INTRODUCTION:
ADAMTS-12 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, 12) expression is important for normal chondrogenesis during development, with increasing expression as cells progress towards hypertrophy (1). In adult cartilage, elevated ADAMTS-12 correlates with cartilage degradation and arthritis progression. ADAMTS-12 degrades COMP (cartilage oligomeric matrix protein), a prominent noncollagenous component of cartilage matrix that is a biomarker for osteoarthritis progression (2).

Regulation of ADAMTS-12 expression is still poorly understood, and the promoter of the ADAMTS-12 gene is not well characterized. In this study, we analyzed the ADAMTS12 promoter and identified several potential c-Maf binding sites. c-Maf is an important transcription factor regulating chondrogenesis and hypertrophy (3). Here we report that c-Maf significantly upregulates ADAMTS-12 promoter and that the upregulation of ADAMTS-12 by c-Maf occurs via a proximal c-Maf binding site in ADAMTS-12 promoter.

METHODS:
Chondrogenic differentiation of isolated passage-2 human bone marrow stem cells (hBMSC) was performed in three-dimensional pellet culture in a defined serum-free medium containing 10 ng/ml recombinant human TGFβ-1 (PeproTech), and 500 ng/ml recombinant human IGF-I (R&D Systems) for 14 days. Gene expression was measured by real time quantitative PCR using Assays-on-Demand Taqman primers and probes. Transcription factor binding analysis was performed in-silico using TRANSFAC MatInspector. The 3-kb proximal promoter of human ADAMTS-12 was obtained by PCR from BAC clone RP11-669P5, and subcloned into pGL4.10 luciferase vector (Promega). A series of 5' truncations and specific 13-nt deletions were also subcloned into pGL4.10 vector. cDNA clone of human c-Maf (Accession BC081542 Open Biosystems) was subcloned into pFlagCMV2 expression vector. SW1353 chondrosarcoma cells were transfected with ADAMTS-12 promoter constructs ± pFlagCMV2-c-Maf. Luciferase activity was measured at 48 hours post-transfection. Transfection efficiency was normalized with Renilla luciferase activity expressed from pRL-TK.

RESULTS:
Co-expression of endogenous c-Maf and ADAMTS-12 during chondrogenic differentiation. Sequence analysis of the 3kb proximal promoter of ADAMTS-12 revealed seven predicted Maf and AP1 related factor binding sites (Fig 2A), raising the possibility that c-Maf regulates ADAMTS-12 expression. We therefore tested for co-expression of the two molecules during stem cell chondrogenesis in pellet culture. We found c-Maf and ADAMTS-12 expression coincidently increased during in vitro chondrogenic differentiation of human stem cells (Fig 1).

Identification of active c-Maf element in the ADAMTS-12 promoter. Analysis of 5' sequential deletion constructs revealed that the proximal 315bp region contains full basal activity and is sufficient for c-Maf-specific transactivation (Fig 2B). The proximal 350bp region was also sufficient to direct c-Maf-specific activation, suggesting the importance of the proximal promoter region for c-Maf transactivation (Fig2B).

Since the proximal 350bp region conferred transactivation by c-Maf, and sequence analysis revealed a consensus Maf binding site at -61 position, we generated the 3 kb promoter constructs with a deletion of 13 nucleotides at either the -61 (Δproximal), or the -2311 (Δdistal) positions as a control. The Δproximal construct abrogated c-Maf-induced ADAMTS-12 promoter activation but the deletion of 13 nucleotides at -2311 (Δdistal) did not affect c-Maf-induced transactivation (Fig2C).

DISCUSSION: In cartilage, ADAMTS-12 metalloproteinase is involved in both normal chondrogenesis and pathological progression of arthritis, yet the regulation of ADAMTS-12 expression is not known in either context. Similarly, c-Maf transcription factor is important in both normal chondrogenesis and pathological progression of arthritis, yet many of its target genes remain to be identified.

Through expression studies and in vitro reporter assays, we report that c-Maf significantly up-regulates ADAMTS-12 promoter transcription, and that the upregulation of ADAMTS-12 by c-Maf occurs via a proximal c-Maf binding site in ADAMTS-12 promoter. This may indicate that c-Maf transcription factor is involved in chondrocyte differentiation and may be involved in OA pathology, at least in part, through the regulation of ADAMTS-12 expression.

References: