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Ribozymes as Tools for Therapeutic Target Validation in Arthritis

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In this paper we describe a method for validating therapeutic gene targets in arthritic disease. Ribozymes are catalytic oligonucleotides capable of highly sequence-specific cleavage of RNA. We designed ribozymes that cleave the mRNA encoding stromelysin, a matrix metalloproteinase implicated in cartilage catabolism. Ribozymes were initially screened in cultured fibroblasts to identify sites in the mRNA that were accessible for binding and cleavage. Accessible sites for ribozyme binding were found in various regions of the mRNA, including the 5′ untranslated region, the coding region, and the 3′ untranslated region. Several ribozymes that mediated sequence-specific and dose-dependent inhibition of stromelysin expression were characterized. Site selection in cell culture was predictive of in vivo bioactivity. An assay for measuring cartilage catabolism in rabbit articular cartilage explants was developed. Ribozymes inhibited IL-1-stimulated stromelysin mRNA expression in articular cartilage explants, yet failed to inhibit proteoglycan degradation. This indicated that up-regulation of stromelysin was not essential for IL-1-induced cartilage catabolism. Broad applications of this approach in therapeutic target validation are discussed. The Journal of Immunology, 2000, 165: 493–498.

A key factor in arthritic disease progression is the excessive degradation of articular cartilage leading to loss of joint function (1). In the past, considerable attention has been focused on the role of particular matrix metalloproteinases (MMPs),2 which are overexpressed in diseased joints relative to normal joints (2). In particular, stromelysin-1 expression is highly elevated in osteoarthritis and rheumatoid arthritis patients and has been the focus of extensive drug discovery efforts (3, 4). Of course, expression levels alone do not necessarily define cause vs effect. Knowledge of which factors actually play a causative role in cartilage degradation would provide a powerful advantage in arthritis drug discovery.

Ribozymes are catalytic oligonucleotides that bind and cleave specific RNA sequences (5). Numerous ribozyme motifs have been found in nature, and additional catalytic motifs have been identified via in vitro selection (6, 7). The hammerhead ribozyme motif can be engineered to cleave an RNA substrate in trans, making it a useful tool for selective inhibition of specific mRNAs (6, 8). The hammerhead is also the smallest naturally occurring ribozyme motif, thereby making it particularly amenable to chemical synthesis.

Synthetic hammerhead ribozymes have been reported to modify target mRNA levels and/or alter cellular phenotype in a number of mammalian cell culture systems (9–12). Beigelman et al. (13) have shown that appropriate nucleotide modifications of chemically synthesized hammerhead ribozymes result in highly nucleoside-resistant molecules that retain catalytic activity. These and other modifications have been shown to be efficacious in cell culture (10) and in vivo (14, 15, 24, 25).

In this paper, we describe a systematic method for identifying efficacious ribozymes against stromelysin-1 (MMP-3) using a rapid cell culture screen to identify accessible sites for ribozyme binding and cleavage. We show that ribozyme site selection in cell culture is predictive of activity in a rabbit knee model of arthritis. Using rabbit articular cartilage explants, we show that ribozymes are readily taken up by chondrocytes and mediate inhibition of IL-1-induced stromelysin expression. The complete lack of concomitant inhibition of cartilage catabolism indicates that stromelysin is not a major contributor to cartilage degradation in this model. Based on this analysis, specific stromelysin inhibitors may not be optimal therapeutics in inflammatory arthritis. The potential for broad applications of this technique in therapeutic target validation are discussed.

Materials and Methods

Ribozyme synthesis

Ribozymes were synthesized and purified as described by Wincott et al. (16). Sequences and modifications were as described by Flory et al. (14). Site numbers indicate the position of cleavage based on the human sequence (GenBank accession no. X05232). The 5′-untranslated region (UTR) extends from 1 to 43, the coding region from 44 to 1477 and the 3′-UTR from 1478 to 1801.

Cell culture assays

HS-27 human foreskin fibroblasts were obtained from American Type Culture Collection (Manassas, VA) and were cultured in DMEM (BioWhittaker, Walkersville, MD) supplemented with 10% FBS (HyClone, Logan, UT). Cell assays were performed between passages 19 and 30. Ribozyme uptake was enhanced by complexing with Lipofectamine (Life Technologies, Rockville, MD) as described previously (10). Cells were seeded in 24-well tissue culture plates at 50,000 cells/well. After 24 h, cells were washed two times with Dulbecco’s phosphate-buffered saline (DPBS)1 and were treated with ribozymes complexed with Lipofectamine in serum-free DMEM. After 3 h, ribozyme/lipid complexes were removed, and 1 ml DMEM plus 10% FBS and 50 U IL-1 (IL-1α, Genzyme, Cambridge, MA) was added to each well. IL-1 stimulation resulted in a 5- to 10-fold increase in stromelysin expression. Time course studies showed that stromelysin mRNA expression reached maximal levels at 12 h after IL-1

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1 Abbreviations used in this paper: MMP, matrix metalloproteinase; UTR, untranslated region; DPBS, Dulbecco’s phosphate-buffered saline.

