Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 345 (2006) 197–204
BBRC
www.elsevier.com/locate/ybbrc

Regulation of the human ADAMTS-4 promoter by transcription factors and cytokines

Kannan Thirunavukkarasu *, Yong Pei, Terry L. Moore, He Wang, Xiao-peng Yu, Andrew G. Geiser, Srinivasan Chandrasekhar

Musculoskeletal Research, Lilly Research Labs, Eli Lilly and Company, Indianapolis, IN 46285, USA

Received 31 March 2006
Available online 4 May 2006

Abstract

ADAMTS-4 (aggrecanase-1) is a metalloprotease that plays a role in aggrecan degradation in the cartilage extracellular matrix. In order to understand the regulation of ADAMTS-4 gene expression we have cloned and characterized a functional 4.5 kb human ADAMTS-4 promoter. Sequence analysis of the promoter revealed the presence of putative binding sites for nuclear factor of activated T cells (NFAT) and Runx family of transcription factors that are known to regulate chondrocyte maturation and differentiation. Using promoter–reporter assays and mRNA analysis we have analyzed the role of chondrocyte-expressed transcription factors NFATp and Runx2 and have shown that ADAMTS-4 is a potential downstream target of these two factors. Our results suggest that inhibition of the expression/function of NFATp and/or Runx2 may enable us to modulate aggrecan degradation in normal physiology and/or in degenerative joint diseases. The ADAMTS-4 promoter would serve as a valuable mechanistic tool to better understand the regulation of ADAMTS-4 expression by signaling pathways that modulate cartilage matrix breakdown.

Keywords: Aggrecanase-1/ADAMTS-4; Promoter; IL-1; Oncostatin M; NFATp; Runx2; β-gal reporter assay

The articular cartilage is a smooth weight-bearing tissue that covers the ends of long bones and facilitates the frictionless movement of one bone over the other enabling proper joint function. Maintenance of integrity of the extracellular matrix (ECM) is critical for proper functioning of the cartilage. Degradation of aggrecan and type II collagen by proteases is a major factor that leads to cartilage erosion in osteoarthritis (OA). Aggrecanases, the primary proteases responsible for aggrecan cleavage, are matrix metalloproteases of the a disintegrin and metalloprotease with thrombospondin motifs (ADAMTS) family that are involved in normal aggrecan turnover and in the enhanced degradation of aggrecan seen in pathologic conditions. Aggrecan fragments containing aggrecanase-generated neoepitopes are found in synovial fluids from patients with osteoarthritis, joint injury, and inflammatory joint disease [1], suggestive of the involvement of aggrecanases in the pathogenesis of these joint disorders.

Although many members of the ADAMTS family cleave aggrecan in vitro, ADAMTS-4 (aggrecanase-1) and ADAMTS-5 (aggrecanase-2) show the highest specific activity for cleaving aggrecan [2]. In vitro studies using small molecule inhibitors of aggrecanase activity and in vivo studies using knockout mice have shed light on the importance of these proteases in cartilage matrix turnover in normal physiology and in cartilage degradation in pathological states [3–6]. In a surgical model of OA, ADAMTS-4 knockout animals are not protected from cartilage degradation [3] whereas ADAMTS-5 knockout mice are significantly protected from cartilage degradation in the knee joint [4], demonstrating ADAMTS-5 to be the critical enzyme involved in pathological aggrecan degradation in mice. However, the relative contribution of these two enzymes to physiological/pathological aggrecan degradation in humans is not known.

* Corresponding author.
E-mail address: kannan@lilly.com (K. Thirunavukkarasu).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.04.023

The expression of ADAMTS-4 mRNA has been demonstrated in a number of tissues in the body [6] and ADAMTS-4 is thought to play a role in growth plate remodeling during normal skeletal development [3,4]. The expression of ADAMTS-4 is induced by catabolic cytokines in cartilage explants [5,7,8]. However, the pathways involved in the regulation of aggrecanase expression have not been elucidated in detail. To better understand the regulation of ADAMTS-4 gene expression, we have cloned a functional 4.5 kb human ADAMTS-4 gene promoter that is responsive to cartilage-active cytokines like the endogenous gene, and investigated its regulation by chondrocyte-expressed transcription factors.

The transcription factor nuclear factor of activated T cells-p (NFATp) has been shown to function as a repressor of cartilage growth and differentiation [9]. Analysis of the 4.5 kb ADAMTS-4 promoter showed the presence of 2 putative binding sites for NFAT transcription factors. Using reporter gene assays, we have shown that overexpression of NFATp results in a significant increase in ADAMTS-4 promoter activity, suggesting ADAMTS-4 as a potential target for NFATp transactivation.

The transcription factor Runx2 plays a key role in chondrocyte maturation and osteoblast differentiation [10–13]. The 4.5 kb ADAMTS-4 promoter contains 8 putative binding sites for the Runx family of transcription factors. We show that overexpression of Runx2 results in a significant increase in transactivation of the ADAMTS-4 promoter, and a corresponding increase in endogenous ADAMTS-4 mRNA expression. Our studies provide evidence for the potential involvement of NFATp and Runx2 in the transcriptional regulation of ADAMTS-4 expression.

Methods

Cloning of the human ADAMTS-4 promoter. The genomic sequences immediately 5′ to the coding region of the human ADAMTS-4 gene were cloned using the Genome Walker kit (Clontech). Gene-specific primers (GSPs) were designed from the published human ADAMTS-4 coding sequence (GenBank # AY044847) [GSP-1 (aggr1R) 5′-ccacagccagcgccct gccaagccccctc-3′ and nested GSP-2 (aggr1Rnest) 5′-ggcactggtactgcagctgg-gaggga-3′]. These primers were used in combination with primers specific to the Genome Walker library adapter to amplify 4515- and 1965-bp fragments of DNA flanking the 5′ end of the ADAMTS-4 coding region. The two fragments were cloned into pCRII-TOPO vector (Invitrogen). Sequence analysis confirmed that these were overlapping proximal fragments of the 5′-flanking region of the ADAMTS-4 gene. The promoter sequence was analyzed for the presence of consensus transcription factor binding sites using the MatInspector program (Genomatix Software GmbH, Germany).

Plasmid construction. The 4515 bp fragment of the ADAMTS-4 promoter containing sequences from −4109 to +406 relative to the transcriptional start site was used as a template to PCR amplify the full-length promoter and five different 5′-deletion fragments (−4109 to +406; −3109 to +406; −1559 to +406; −1180 to +406; −749 to +406; −405 to +406). *Xho*I and *Hin*dIII restriction sites were incorporated into the forward and reverse primers, respectively, and the resulting PCR products were cloned into the corresponding sites in pβ-gal-Basic reporter vector (Clontech).

pEF/myc/cyto (control vector) was purchased from Invitrogen. The NFATp cDNA (isoform C) [14] was cloned from rat pancreas and inserted into the *Nco*I and *Not*I sites of pEF/myc/cyto to generate pEF-NFATp. The Runx2 expression construct (pEF-Runx2) has been described previously as pEF-Cbfa1 [15].

Cell culture, DNA transfection, and cytokine treatments. COS1, C3H10T1/2, and SW1353 cells were grown in DMEM supplemented with 10% FBS. Bovine articular chondrocytes (BACs) were isolated from bovine knee joints (Covance Research, Denver, PA) through protease/collagenase digestion and grown in DMEM containing sodium pyruvate + 10% FBS.

Promoter transfection and cytokine treatment of BACs: BACs were plated in 6-well plates at a density of 4×10^5 cells/well and allowed to grow for 4–5 days. The day before the transfection, the monolayer culture was replenished with fresh culture medium. The ADAMTS-4 promoter-β-gal construct was transiently transfected into the cells using Fugene6 reagent (Roche). The next day, the cells were treated with cytokines (10 ng/ml of human IL-1α or 50 ng/ml OSM; R&D Systems, Minneapolis, MN), either alone or in combination, in 0.1% serum-containing medium for a period of 24 h. At the end of the study, the cells were washed with PBS and cell lysates were prepared. β-gal activity in cell extracts was measured using a luminescent β-gal assay kit (Roche). Data from a representative experiment are shown as fold change in β-gal activity ± standard error of mean.

For NFATp transactivation studies, 1×10^5 cells were plated per well in 6-well plates and incubated for 24 h. Cells were then cotransfected with 1μg each of the reporter plasmid (ADAMTS-4 promoter-β-gal or the control promoterless vector pβ-gal-Basic) and the effector plasmid (pEF-NFATp or the control expression vector pEF/myc/cyto), using Fugene6 transfection reagent. The same procedure was followed for Runx2 transactivation studies, using pEF-Runx2 as the effector plasmid. The total amount of DNA transfected per well was maintained constant. β-gal activity in cell lysates was measured 36–48 h post-transfection. All the experiments were performed at least twice in triplicate wells, using different plasmid preparations. β-gal activity values are expressed as fold induction over basal (control vector transfected) levels.

Runx2 overexpression for mRNA analysis. SW1353 cells were plated in T150 flasks and allowed to grow for two days (~70% confluence). For infection, 100 μl of adenovirus (titer: 2.2×10^9 pfu/ml) encoding either Runx2 (Ad-Runx2) [16] or GFP (Ad-GFP) was diluted in 10 ml of complete medium and incubated with the cells overnight. The next day, 10 ml more fresh medium was added to the pre-existing medium. Cells were harvested for RNA purification on the following day.

Real-time PCR. Total RNA was extracted with Tripure Reagent (Roche) and mRNA was purified using the Oligotex kit (Qiagen). cDNA was synthesized from mRNA using the SuperScript first-strand synthesis system for RT-PCR (Invitrogen). Real-time PCR was performed with an ABI Prism7900 Sequence Detection System.

Results

Cloning of the 4.5 kb ADAMTS-4 promoter and analysis of putative transcription factor binding sites in the promoter

Using the genome walking PCR strategy, we have cloned a 4.5 kb fragment of the 5′-flanking region of the human ADAMTS-4 gene (−4109 to +406). Sequence analysis of the 4.5 kb promoter, schematically shown in Fig. 1, revealed the presence of a TATA box-like sequence (TTAAAA, at position −49), a CCAAT-box sequence, and an Sp1-binding site adjacent to the transcription site, as described in the previously published 1.1 kb promoter sequence [17]. We also noted the presence of putative binding sites for the NFAT and Runx family of transcription factors. The consensus NFAT-binding elements (NBE) containing the sequence 'GGAAAA' were located at positions (−707 to −702) and (−3098 to −3093) upstream of

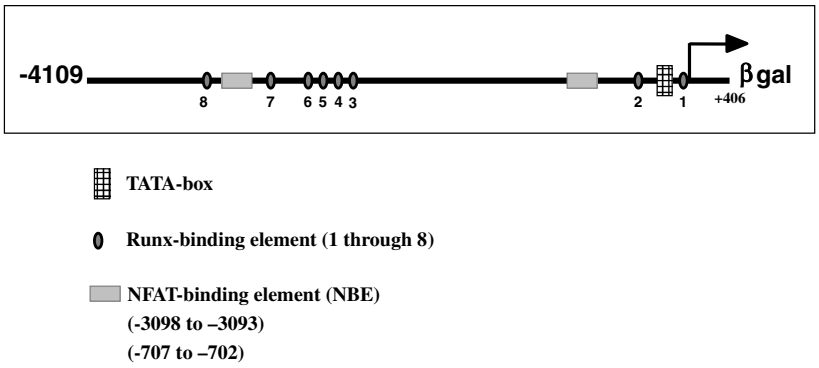

Fig. 1. Schematic representation of the 4.5 kb human ADAMTS-4 promoter. The 4.5 kb promoter (−4109 to +406) was cloned upstream of the β-gal coding sequence in the vector pβ-gal-Basic (Clontech). The putative NFAT- and Runx-binding elements are shown. The arrow represents the transcription start site [17].

Table 1
Location and sequence of putative Runx-binding elements in the ADAMTS-4 promoter

Runx-binding element #	Sequence	Position relative to transcription start site
8	5′-TCCCACA-3′	−3136 to −3130
7	5′-TGTGGGT-3′	−2678 to −2672
6	5′-TGTGGGA-3′	−2155 to −2149
5	5′-TGCCACA-3′	−2116 to −2110
4	5′-TGTGGCC-3′	−2024 to −2018
3	5′-CCCCACA-3′	−1839 to −1833
2	5′-TGTGGGG-3′	−240 to −234
1	5′-ACCCACA-3′	−15 to −9

the transcription start site. These NBEs were identical to the ones found in the regulatory regions of various cytokine genes that are targets of NFAT proteins in T lymphocytes [18]. The location and sequence of the eight putative Runx-binding elements in the promoter are shown in Fig. 1 and Table 1. These putative Runx-binding elements were identical or similar to the ones found in the regulatory regions of several known targets of Runx2 that are expressed in osteoblasts and chondrocytes [19–22].

Analysis of the functionality of the ADAMTS-4 promoter

In order to determine the transcriptional functionality of the promoter, we cloned the promoter upstream of the β-gal reporter gene in the vector pβ-gal-Basic (p4.5Aggr1-β-gal). The ability of the promoter to drive expression of β-gal was tested in transient transfection assays in COS1, a monkey kidney cell line, C3H10T1/2, a mouse mesenchymal cell line, and SW1353, a human chondrosarcoma cell line. The 4.5 kb promoter directed a higher level of expression of the β-gal reporter gene compared to the promoterless vector (pβ-gal-Basic) in all four cell lines, indicative of the transcriptional functionality of the promoter in these cell lines (Fig. 2A). As a positive control and to verify transfection efficiency, separate plates were transfected with a β-gal expression plasmid (pβ-gal-Promoter, Clontech), that has the β-gal reporter sequence under the control of the SV40 early promoter (data not shown). This was done to avoid possible squelching of factors that could arise when cotransfecting multiple plasmids [23].

To further confirm the functionality of the promoter, we tested whether the promoter would respond to cytokines like the endogenous ADAMTS-4 gene [24]. Bovine articular chondrocytes were transfected with the ADAMTS-4 promoter-β-gal construct or the promoterless vector pβ-gal-Basic, and were subsequently treated with IL-1α and oncostatin M, either alone or in combination, for a period of 24 h. As shown in Fig. 2B, both cytokines showed some induction of ADAMTS-4 promoter activity on its own, but had a synergistic effect when used in combination. This is reflective of the synergism observed in inducing endogenous ADAMTS-4 expression [24] and is suggestive of the presence of functional elements in the promoter that mediate responsiveness to known catabolic factors.

Transactivation of the ADAMTS-4 promoter by NFATp

NFAT proteins are known to bind preferentially to the purine-rich core sequence (A/T)GGAAAA [18]. The presence of two putative NBEs in the 4.5 kb ADAMTS-4 promoter, combined with the suggested role of NFATp in chondrogenesis and cartilage degradation [9,25,26], prompted us to analyze whether ADAMTS-4 is one of the transcriptional targets of NFATp. In order to test the ability of NFATp to transactivate the ADAMTS-4 promoter, COS1, C3H10T1/2, and SW1353 cells were cotransfected with the 4.5 kb Aggr1-β-gal construct along with either the NFATp expression construct (pEF-NFATp) or the empty vector (pEF/myc/cyto). Cotransfections with the promoterless β-gal construct (pβ-gal-Basic) served as a negative control. Overexpression of NFATp led to a 20-fold increase in ADAMTS-4 promoter activity in COS1 cells, a 46-fold in C3H10T1/2 cells, and a 3.5-fold increase in SW1353 cells, when compared to empty vector (pEF/myc/cyto) control (Fig. 3A–C). Transactivation of the promoter by NFATp in multiple cell types suggests ADAMTS-4 as a downstream target of NFATp action.

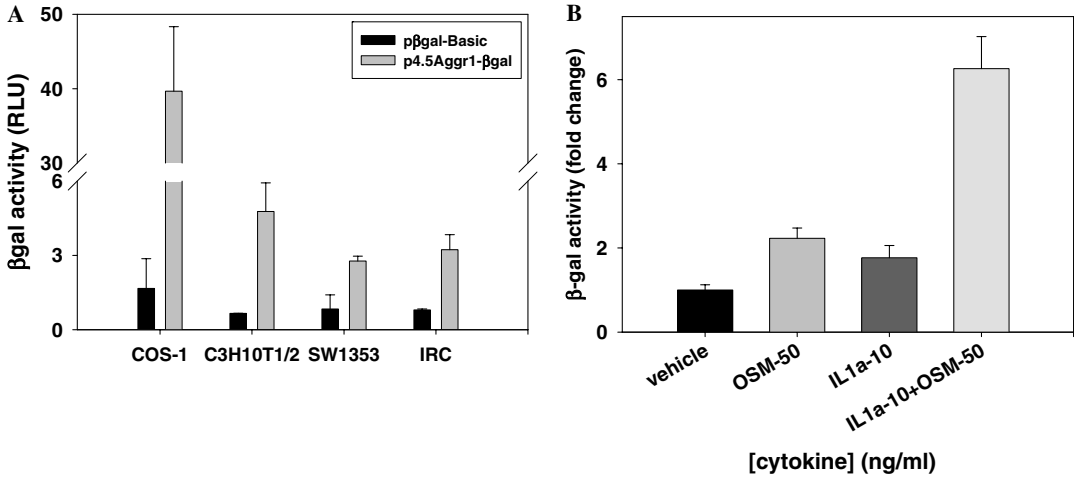

Fig. 2. Functionality of the ADAMTS-4 promoter in fibroblastic, mesenchymal, and chondrosarcoma cell lines. (A) The p4.5Aggr1-β-gal construct was transfected into COS1, C3H10T1/2, and SW1353 cell lines. β-gal activity in cell extracts, that is indicative of basal promoter activity, was measured ~48 h after transfection. Three independent transfection experiments were done in triplicate and the mean ± standard error of β-gal activity from a representative experiment is shown. (B) Bovine articular chondrocytes were transfected with p4.5Aggr1-β-gal construct and were subsequently treated with vehicle, IL-1α (10 ng/ml), oncostatin M (OSM, 50 ng/ml), or a combination of the two cytokines. β-gal activity in cell extracts was measured 24 h post-cytokine treatment.

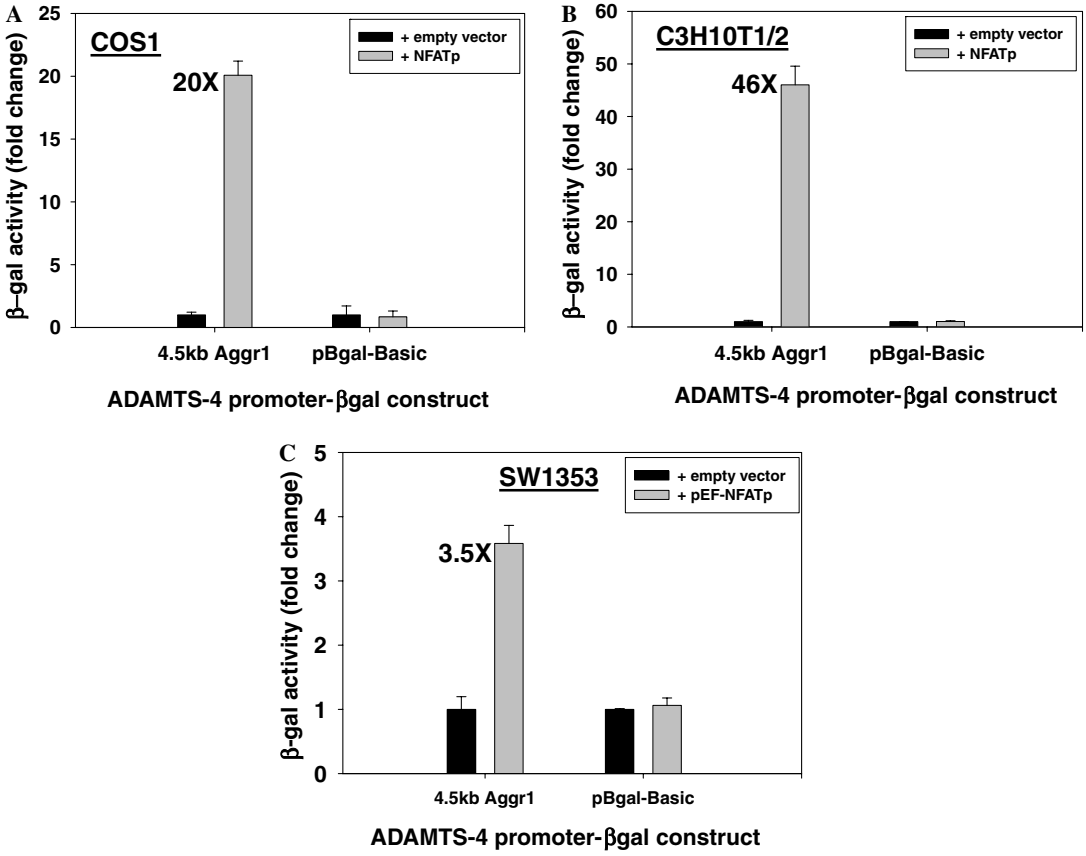

Fig. 3. Transactivation of the ADAMTS-4 promoter by NFATp. The p4.5Aggr1-β-gal construct was cotransfected along with either the NFATp expression construct (pEF-NFATp) or the empty vector (pEF/myc/cyto) into COS1 (A), C3H10T1/2 (B), and SW1353 cells (C), and the β-gal activity was measured in cell extracts. Values represent the fold increase in β-gal activity (means ± standard error) in NFATp-transfected cell extracts compared to that in empty vector-transfected cell extracts.

Transactivation of the ADAMTS-4 promoter by Runx2

The transcription factor Runx2 plays a crucial role in chondrocyte maturation and osteoblast differentiation [10–13]. The presence of 8 putative binding sites for the Runx family of transcription factors in the ADAMTS-4 promoter (Fig. 1 and Table 1) suggested a possible role for regulation of this promoter by Runx2. To determine whether ADAMTS-4 could be a potential downstream target of Runx2, cotransfection experiments similar to those with the NFATp expression construct were performed using a Runx2 expression construct (pEF-Runx2) in COS1 fibroblastic cells and in SW1353 human chondrosarcoma cells. Runx2 overexpression led to a 13-fold increase in ADAMTS-4 promoter activity in COS1 cells and a 24-fold increase in SW1353 cells (Fig. 4A and B), suggestive of a role for Runx2 in ADAMTS-4 transcriptional regulation.

Effect of sequential 5′-deletions in the ADAMTS-4 promoter on NFATp and Runx2 transactivation

In order to assess the involvement of the different NFATp- and Runx-binding elements in mediating transactivation and to assess the relative contribution of the various elements (and the promoter regions in which they occur) to overall promoter activity, we generated five sequential 5′-deletion constructs that contain varying numbers of consensus NBE- and Runx-binding elements (Fig. 5).

Analysis of NFATp transactivation of the promoter deletions in COS1 and C3H10T1/2 cells demonstrated that the two large promoter fragments (−4109 to +406 and −3109 to +406) that include the upstream NBE were robustly transactivated by NFATp (Fig. 6A and B). However, deletion of the upstream NBE-containing region led

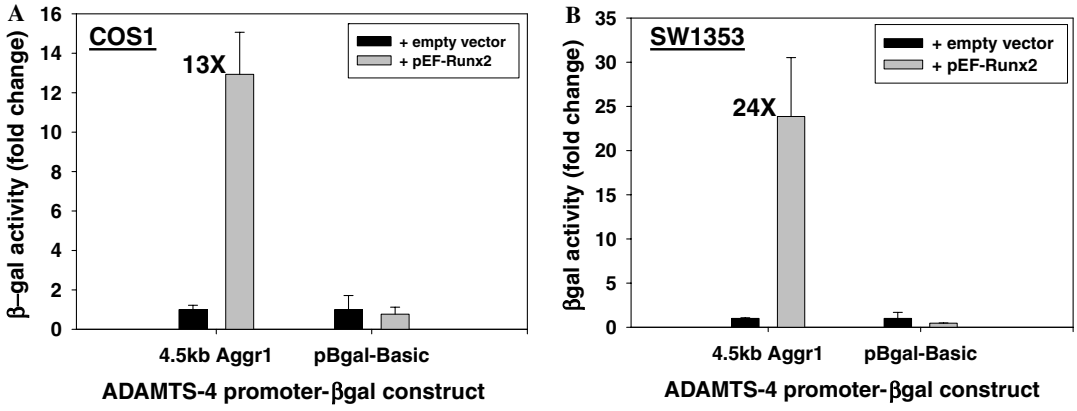

Fig. 4. Transactivation of the ADAMTS-4 promoter by Runx2. The p4.5Aggr1-β-gal construct was cotransfected along with either the Runx2 expression construct (pEF-Runx2) or the empty vector (pEF/myc/cyto) into COS1 (A) and SW1353 cells (B) and the β-gal activity in cell extracts was measured ~48 h post-transfection.

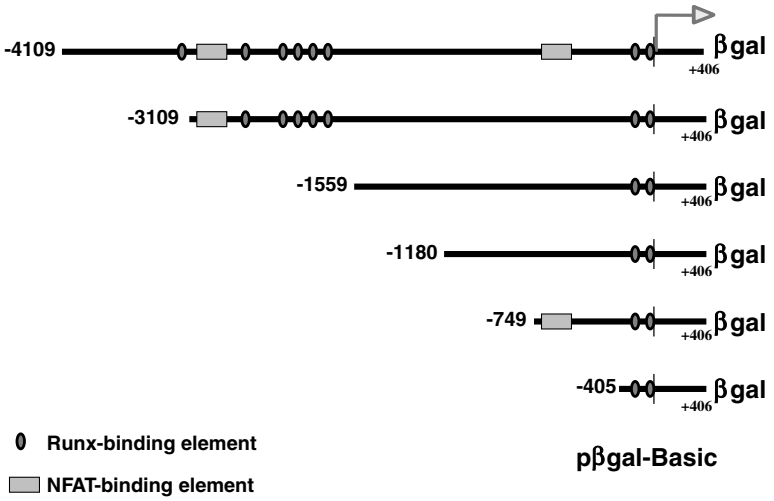

Fig. 5. Schematic representation of sequential 5′-deletions of the ADAMTS-4 promoter. 5′-Deletion fragments of the ADAMTS-4 promoter were generated by PCR and were ligated upstream of the β-gal reporter gene in the vector pβ-gal-Basic. The locations of the NBE- and Runx-binding elements are shown.

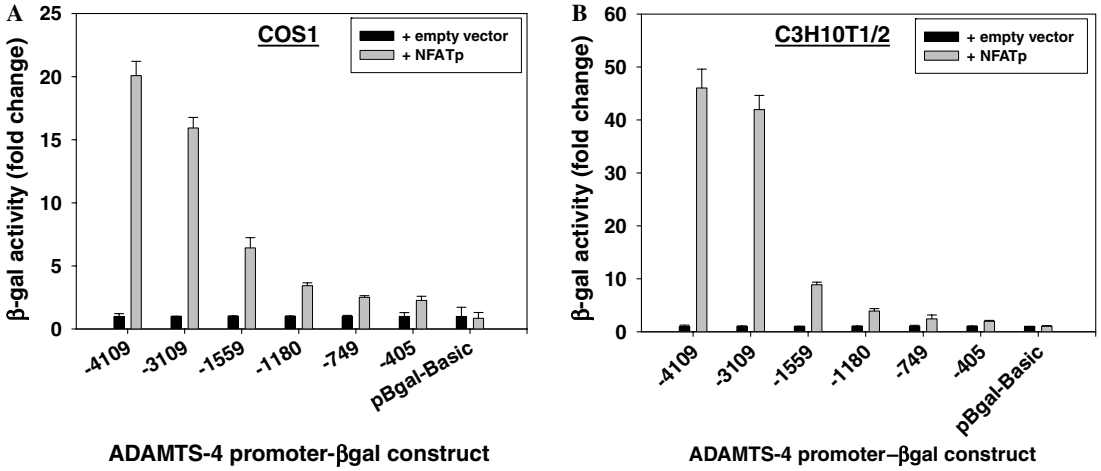

Fig. 6. Effect of 5′-deletions in the ADAMTS-4 promoter on NFATp transactivation. The ADAMTS-4 promoter deletion constructs were cotransfected with either pEF-NFATp or pEF/myc/cyto into COS1 (A) and C3H10T1/2 cells (B) and the β-gal activity in cell extracts was measured ~48 h post-transfection.

to a substantial loss in NFATp transactivation, suggesting the involvement of the upstream region in NFATp regulation of the promoter (Fig. 6A and B).

With regard to Runx2 transactivation, the promoter deletions showed minimal effect in COS-1 cells, whereas in SW1353 cells, there was some decrease in transactivation upon removal of the upstream promoter regions (Figs. 7A and B). However, in both cell lines, the shortest promoter fragment (−405 to +406) still showed substantial Runx2 inducibility (8-fold over control) (Fig. 7A and B) suggestive of the involvement of the proximal promoter region in mediating Runx2 effects.

Overexpression of Runx2 increases endogenous ADAMTS-4 mRNA levels in SW1353 cells

Since Runx2 overexpression resulted in a robust stimulation of ADAMTS-4 promoter activity, we analyzed whether Runx2 would up-regulate the transcriptional activity of the endogenous ADAMTS-4 gene. We utilized a Runx2-overexpressing adenovirus [16] to infect SW1353 cells and a GFP-overexpressing adenovirus as a control. Real-time PCR analysis showed a substantial (14-fold) increase in ADAMTS-4 mRNA levels in Runx2-overexpressing cells (Fig. 8). We also measured the mRNA levels of MMP-13, a known transcriptional target of Runx2 [19,21], to serve as a positive control, and observed a 16-fold increase in the levels of MMP-13 mRNA (Fig. 8). These observations implicate the ADAMTS-4 gene to be a downstream transcriptional target of Runx2.

Discussion

Here, we have reported the isolation and characterization of a 4.5 kb human ADAMTS-4 promoter. The activity of the promoter in fibroblastic, mesenchymal, and chondrosarcoma cell lines is in good agreement with the expression of the gene in multiple cell types [6,27]. The response

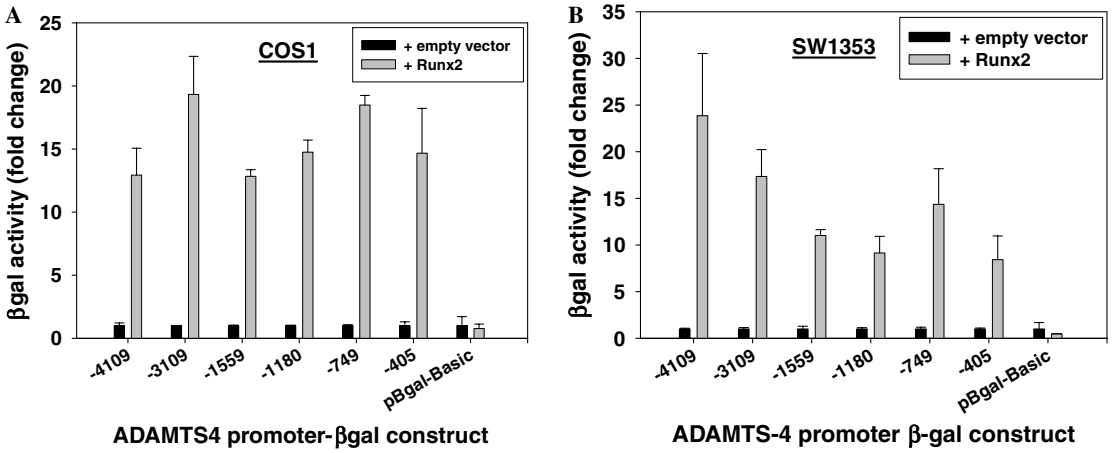

Fig. 7. Effect of 5′-deletions in the ADAMTS-4 promoter on Runx2 transactivation. The ADAMTS-4 promoter deletion constructs were cotransfected with either pEF-Runx2 or pEF/myc/cyto into COS1 (A) and SW1353 cells (B) and the β-gal activity was measured in cell extracts ~48 h post-transfection.

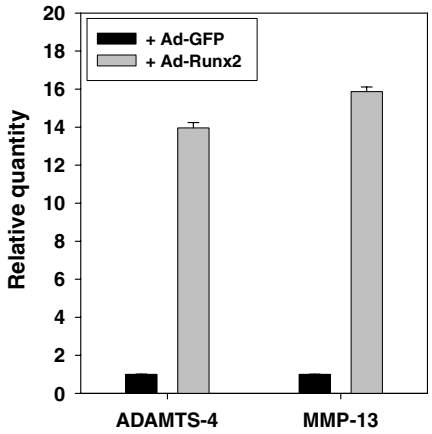

Fig. 8. Overexpression of Runx2 increases endogenous ADAMTS-4 mRNA levels in SW1353 cells. SW1353 cells were infected with an adenovirus-overexpressing Runx2 (Ad-Runx2) or GFP (Ad-GFP: control). Messenger RNAs were isolated ~48 h post-infection and analyzed by real-time PCR. Twenty-seven nanograms mRNA was used per reaction and all genes were analyzed in triplicate using assay-on-demand primer-probe sets (Applied Biosystems). The relative quantities of each gene were determined by the $\Delta\Delta C_t$ method.

of the promoter to catabolic cytokines in a fashion similar to the endogenous ADAMTS-4 gene suggests that we have cloned a functional promoter that would potentially be regulated by factors that modulate endogenous gene activity. Consistent with the presence of putative binding sites for chondrocyte-expressed transcription factors, the ADAMTS-4 promoter was transactivated by NFATp and Runx2.

Sequential 5′-deletions in the promoter led to a gradual loss of transactivation by NFATp, implicating the involvement of the distal promoter region in mediating responsiveness to NFATp. NFATp transactivation could be mediated directly by the NBE sites in the promoter or it could reflect an indirect effect of NFATp on ADAMTS-4 promoter activity. Indirect activation could occur either via upregulation of other transcription factors that in turn bind to cognate sites in the promoter and activate transcription, or via interaction of NFATp with other promoter-binding factors that in turn drive promoter activity.

In NFATp knockout mice, cells in the extra-articular tissues spontaneously differentiate to form cartilage [9] suggesting a role for NFATp in cartilage regeneration. In addition, our studies implicate a role for NFATp in catabolic processes in cartilage via the upregulation of ADAMTS-4 gene expression. Further evidence for a role for NFAT proteins in ADAMTS-4 gene regulation comes from studies by Little et al. [25] showing that cyclosporin A blocks the IL-1α-stimulated increase in ADAMTS-4 expression and cartilage degradation. Taken together, these data would suggest the possibility that inhibition of NFATp expression/function may have protective effects on the cartilage matrix in addition to potential reparative effects.

