EXPERIMENTAL and MOLECULAR MEDICINE, Vol. 30, No 2, 59-64, June 1998

Interferon-γ upregulates the stromelysin-1 gene expression by human skin fibroblasts in culture

Kyu-Suk Lee,[1,2] Young-Wook Ryoo[1] and Joon-Young Song[1]

1 Department of Dermatology, Keimyung University School of Medicine, Taegu 700-310, Korea

2 Corresponding author

Accepted 7 March 1998

Abbreviations: ECM, extracellular matrix; MMP, matrix metalloproteinase; IFN-γ, interferon-γ; TGF-β, transforming growth factor-β; CAT, chloramphenicol acetyl transferase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; TRE, tumor promoter responsive element

Abstract

The equilibrium between deposition and degradation of extracellular matrix(ECM) is essential to normal tissue developement and repair of wound or inflammatory responses. It has recently become apparent that several cytokines and growth factors are capable of modulating fibroblast proliferation and biosynthetic activity. To understand the role of these factors in connective tissue regulation, we examined the effect of interferon-γ (IFN-γ) on stromelysin-1 gene expres-sion in cultured human dermal fibroblasts. The steady-state levels of stromelysin-1 mRNA were increased in IFN-γ treated cultured dermal fibroblasts. In the CAT assay, the stromelysin-1 promoter activity was increased 2.8-fold compared with untreated control. Therfore IFN-γ stimulates the stromelysin-1 promoter activity, resulting in transcriptional enhance-ment of gene expression. Transforming growth factor-β (TGF-β) showed the antagonistic action to the effects of IFN-γ in cultured dermal fibroblasts. Furthermore, gel mobility shift assays demonstrated enhanced AP-1 binding activities in nuclear extracts from cells incubated with IFN-γ. These data suggest that IFN-γ is an up-regulator and TGF-β is a down regulator on the stromelysin-1 gene expression, respectively, and the AP-1 binding site may be necessary for gene response.

Keywords: stromelysin-1, interferon-γ, AP-1, gene expression

Introduction

The matrix metalloproteinase (MMP)familly, which includes collagenase (MMP-1), gelatinase (MMP-2), stromelysins (MMP-3), matrilysin and metalloelastase, were structurally related zinc-dependent proteolytic enzymes, yet specific activities are divergent (Woessner, 1990; McCachren, 1991; Mauviel, 1993). Stromelysin-1 and -2 are closely related, yet distinct from metalloproteinases, and both can degrade many non-collagenous connetive tissue macromolecules such as fibronectin, laminin, elastin, IgG, and proteoglycans (Chin *et al*,, 1985). Stromelysin-1-producing keratinocytes resided on the basement membrane, whereas stromelysin-2-producing keratinocytes were in contact with the dermal matrix (Saarialho-Kere *et al*., 1993). Furthermore, stromelysin-1 expression was prominent in dermal fibroblasts, whereas no signal for stromelysin-2 was observed in any dermal cells (Saarialho-kere *et al*., 1993). Beside participating in normal connective tissue homeostasis, development and remodeling, the proteolytic activity of matrix metalloproteinases contributes significantly to the tissue damage that occurs in chronic inflamatory disease, such as rheumatoid arthritis and osteoarthrtis (Pelletier *et al*., 1993), as well as tumor invasion (Martrisian, 1990).

Metalloproteinases are produced by multiple cell types and typically, their expressions are tightly regulated and limied to periods of active remodelling. The cell type-specific expression of metalloproteinases is regulated by various factors, including cell-matrix interaction, growth factors and cytokines, lipid mediator, tumor promotors, and inflammatory agent such as bacterial lipopolysaccharide (Saarialho-Kere *et al*., 1993). One of the cytokines previously shown to modulate the activity of MMP-1 and MMP-3 is interferon-γ (IFN-γ), a physiologic mediator synthesized and released primarily by helper T-lymphocytes (Sen, 1992). It has been demonstrated that IFN-γ decreases the activity of MMP-1 in rheumatoid synovial fibroblasts, human articular chondrocytes or human alveolar macrophages in culture (Andrew *et al*., 1990; Shaprio *et al*., 1990; Unemori *et al*., 1991). But the expression of MMP-1 and MMP-3 in cultured human skin keratinocytes cultures were found to be upregulated by the addition of IFN-γ. IFN-γ also suppressed lipopoly-saccharide-induced production of srtromelysin in human macrophage, whereas inhibition of tissue inhibitor of metalloproteinase synthesis required 50 to 100 fold higher concentrations of this cytokine (Shaprio *et al*., 1990). Thus, these previous studies suggest that IFN-γ affect MMP activities of a variety of different cell types *in vitro* culture systems. But, few

reports are available about the effect of this cytokine on MMP activities in skin dermal fibroblasts.