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stimulation and that secreted stromelysin protein reached maximal levels at 18 h after IL-1 stimulation (data not shown). IL-6 secretion was strongly induced after IL-1 stimulation. Supernatants were harvested at 18 h after IL-1 stimulation and stored at −70°C before ELISA analysis. Human synovial fibroblasts derived from arthritis patients undergoing joint replacement surgery were obtained from Dr. V. Baragi (Parke-Davis Pharmaceuticals) and were assayed as described for HS-27 cells.

**Stromelysin and IL-6 ELISA**

Stromelysin protein levels were quantified by sandwich ELISA. A mAb I4D IIB-specific for human stromelysin was obtained from Drs. L. Jack Windsor and Jeffrey Engler (University of Alabama, Tuscaloosa, AL). Unless otherwise indicated, the buffer used was DPBS, 0.1% BSA, 0.05% Tween 20, and all incubations were conducted at 37°C for 1 h with four 200-μl washes between steps. 1) Immulon I plates (Dynatech Laboratories, Chantilly, VA) were coated with 100 μl of 1 μg/ml IID4 mAb (capture Ab) in 100 mM Na-carbonate buffer (pH 9.0) overnight at 4°C. 2) Wells were blocked with DPBS, 1.0% BSA. 3) One hundred microliters of sample or standards diluted in DPBS/0.1% BSA was added to the coated wells. 4) One hundred microliters of a 1/500 dilution of goat anti-rabbit IgG (H+L)-AP (The Jackson Laboratory, Bar Harbor, ME) was added. 6) One hundred microliters of 1 ng/ml p-nitrophenyl phosphate in 100 mM diethanolamine (pH 9.5), 5 mM MgCl2 was added. Absorbance was read at 405 nm. 7) One hundred microliters of IID4 IIB-specific streptavidin-AP (capture Ab) was added as a standard. 8) As a negative control, a gift was a gift from Dr. V. Baragi. Purified IL-6 protein was obtained from R&D Systems (Minneapolis, MN). The threshold of sensitivity was ≤40 pg/ml human stromelysin. IL-6 protein levels were quantified by sandwich ELISA. The protocol was identical with that of the stromelysin sandwich ELISA, with the following modifications: all incubations were conducted at room temperature. 1) One hundred microliters of IID4 mAb (Biogenesis, Bournemouth, U.K.; #5980-0307) was added to the wells. 5) One hundred microliters of a 1/500 dilution of rabbit anti-β-actin IgG (H+L)-L-AP (The Jackson Laboratory, Chantilly, VA) were coated with 100 μl of 1 mg/ml β-actin antibody in 0.2 M HEPES buffer (pH 8.0). 6) One hundred microliters of 1 mg/ml L)-AP (The Jackson Laboratory, Bar Harbor, ME) was added. 7) One hundred microliters of a 1/500 dilution of goat anti-rabbit IgG (H+L)-L-AP (The Jackson Laboratory, Bar Harbor, ME) was added. After the incubation, the cartilage fragments were washed in saline, embedded in PolyFreeze, and snap frozen in liquid nitrogen. Twelve-micro- meter frozen sections were prepared on a freezing microtome and then photographed on a Leica microscope equipped with epifluorescence.

**RNA extraction from articular cartilage explants**

Total cartilage from each rabbit knee was homogenized in 5 ml Trizol (Life Technologies; phenol and guanidine isothiocyanate) for 60 s. Samples were incubated at 22°C for 30 min, and then 1 ml chloroform was added, samples were shaken vigorously for 15 s and centrifuged at 4500 × g for 20 min at 4°C, and the aqueous phase was removed to a fresh tube. Phenol:chloroform:isoamyl alcohol (25:24:1) was added (2.25 ml), the samples were centrifuged at 12,000 × g for 12 min at 4°C, and the aqueous phase was removed to a fresh tube. Isopropanol was added (2.5 ml), and samples were placed at −20°C for 1–24 h. Samples were then centrifuged at 12,000 × g for 12 min at 4°C, the supernatant was removed, pellets were washed with 75% EtOH and recenterfuged, the supernatant was removed, and the pellets were air dried. Stromelysin RNA levels were measured by Northern analysis as described (14).

**Statistical methods**

Statistical analysis was performed using JMP software version 3.1 (SAS Institute, Cary, NC). The cartilage explant data were analyzed by first verifying that they were normally distributed before a one-way ANOVA. Group means were then compared using either Dunnett’s (against control, α = 0.05) or Tukey-Kramer (against each other, α = 0.05) post hoc tests as indicated in figures.

**Results**

**Site selection in cultured human fibroblasts**

The hammerhead ribozyme consensus sequence occurs frequently in a typical mRNA, but only a limited number of sites are expected to be accessible to intracellular ribozyme binding, owing to RNA secondary structure and protein binding. Thus, we elected an empirical approach to assess the relative cleavage activity of stromelysin ribozymes targeting a panel of hammerhead cleavage sites. The panel was chosen based on homology between human and rabbit stromelysin mRNA sequences and on the predicted propensity of the ribozyme to fold into a catalytically active conformation (18). Ribozymes targeting 24 cleavage sites in human stromelysin mRNA, including sites in the coding and UTRs, were synthesized using nucleotide modifications that confer resistance to nucleolytic degradation (13). All of the ribozymes showed catalytic cleavage activity against a matched substrate in test tube cleavage assays (data not shown). The ribozyme panel was tested for inhibition of stromelysin mRNA secrletion in IL-1-stimulated HS-27 human foreskin fibroblasts (data not shown). Based on this analysis, ribozymes targeting the most accessible sites were selected for further study.

**Mechanism of inhibition, specificity, and dose dependence**

Active ribozymes were tested in parallel with inactive controls containing identical binding arms but with mutations in the catalytic core known to impair cleavage activity. As seen in Fig. 1A, active ribozymes targeting sites 21, 1049, 1363, 1366, and 1489 showed inhibition of stromelysin secretion relative to their inactive controls, demonstrating that the inhibition was mediated by ribozyme cleavage. Inhibition by the ribozyme targeting site 1410 was not statistically different from control stromelysin levels. The ribozyme targeting site 883 was ineffective in the initial screens, probably due to lack of binding site accessibility, and was included here as a negative control. In addition, ribozymes targeting human sites 463 and 1403 also showed efficacy in cell culture relative to their respective inactive controls (data not shown). These ribozymes were not pursued due to lack of homology with rabbit...
stromelysin. Interestingly, efficacious ribozymes were identified at sites within the 5′-UTR (site 21), the coding region (sites 463, 1049, 1363, 1366, and 1403), and the 3′-UTR (site 1489).

IL-1-induced IL-6 secretion was measured as an additional control for specificity to ensure that active ribozymes were not simply causing inhibition of secretion or general cytotoxicity. At this dose, there was little effect of the ribozyme treatment on IL-6 induction and no statistical difference between active vs inactive ribozymes (Fig. 1B). Thus, the ribozyme effect appears to be specific for stromelysin expression.

The ribozyme targeting site 1366 was further tested in dose-response experiments. As shown in Fig. 2, the active ribozyme inhibited stromelysin expression in a dose-dependent manner, with an IC50 of ~240 nM. Again, the inactive control did not inhibit stromelysin expression, indicating that the inhibition was mediated by ribozyme cleavage of the target RNA. This ribozyme was also tested against three isolates of human synovial fibroblasts obtained from osteoarthritis patients undergoing joint replacement surgery and showed an IC50 ranging from 250 to 400 nM (data not shown). Stromelysin RNA levels in IL-1-stimulated human synovial fibroblasts treated with ribozyme were measured using an RNase protection assay, and a good correlation was observed between the level inhibition at the protein and RNA level (data not shown).

Correlation between efficacy in cell culture and in vivo

We have previously shown that intraarticular administration of ribozymes results in uptake into synovial fibroblasts and a tissue half-life of ~3 days (14). Furthermore, we have observed ribozyme-mediated inhibition of stromelysin mRNA expression in rabbit synovium in an IL-1-induced arthritis model using several of the ribozymes described here. Table I shows a comparison between cell culture and in vivo efficacy. Three of the four sites that show efficacy in cell culture (1049, 1363, and 1366) also show efficacy in vivo. These three sites are all in well-conserved parts of the coding region (≥84% homology at the nucleotide level in the surrounding region). To assess the predictive capability of the cell culture site selection screen, it is also important to evaluate the in vivo performance of ribozymes that failed in the cell culture screen. In this case, the correlation also holds, with site 883 showing negative results in cell culture and in vivo. In addition, one other ribozyme site that gave negative results in cell culture was tested in vivo and also failed to show efficacy (data not shown). Site 1410 showed marginal inhibition (not statistically significant) both in cell culture and in vivo.

The only exception to the correlation between cell culture and in vivo results was with the ribozyme targeting site 1489. This ribozyme showed efficacy in human cell culture but not in the rabbit model. This site resides in the poorly conserved 3′-UTR (only 66% homology in the surrounding region), raising the possibility that the mRNA in that region adopts a different secondary structure in the rabbit mRNA compared with the human mRNA, rendering the site inaccessible to ribozyme binding. In addition, three base changes were required in the binding arms of the ribozyme itself to make the rabbit-specific version, thus raising the possibility that the rabbit-specific ribozyme adopts a less favorable conformation compared with the human-specific ribozyme.

The role of stromelysin in cartilage catabolism

Ribozymes targeting sites 1049 and 1366 were tested for their ability to inhibit IL-1-induced loss of cartilage proteoglycan in the rabbit knee arthritis model. No efficacy was observed, despite reductions in stromelysin mRNA levels in the synovium (data not shown). Interpreting this result with respect to the role of stromelysin in cartilage catabolism was complicated by delivery issues. After intraarticular administration into healthy rabbit knees, synthetic ribozymes were readily taken up by synovial fibroblasts, but penetration into cartilage was extremely low (Fig. 3, a and b). Similar results were observed with radiolabeled ribozyme (data not shown). Using in situ hybridization, we confirmed literature reports (19) that both synovial fibroblasts and cartilage chondrocytes express stromelysin RNA in response to IL-1 (data not shown). Thus, although stromelysin could originate from either tissue, ribozymes were only present in the synovium. Interestingly, similar fluorescent ribozyme studies in a rabbit partial meniscectomy injury model show ribozyme uptake by cartilage chondrocytes in osteoarthritic-like lesions on the articulating surface (C. M. Flory and R. Moskowitz, unpublished data). This indicates that the compromised cartilage surface in a disease state allows ribozyme penetration.

To address unambiguously the role of stromelysin in IL-1-induced cartilage catabolism, we utilized a cartilage explant model.
In contrast to the impermeable barrier presented by a healthy articulating surface, cultured articular cartilage explants take up ribozymes readily, as shown in Fig. 3, c and d. Comparison of the 4-h and 24-h samples suggests that uptake progresses via diffusion of the ribozyme from the cut edge of the cartilage.

Cartilage explants were cultured in serum-free DMEM and treated with IL-1 to induce cartilage catabolism. Cartilage samples were analyzed for RNA content before and after IL-1 stimulation as shown in Fig. 4A. The active stromelysin ribozyme targeting site 1366 clearly inhibited the IL-1-induced stromelysin mRNA expression, whereas the inactive control did not. It is interesting to note that efficacy was observed in this ex vivo model with free ribozyme (i.e., no lipid formulation). Cell culture systems generally require the use of a cationic lipid vehicle to enhance cellular uptake of oligonucleotide, as used in Figs. 1 and 2. The same ribozymes injected without a lipid vehicle in vivo were taken up into synovial cells and demonstrated down-regulation of the target RNA (see Flory et al. (14) and Table I). This may reflect differences in oligonucleotide uptake pathways in vitro vs in vivo. The freshly harvested cartilage explants may represent an intermediate state that more closely mimics in vivo uptake conditions.

Table I. Correlation between cell culture and in vivo efficacy of stromelysin ribozymesa

<table>
<thead>
<tr>
<th>Ribozyme Site</th>
<th>Inhibition in Human Cell Culture</th>
<th>Inhibition in Rabbit Synovium</th>
<th>Binding Site Homology</th>
<th>Context Homology (50 nt)</th>
</tr>
</thead>
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<td>883</td>
<td>No</td>
<td>No</td>
<td>100%</td>
<td>78%</td>
</tr>
<tr>
<td>1049</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>90%</td>
</tr>
<tr>
<td>1363</td>
<td>Yes</td>
<td>Yes</td>
<td>100%</td>
<td>84%</td>
</tr>
<tr>
<td>1410</td>
<td>No</td>
<td>No</td>
<td>100%</td>
<td>92%</td>
</tr>
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<td>Yes</td>
<td>93%</td>
<td>86%</td>
</tr>
<tr>
<td>1489</td>
<td>Yes</td>
<td>No</td>
<td>79%</td>
<td>66%</td>
</tr>
</tbody>
</table>

a Human sequence-specific ribozymes were tested in an IL-1-stimulated human foreskin fibroblast model in culture, and inhibition of stromelysin protein expression was measured by ELISA (Figs. 1 and 2). Rabbit-specific versions of the ribozymes were tested in an IL-1-stimulated rabbit knee model of arthritis, and inhibition of stromelysin mRNA in synovium was measured by Northern analysis (14). Site homology refers to the nucleotide sequence conservation between human and rabbit stromelysin in the binding domain of the ribozyme. Context homology refers to the nucleotide sequence conservation between human and rabbit stromelysin in the 50-nt region surrounding the cleavage site. Sites 883, 1049, 1363, and 1410 target sites having complete homology between human and rabbit stromelysin, and therefore identical ribozymes were used in both the cell culture and in vivo experiments. In the case of sites 1366 and 1489, the human-specific versions were used for the human cell culture experiments, and rabbit-specific analogs were used in the in vivo experiments.

FIGURE 3. a. Uptake of rhodamine-conjugated ribozyme by rabbit synovium at 24 h after intraarticular injection into the knee (×400 magnification). b. Uptake of rhodamine-conjugated ribozyme by femoral condyle cartilage at 24 h after intraarticular injection into rabbit knee (×200 magnification). Uptake of rhodamine-conjugated ribozyme by chondrocytes in cultured articular cartilage explants at 4 h (c) and 24 h (d) postadministration (×200 magnification). In c and d, the cut surface is oriented to the left, and the intact articulating surface is to the right.
To assess catabolic activity in the explants, proteoglycan levels were measured in the culture medium and in cartilage tissue using a spectrophotometric assay. A statistically significant loss of proteoglycan could be measured in response to IL-1 stimulation, as shown in Fig. 4B. This IL-1-induced catabolism could be completely inhibited by a broad-spectrum synthetic hydroxamate MMP inhibitor, batimastat (BB-94) (20). Actinomycin D also inhibited IL-1-induced catabolism, indicating that the bulk of the catabolic activity resulted from transcriptionally induced gene expression rather than from posttranscriptional activation. Culturing in the absence of IL-1 but with exogenously added activated stromelysin protein resulted in dramatic proteoglycan loss. Further characterization of the explant assay showed that the basal level of catabolism in the absence of IL-1 could be reduced by culturing the cartilage for 24 h after tissue harvest and then washing to remove trauma-induced catabolic enzymes (data not shown).

Despite the fact that ribozyme treatment resulted in inhibition of the IL-1-induced stromelysin mRNA levels, we were not able to observe any ribozyme-mediated inhibition of IL-1-induced cartilage catabolism using a ribozyme targeting site 1366, as shown in Fig. 4C. Ribozymes targeting site 1049 were also tested and failed to inhibit cartilage catabolism (data not shown). Also, inhibition of cartilage catabolism was not observed with rhodamine-conjugated ribozyme or when 10-fold higher ribozyme concentrations were used or when ribozyme was delivered with LipofectAMINE (data not shown). The fact that ribozymes inhibited induction of stromelysin expression in IL-1-stimulated explants yet failed to inhibit catabolism suggests that induction of stromelysin expression is not a critical event in IL-1-stimulated proteoglycan loss in rabbit articular cartilage.

**Discussion**

By testing ribozymes targeting both accessible and inaccessible sites within the stromelysin message in cell culture and in vivo, we have established a clear correlation between inhibition of target RNA in cell culture and in vivo. Thus, cell culture screening provides a rapid method for identifying optimal ribozymes for target validation studies.

The ribozymes utilized in this study were highly specific for stromelysin-1 and, based on homology alignments and hammerhead ribozyme in vitro cleavage specificity, would not be expected to affect levels of related MMPs such as collagenase or stromelysin-2. In fact, the nucleotide sequences of stromelysin-1 and collagenase-1 are sufficiently different that only one hammerhead ribozyme site is conserved between the two RNAs; this site was not chosen for this study.

