known to have a binding site in the MMP-13 promoter region (4), inconsistencies between Runx2 mRNA levels and transcriptional activity of MMP-13 suggest that post-translational modification of Runx2, such as phosphorylation, is necessary to alter its transcriptional regulation of MMP-13.

In contrast to the preferential inhibition of MMP-13 expression by ERK and JNK inhibitors, the p38 inhibitor did not affect the expression of MMP-13 in these cells as it has been shown to do in chondrosarcoma cells (3). Xiao et al has shown that the ERK1/2-dependent phosphorylation cascade regulates Runx2 activity in murine preosteoblast and rat osteosarcoma cells (5). Together with our results, these findings suggest that regulation of MMP-13 expression in GCT stromal cells involves Runx2 phosphorylation through the ERK and JNK signal transduction network.

In summary, we have demonstrated that 1. The inflammatory cytokines IL-1β and TNF-α are expressed by the giant cells of GCT and induce a substantial upregulation of MMP-13 mRNA expression in GCT stromal cells, and 2. Cytokine-induced MMP-13 expression is strongly suppressed by ERK and JNK kinase inhibitors. Our results suggest the involvement of Runx2 in mediating the effects of the ERK and JNK pathways on MMP-13 expression in GCT mesenchymal stromal cells. These signaling pathways may therefore serve at targets in treatment strategies for this destructive tumor.


Fig. 1. Immunolocalization of A. IL-1β, B. TNF-α, and C. Runx2 in human specimens of GCT formalin-fixed-paraffin-embedded tissue.

Fig. 2. Relative mRNA expression of MMP-2, -13 and Runx2 after exposure to cytokines based on real-time PCR.

Fig. 3. Down-regulation of IL-1β-induced MMP-13 expression by ERK & JNK inhibitors in GCT stromal cells based on real-time PCR.

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