In this study, we have examined the expression of the stromelysin-1 and collagenase in cultured human dermal fibroblasts treated with IFN-γ and/or TGF-β.

Materials and methods

Cell Cultures

The cell line used in the present experiment was established from foreskin of infant. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum, penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericine B (1μg/ml) at 37˚C in a humidified atmosphere of 5% CO_2. Trypan blue test was used for checking cell viability. Confluent fibroblast in 100-mm petri dishes were treated with IFN-γ (Sigma Co., MO) or TGF-β (Genzyme Co., CA) in the concentrations indicated for 24 h.

Northern blot analysis

Total celluar RNA was isolated by the methods of Chomzynski and Sacchi from cultured normal skin fibroblasts. An equal amount of total RNA (25 μg) was fractionated by 1% agarose gel electrophoresis (100 volts, 5 h) after denaturating the samples with formaldehyde and formamide and stained with ethidium bromide (Whal *et al*., 1979). RNA transcripts obtained were transferred to Zeta probe filters (BioRad, Richmond, USA) by vacuum transfer and immobilized by heating at 80˚C for 30 min. Each filter was prehybridized for 12-18 h and subsequently hybridized for 24 h with cDNAs labeled by [^{32}P]dCTP (NEG 036H, New England Nuclear, Boston, USA) by nick translation (Rigby *et al*., 1977). Following hybridization, the filter was washed and autoradiography was performed (Thomas P., 1980). The following human-sequence-specific cDNAs were utilized this study: for stromelysin-1, a 1.5-kb stromelysin cDNA (Saus *et al*., 1988); for collagenase, a 2.0-kb collagenase cDNA (Goldberg *et al*., 1986); for glyceraldehyde-3-phosphate dehydrophate dehydrogenase (GAPDH); a 1.2-kb GAPDH cDNA (Fort *et al*., 1985).

Transient cell transfection and CAT assay

Human foreskin fibroblasts were transfected with 10 or 20 μg of construct DNA (ST-56/CAT), which contains 0.5 kb of 5'-flanking DNA of stromelysin-1 gene linked to the CAT reporter gene (Buttice *et al.*, 1991). This construct has been derived from a 560 bp XhoI/HindIII genomic subclone that spans from position -560 to +6 of the stromelysin-1 promoter (Saus *et al*., 1988).

Transfection was performed with the calcium phosphate/DNA co-precipitation method (Graham and Van der Eb, 1973), followed by a 1 min glycerol (15%) shock. After 40 h incubation with or without the IFN-γ or TGF-β, the cells were harvested and lysed by three cycles of freezing and thawing in 100 μl of 0.25 M Tris-HCl, pH 7.8. The protein concentration of each extract was determined with a protein assay kit (Bio-Rad) and the same amount (5-10 μg per assay) of protein from each cell extract was used for parallel determinations of CAT activity using [^{14}C]chroramphenicol as substrate (Gorman *et al*., 1982). The acetylated and non-acetlated forms of radioactive chloramphenicol were seperated by thin-layer chromatography and visualized by autoradiography. The enzyme activity was quantified by cutting out pieces of thin layer chromatography plates containg different forms of [^{14}C]chroramphenicol converted to its acetlated forms, after correction for β-galactosidase activity in each as a control of transfection efficiency (Sambrook *et al*., 1989).

Gel mobility shift assay

Nuclear proteins were isolated from cultured skin fibroblasts incubated with or without IFN-γ or TGF-β for 24 h using a small scale preparation technique (Andrews and Faller, 1991). For DNA binding assay, a 21-bp, 5'-CGCTT GATGAGTCCAGCCGGAA-3', double-stranded oligomer containing the stromelysin-1 AP-1 binding site was used. The end-labeled oligomer (total radioactivity per reaction, 5 X 10^4 cpm) was incubated with 8 μg of the nuclear extract for 30 min on ice in 20 μl of binding reaction buffer (12 mM HEPES, pH 7.9; 4 mM Tris, pH 7.9; 60 mM KCl; 1 mM EDTA; 1 mM dithiothreitol; 12% glycerol) in the presence of 0.2 μg of poly(dI-dC), and the DNA-protein complexs were fractionated on 4% polyacrylamide gel containing 0.4X Tris-borate-EDTA (TBE) buffer under non-denaturing conditions, as described previously (Tamai *et al*., 1994). As a competitor for the binding, a hundred-fold molar excess of the same oligomer was added to the binding reaction. The oligomer-protein complexes were visualized by autoradiography by exposure of the gels to X-ray films at -70˚C overnight.

Results

Effect of IFN-γ and TGF-β on stromelysin-1 mRNA expression

The expression of the stromelysin-1 and collagenase genes was examined in cultured fibroblasts by determining the steady-state levels of mRNA by Northern analysis. The size of stromelysin-1, collagenase, and GAPDH mRNA transcripts, was 2.2 kb, 2.4 kb, and 1.4 kb, respec-tively (Figure 1A). The levels of stromelysin-1 mRNAs were 160 ± 12.5 densitometric abundance units (mean ± S.E.M.) in control, 430 ± 20.5 units in IFN-γ treated group, 280 ± 18.4 units in both IFN-γ and TGF-β

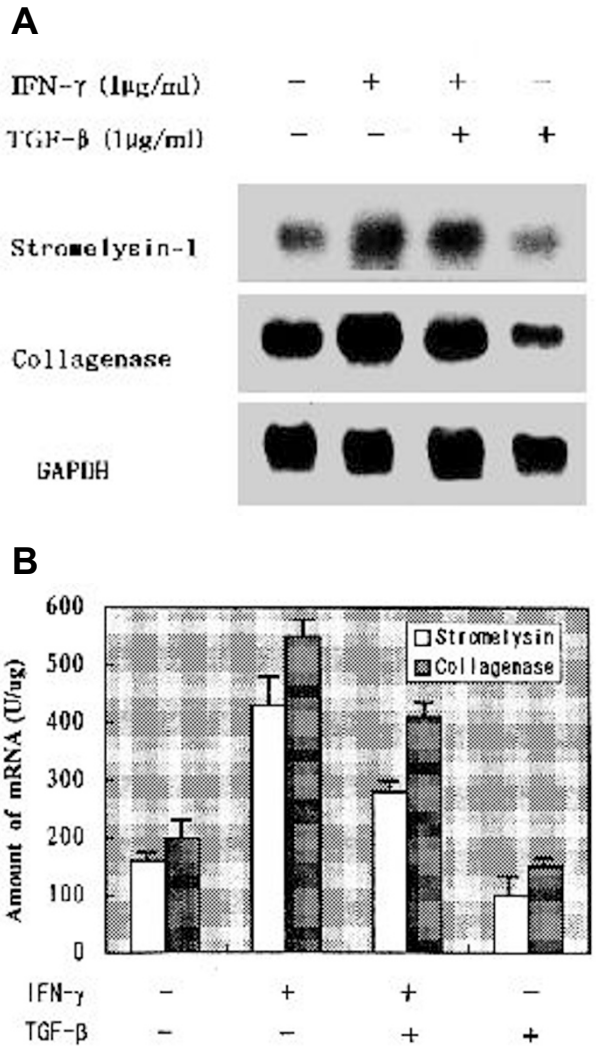

Figure 1. Effect of interferon-γ (IFN-γ) and transforming growth factor-β (TGF-β) on collagenase and stromelysin-1 gene expression. IFN-γ and TGF-β, both at 1 μg/ml, were added to confuluent fibroblast cultures maintained in medium containg 1% fetal bovine serum. Total RNA (15 μg/lane) was extracted after a 24 h incubation and analyzed by Northern hybridization with cDNA probes for stromelysin-1, collagenase and GAPDH(A). Quantitation of stromelysin-1 and collagenase mRNA levels by densitometric scanning of the X-ray film in A. The mRNA levels were corrected for GAPDH mRNA level in the same RNA samples Experiments were performed in triplicate and repeated twice. Bars represent mean ± S.E.M.(B).

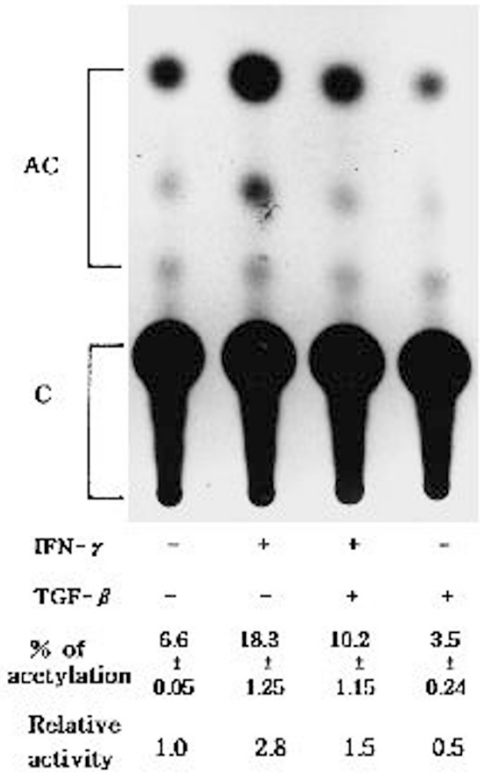

Figure 2. Enhancement of stromelysin-1 promotor activity by IFN-γ in transient cell transfections. Confluent cultures of skin fibroblasts were transfected with the stromelysin-1 promotor/CAT gene construct ST-56/CAT. Three hours after the glycerol shock, the cells were exposed to IFN-γ and TGF-β in medium containg 0.5% calf serum. After 24-48 h of additional incubation, the cells were harvested and CAT activity was determined. CAT assay was performed by separation of acetylated (AC) and unacetylated (C) forms of [^{14}C]chloramphenicol by thin-layer chromatography.

(1 μg/ml) treated, 103 ± 8.3 units in TGF-β treated group. The expression of stromelysin-1 gene was significantly enhanced by IFN-γ, 430 ± 20.5 units compared with 160 ± 12.5 units in control, and maximum effect was noted at 1 μg/ml (data not shown) and its effect was decreased by TGF-β. IFN-γ coordinately upregulates stromelysin-1 and collagenase gene expression in cultured fibroblasts (Figure 1A and 1B).

Tanscriptional regulation of the stromelysin-1 gene at the promoter level

To examine the possibility that the elevated mRNA level for stromelysin-1 is resulted from enhanced transcriptional activity of the corrresponding genes, 566-bp stromelysin-1 CAT constructs were used in transient transfections of cultured humam skin fibroblasts. Fifteen hours following the transfection with strmelysin-1/CAT construct, 1 μg/ml IFN-γ was added to parallel duplicate cultures for 24 h. As the result of CAT assay, the percentage of acetylation were 6.6 ± 0.05% in control group, 18.3 ± 1.25% in IFN-γ treated group, 10.2 ± 1.15% in both IFN-γ and TGF-β, 3.5 ± 0.24% in TGF-β treated group. Therefore, the promoter activity was increased 2.8-fold compared to untreated control (Figure 2, Table 1). IFN-γ up-regulated stromelysin-1 promotor activity, suggesting transcriptional enhancement of gene expression, and TGF-β antagonize

the effect of IFN-γ.

IFN-γ-treated nuclear extract leads to increased AP-1 binding activity

Ap-1 binding activity to the [^{32}P]-labeled AP-1-stromelysin DNA fragment derived from the strmelysin-1 promoter was increased in IFN-γ-treated nuclear extract. The specificity of the binding was demonstrated by competetion of the binding protein with unlabeled 100 molar excess AP-1 binding element. These data empha-size the possibility of transcriptional level of regulation of stromelysin-1 expression by interferon-γ in cultured human skin fibro-blasts (Figure 3).

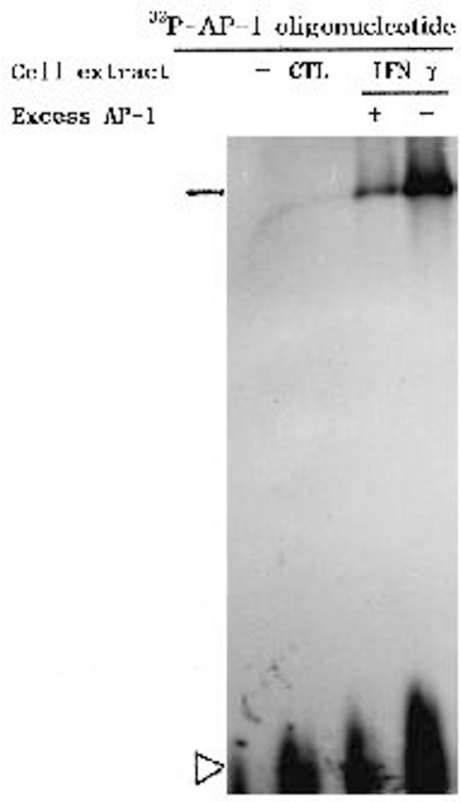

Figure 3. IFN-γ enhances nuclear protein binding to the collagenase/stromelysin-1 AP-1 *cis*-elements, as analyzed by gel mobility shift assay. A radioactively labeled double-stranded oligonucleotide containing the AP-1 binding sequence, was incubated with nuclear extracts from foreskin fibroblasts treated with or without IFN-γ for 5 h in medium containg 10% FCS. The reaction mixture was subsequently fractionated by electrophoresis on 4% non-denaturing polyacrylamide gels. Competition assay was performed with a hundred-fold molar excess of unlabeled AP-1-containg oligonucleotide. CTL: control.

Discussion

Maintenance of the equilibrium between deposition and degradation of ECM is essential to normal tissue development and tissue repair processing. Matrix metalloproteinases form a family of enzymes capable of degrading various ECM components that are expressed in normal remodeling of connective tissue, such as that occurring during embryonic development, uterine resorption and wound healing as well as in disease states, such as in rheumatoid arthritis or tumor invasion (McCachren, 1991; Mauviel, 1993).

Stromelysin is critical for degradation of ECM of connective tissue, such as collagen III, IV and IX, fibronectin, gelatin, proteoglycans, and laminin, and for collagenase activation. These modulation of ECM is often selective and different cytokines can alter the balance between the active protease and their inhibitors (Mauviel, 1993).

It has been previously demonstrated that IFN-γ, an important lymphocyte product, is capable of inhibiting fibroblast collagen synthesis *in vitro* and that IFN-γ is the most potent inhibitor among other lymphokines (Jimenez *et al*., 1984). It was shown that inhibitory effects of IFN-γ on collagen synthesis were accompanied by decreased levels of type I, II and III procollagen mRNA in dermal and synovial fibroblasts (Unemori *et al*., 1991) and articular and costal chondrocytes (Adrew *et al*., 1990). Similary, the expression of MMP-3 (stromelysin) in fibroblasts cultures was found to be inhibited by the addition of IFN-γ (Mauviel *et al*., 1993). These results suggests that INF-γ may play an important role in preventing excessive connective tissue degradation in physiologic condition. But, these findings are not consistent with other report that IFN-γ upregulates the expression of the collagenase and stormelysin in cultured skin keratinocytes (Tamai *et al*., 1995). This report further emphasizes the cell-type specificity with respect to IFN-γ effects on the regulation of MMPs. TGF-β showed little effect on the expression of stromelysin gene, whereas it suppressed the collagenase activity in cultured fibroblasts (Mauviel *et al*., 1993).

In this study, we examined the effects of IFN-γ and/or TGF-β on stromelysin-1 gene transcription in cultured foreskin fibroblasts. It was revealed on Northern blot analysis that IFN-γ increased the gene expression of stromelysin-1 up to 2.5 fold, while TGF-β counteracted the effect of IFN-γ. The regulation point of stromelysin-1 gene expression by IFN-γ and TGF-β appeared to be at the transcriptional stage. CAT assay after transient transfection of stromelysin-promoter/CAT gene construct showed that the CAT activity was enhanced by 2.8-fold in the presence of IFN-γ and decreased by 1.5-fold with TGF-β. This suggests that IFN-γ may be an up-regulator of stromelysin-1 promoter activity and TGF-β antagonize the effect of IFN-γ.