Tomita et al. [26] have shown that NFAT4 induces chondrogenic differentiation of a mesenchymal cell line via the upregulation of BMP2 expression. The NBE we have identified in the ADAMTS-4 promoter is a consensus binding site for all members of the NFAT family. Although we have focused our studies on NFATp because of the reported role of NFATp in chondrocyte biology, it would be interesting to test whether NFAT4 affects ADAMTS-4 gene expression.

Consistent with the presence of multiple Runx-binding elements in the human ADAMTS-4 promoter, we have shown that the promoter is transactivated by Runx2. Sequential 5′-deletion analysis showed that the shortest proximal promoter fragment (−405 to +406) that harbors two putative Runx-binding elements still retains Runx2 responsiveness. Previous studies have demonstrated the ability of Runx2 to bind to Runx-binding sequences identical to the ones reported here [20–22], suggesting a role for one or more of the Runx-binding elements in mediating Runx2 responsiveness of the ADAMTS-4 promoter. However, based on our studies, we cannot rule out an indirect effect of Runx2 on ADAMTS-4 transactivation.

Concomitant with the activation of the ADAMTS-4 promoter, overexpression of Runx2 also resulted in a significant increase in ADAMTS-4 mRNA levels in SW1353 cells, implicating ADAMTS-4 as a downstream target of Runx2 transactivation. Runx2 is known to play a role in chondrocyte maturation and hypertrophy [12,19,21,28], and Runx2 overexpression is associated with cartilage degeneration [19]. This, combined with the potential role of Runx2 in stimulating ADAMTS-4 gene expression, would suggest Runx2 as a potential therapeutic target for the inhibition of degenerative changes associated with OA.

In summary, we have cloned and characterized a 4.5 kb human ADAMTS-4 promoter containing functional response elements to specific transcriptional regulators. Our studies provide evidence for the involvement of NFATp and Runx2 in the regulation of ADAMTS-4 gene transcription and suggest that inhibition of the expression/function of NFATp and/or Runx2 may enable us to modulate aggrecan degradation. This promoter would serve as a valuable mechanistic tool to better understand the regulation of ADAMTS-4 expression by chondrotrophic factors and signaling pathways that modulate cartilage matrix breakdown. The promoter–reporter construct may also be used as a high-throughput screening tool to identify inhibitors of ADAMTS-4 expression.

References

[1] L.S. Lohmander, P.J. Neame, J.D. Sandy, The structure of aggrecan fragments in human synovial fluid. Evidence that aggrecanase mediates cartilage degradation in inflammatory joint disease, joint injury, and osteoarthritis, Arth. Rheum. 36 (1993) 1214–1222.

[2] W. Zeng, C. Corcoran, L. Collins-Racie, E. LaVallie, Z. Yang, E. Morris, C. Flannery, Comparative aggrecanolytic activities of ADAMTS-7, -9, -10, -16 & -18 versus ADAMTS-4 & -5, Trans. Orth. Res. Soc. (2005), poster# 1706.

[3] S.S. Glasson, R. Askew, B. Sheppard, B.A. Carito, T. Blanchet, H.L. Ma, C.R. Flannery, K. Kanki, E. Wang, D. Peluso, Z. Yang, M.K.