We have shown that IL-1-stimulated proteoglycan catabolism in cartilage explants can be inhibited by a broad-spectrum protease inhibitor (BB-94) as well as by an inhibitor of transcription (Actinomycin D). Despite the fact that ribozymes targeting stromelysin were able to inhibit IL-1-induced stromelysin mRNA expression, no inhibition of proteoglycan degradation was observed. In this explant model, basal stromelysin RNA expression (i.e., without IL-1 stimulation) is relatively high, and the increase in stromelysin RNA expression in response to IL-1 is modest (roughly a 30% increase) as shown in Fig. 4. Although the ribozyme treatment only reduced total stromelysin RNA by ~30%, this represents a virtually complete inhibition of IL-1-induced stromelysin expression. Because some stromelysin expression is still detectable after ribozyme treatment, we can’t rule out a role for this basal level of stromelysin in cartilage catabolism. However, it’s clear that inhibiting all new transcription with Actinomycin D eliminates IL-1-induced cartilage catabolic activity; in contrast, specifically inhibiting new stromelysin transcription with a ribozyme shows no effect on cartilage catabolism. This result focuses interest on other IL-1-induced activities in these explants. Aside from stromelysin, there must be one or more genes that 1) are IL-1-induced at the transcriptional level (Actinomycin D result), 2) are themselves MMPs or have downstream catabolic consequences that are mediated through MMPs (BB94 result), and 3) are distinct from stromelysin (ribozyme result). It’s possible, for example, that the IL-1 treatment induces the expression of some unknown “Factor A” at the transcriptional level, which in turn activates the pro-form of constitutively present stromelysin protein, which then contributes to catabolic activity either through direct cleavage of cartilage or through activation of the pro-forms of other proteases. Thus, stromelysin could play a role in catabolism, yet its expression would not be the key IL-1-responsive activating event (played by “Factor A”) that initiates the catabolic cascade.

**FIGURE 4.** A, One micromolar active (1366A) and inactive (1366I) ribozymes targeting stromelysin site 1366 were added to the culture medium of fresh rabbit articular cartilage explants. After 24 h, IL-1 was added, and 6 h after IL-1 stimulation, RNA was extracted from the cartilage and stromelysin mRNA levels were determined by Northern analysis normalized to 18S rRNA. Error bars represent SEM for n = 3. B, Cartilage explants were cultured in the presence of 10 μg/ml aminophenylmercuric acetate-activated purified human stromelysin, 50 μM IL-1, IL-1 + 15 μM BB-94, or IL-1 + 5 μg/ml Actinomycin D (Sigma). The y-axis shows percent of proteoglycan remaining in the cartilage (amount in cartilage vs total amount in supernatant plus cartilage). Error bars represent SEM for n = 7. C, One micromolar active (1366A) and inactive (1366I) ribozymes were added to the culture medium of cartilage explants. Twenty-four hours later, IL-1 was added, and 48 h after IL-1 stimulation, proteoglycan release was measured. Error bars represent SEM for n = 6. * p < 0.05 vs +IL-1 control by Dunnett’s; ** p < 0.05 vs +IL-1 control by Tukey-Kramer.
Mudgett et al. (21) have recently reported that a stromelysin knockout mouse is susceptible to collagen-induced arthritis, another inflammatory arthritis model. This is consistent with the notion that stromelysin expression is not a critical factor in inflammatory arthritis. Although stromelysin does not appear to be an optimal therapeutic target in inflammatory arthritis, we cannot rule out a role for this MMP in osteoarthritis based on these studies. Osteoarthritis is a complex, multifactorial process of joint degeneration (22). The joint pathology differs histologically and biochemically between inflammatory arthritis models and more osteoarthritis-like injury models. Thus, different molecular targets may be appropriate for therapeutic intervention in each case.

Other methods for target validation include knockout animals, Abs, and specific small molecule inhibitors. In the case of stromelysin, the knockout mouse showed a similar phenotype to the ribozyme treatment. Arguably there are several advantages to the ribozyme approach, including the rapid timeframe and the ability to modulate the dose and magnitude of inhibition. This allows one to assess the degree to which gene expression must be inhibited to elicit the desired effect, which can be very important information for drug development. With Abs, technical hurdles include delivery to the appropriate target tissue, in this case cartilage matrix, as well as the timeframe to develop and characterize the Abs. It has been difficult to develop small molecule inhibitors that are truly selective for stromelysin-1 vs closely related MMPs (23). Ribozymes represent a rapid and specific method for inhibition of selective genes that are implicated in disease pathways. The resulting effect on phenotype (be it positive, negative, or neutral) provides a sensitive measure of the role of the gene in disease.

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11. Mudgett et al. (21) have recently reported that a stromelysin inhibitor blocks cartilage degradation in rabbits. Proc. Natl. Acad. Sci. USA 93:754.