Majumdar, E.A. Morris, Characterization of and osteoarthritis susceptibility in ADAMTS-4-knockout mice, Arth. Rheum. 50 (2004) 2547–2558.
[4] S. Glasson, R. Askew, B. Sheppard, B. Carito, T. Blanchet, H. Ma, C. Flannery, D. Peluso, K. Kanki, Z. Yang, M. Majumdar, E. Morris, Deletion of active ADAMTS5 prevents cartilage degradation in a murine model of osteoarthritis, Nature 434 (2005) 644–648.
[5] A.M. Malfait, R.Q. Liu, K. Ijiri, S. Komiya, M.D. Tortorella, Inhibition of ADAM-TS4 and ADAM-TS5 prevents aggrecan degradation in osteoarthritic cartilage, J. Biol. Chem. 277 (2002) 22201–22208.
[6] H. Stanton, F. Rogerson, C. East, S. Golub, K. Lawlor, C. Meeker, C. Little, K. Last, P. Farmer, I. Campbell, A. Fourie, A. Fosang, ADAMTS5 is the major aggrecanase in mouse cartilage in vivo and in vitro, Nature 434 (2005) 648–652.
[7] B. Caterson, C.R. Flannery, C.E. Hughes, C.B. Little, Mechanisms involved in cartilage proteoglycan catabolism, Matrix Biology 19 (2000) 333–344.
[8] M.D. Tortorella, A.M. Malfait, C. Deccico, E. Arner, The role of ADAM-TS4 (aggrecanase-1) and ADAM-TS5 (aggrecanase-2) in a model of cartilage degradation, Osteoarth. Cartilage 9 (2001) 539–552.
[9] A.M. Ranger, L.C. Gerstenfeld, J. Wang, T. Kon, H. Bae, E.M. Gravallese, M.J. Glimcher, L.H. Glimcher, The nuclear factor of activated T cells (NFAT) transcription factor NFATp (NFATc2) is a repressor of chondrogenesis, J. Exp. Med. 191 (2000) 9–22.
[10] P. Ducy, R. Zhang, V. Geoffroy, A.L. Ridall, G. Karsenty, Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation, Cell 89 (1997) 747–754.
[11] M. Inada, T. Yasui, S. Nomura, S. Miyake, K. Deguchi, M. Himeno, M. Sato, H. Yamagiwa, T. Kimura, N. Yasui, T. Ochi, N. Endo, Y. Kitamura, T. Kishimoto, T. Komori, Maturational disturbance of chondrocytes in Cbfa1-deficient mice, Developmental Dynamics 214 (1999) 279–290.
[12] S. Takeda, J.-P. Bonnamy, M.J. Owen, P. Ducy, G. Karsenty, Continuous expression of Cbfa1 in nonhypertrophic chondrocytes uncovers its ability to induce hypertrophic chondrocyte differentiation and partially rescues Cbfa1-deficient mice, Genes Dev. 15 (2001) 467–481.
[13] C. Ueta, M. Iwamoto, N. Kanatani, C. Yoshida, Y. Liu, M. Enomoto-Iwamoto, T. Ohmori, H. Enomoto, K. Nakata, K. Takada, K. Kurisu, T. Komori, Skeletal malformations caused by overexpression of Cbfa1 or its dominant negative form in chondrocytes, J. Cell Biol. 153 (2001) 87–99.
[14] C. Luo, E. Burgeon, J.A. Carew, P.G. McCaffrey, T.M. Badalian, W.S. Lane, P.G. Hogan, A. Rao, Recombinant NFAT1 (NFATp) is regulated by calcineurin in T cells and mediates transcription of several cytokine genes, Mol. Cell. Biol. 16 (1996) 3955–3966.
[15] K. Thirunavukkarasu, D.L. Halladay, R.R. Miles, X. Yang, R.J.S. Galvin, S. Chandrasekhar, T.J. Martin, J.E. Onyia, The osteoblast-specific transcription factor Cbfa1 contributes to the expression of osteoprotegerin (OPG), a potent inhibitor of osteoclast differentiation and function, J. Biol. Chem. 275 (2000) 25163–25172.
[16] V. Krishnan, T.L. Moore, Y.L. Ma, L.M. Helvering, C.A. Frolik, K.M. Valasek, P. Ducy, A.G. Geiser, Parathyroid hormone bone anabolic action requires Cbfa1/Runx2-dependent signaling, Mol. Endocrinol. 17 (2003) 423–435.
[17] Y. Mizui, K. Yamazaki, Y. Kuboi, K. Sagane, I. Tanaka, Characterization of 5′-flanking region of human aggrecanase-1 (ADAMTS4) gene, Mol. Biol. Reports 27 (2000) 167–173.
[18] E. Serfling, F. Berberich-Siebelt, S. Chuvpilo, E. Jankevics, S. Klein-Hessling, T. Twardzik, A. Avots, The role of NF-AT transcription factors in T cell activation and differentiation, Biochim. Biophys. Acta 1498 (2000) 1–18.
[19] X. Wang, P.A. Manner, A. Horner, L. Shum, R.S. Tuan, G.H. Nuckolls, Regulation of MMP-13 expression by RUNX2 and FGF2 in osteoarthritic cartilage, Osteoarth. Cartilage 12 (2004) 963–973.
[20] M. Sato, E. Morii, T. Komori, H. Kawahata, M. Sugimoto, K. Terai, H. Shimizu, T. Yasui, H. Ogihara, N. Yasui, T. Ochi, Y. Kitamura, Y. Ito, S. Nomura, Transcriptional regulation of osteopontin gene in vivo by PEBP2aA/CBFA1 and ETS1 in the skeletal tissues, Oncogene 17 (1998) 1517–1525.
[21] M.J.G. Jimenez, M. Balbin, J.M. Lopez, J. Alvarez, T. Komori, C. Lopez-Otin, Collagenase 3 is a target of Cbfa1, a transcription factor of the runt gene family involved in bone formation, Mol. Cell. Biol. 19 (1999) 4431–4442.
[22] P. Ducy, G. Karsenty, Two distinct osteoblast-specific cis-acting elements control expression of a mouse osteocalcin gene, Mol. Cell. Biol. 15 (1995) 1858–1869.
[23] A. Farr, A. Roman, A pitfall of using a second plasmid to determine transfection efficiency, Nucleic Acids Res. 20 (1992) 920.
[24] M. Pratta, C.R. Hanning, T. Newman-Tarr, F. Wang, K. Steplewski, J.R. Connor, S. Slathia, M.R. West, L.S. Lohmander, S. Kumar, Synergistic stimulation of aggrecan degradation by IL-1a and oncostatin M in human articular cartilage explants is associated with the specific induction of ADAMTS-4 expression, Trans. Orth. Res. Soc. (2006), abstract #1469.
[25] C.B. Little, C.E. Hughes, C.L. Curtis, S.A. Jones, B. Caterson, C.R. Flannery, Cyclosporin A inhibition of aggrecanase-mediated proteoglycan catabolism in articular cartilage, Arthr. Rheum. 46 (2002) 124–129.
[26] M. Tomita, M. Reinhold, J.D. Molkentin, M. Naski, Calcineurin and NFAT4 induce chondrogenesis, J. Biol. Chem. 277 (2002) 42214–42218.
[27] I. Abbaszade, R.Q. Liu, F. Yang, S.A. Rosenfeld, O.H. Ross, J.R. Link, D.M. Ellis, M.D. Tortorella, M.A. Pratta, J.M. Hollis, R. Wynn, J.L. Duke, H.J. George, M.C. Hillman Jr., K. Murphy, B.H. Wiswall, R.A. Copeland, C.P. Decicco, R. Bruckner, H. Nagase, Y. Itoh, R.C. Newton, R.L. Magolda, J.M. Trzaskos, T.C. Burn, Cloning and characterization of ADAMTS11, an aggrecanase from the ADAMTS family, J. Biol. Chem. 274 (1999) 23443–23450.
[28] H. Enomoto, M. Enomoto-Iwamoto, M. Iwamoto, S. Nomura, M. Himeno, Y. Kitamura, T. Kishimoto, T. Komori, Cbfa1 is a positive regulatory factor in chondrocyte maturation, J. Biol. Chem. 275 (2000) 8695–8702.