The promoter regions of the gene encoding for stromelysin-1 have been sequenced and exhibit common features important for transcriptional regulation (Qui *et al*., 1989). The promoter contain a TATA box, about 30 nucleotides upstream from the transcriptional start site,

a tumor promotor-responsive element (TRE) (Angel *et al*., 1987). The TRE (5'-TGAGTCA-3') binds the transcriptional factor AP-1, composed of dimers of protein product encoded by the families of oncogenes, *fos* and *jun*. In this context, it was of interest to note that the constitutive expression of stromelysin-1 has been suggested to involve AP-1. In this study, the binding activity of nuclear extract to oligonucleotide containing AP-1 sequence was increased during incubation of the dermal fibroblasts treated with IFN-γ. The competition assay was performed with 100-fold molar excess of unlabeled AP-1 containing oligonucelotide.

These data suggest that IFN-γ may be an up-regulator of the stromelysin-1 gene expression at the level of transcriptionin human skin fibroblasts and further provide intriguing evidence for the role of the cytokine net work in regulating the extracellular matrix turnover in physiological and pathological situations.

Acknowledgement

This studies were supplemented in part by the Special Research Fund(1995) of the Dongsan Medical Center.

References

Andrew, H. Bunning, R. Plumpton, T. Clark, I. Russell, R. and Cawston, T. (1990) Inhibition of interleukin-1 induced collagenase production in human articular chondrocytes *in vitro* by recombinant human interferon-gamma. *Arthritis Rheum.* 33: 1733-1739

Andrew, N. C. and Faller, D. V. (1991) A rapid micropreparation technique for extraction of DNA-binding proteins from limiting numbers of mammalian cells. *Nucleic Acids Res*. 19: 2499

Angel, P. Baumann, I. Stein, B. Delius, H. and Rahmsdorf, H. (1987) TPA induction of the human collagenase gene is mediated by an inducible enhancer element located in the 5'-flanking region. *Mol. Cell. Biol.* 7: 2256-2266

Buttice, G. Quinones, S. and Kurkinen, M.(1991) The AP-1 site is required for basal expression but is not necessary for TPA-response of the human stromelysin gene. *Nucleic Acids Res*. 19: 3723-3731

Chin, J. R. Murphy, G. and Werb, Z. (1985) Stromelysin, a connective tissye degrading metalloproteinase secreted by stimulated rabbit synoial fibroblasts in parallel with collagenase. *J. Biol. Chem*. 260: 12367-12376

Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidilm, thiocyanate-phenolchloroform extraction. *Anal. Biochem*. 162: 156-159

Fort, P. Marty, L. Piechaczyk, M. Sabrouty, S. E. I. Dan, D. Jeanteur, P. and Blanchard, J. (1985) Various rat adult tissues express only one major mRNA species from the glyceraldehyde-3-phosphate-dehydrogenase multigenic family. *Natl. Acids. Res*. 13: 1431-1442

Goldberg, G. I., Wilhelm, S. M., Kronberger, A. Bauer, E. A. Grant, G. A. and Eisen A. Z. (1986) Human fibroblas collagenase. Complete primary structure and homology to oncogene transfoemation-induced rat protein. *J. Biol. Chem*. 261: 6600-6605

Gorman, C. Moffat, L. and Howard, B. (1982) Recombinant genomes which express chloramphenicol acethyltransferase in mammalian cells. *Mol. Cell Biol.* 2: 1044-1054

Graham, F. and Van der Eb (1973) A new technique for the assay of infectivity of human adenovirus 5 DNA. *Virology* 57: 456-467

Jimenez, S. Freundhch, B. and Rosenbloom, J. (1984) Selective inhibition of diploid fibroblasts collagen synthesis by interferons. *J. Clin. Invest.* 74: 1116-1121

Martrisian, L. (1990) Metalloproteinases and their inhibitors in connective remodelling. Trens. Genet. 6: 121-125

Mauviel, A. (1993) Cytokine regulation of metalloproteinase gene expres-sion. *J. Cell Biochem.* 53: 288-295

Mauviel, A. Chen, Y. Dong, W. Evans, C. and Uitto, J. (1993) Transcrip-tional interaction of transforming growth factor-β with proinflammatory cytokines. *Current Biol.* 3: 822-831

McCachren, S. S. (1991) Expression of metalloproteinases and metal-loproteinase inhibitor in human arthritic synovium. *Arthritis Rheum*. 43: 1085-1093

Pelletier, J. Faure, M. Dibattista, J. Wilhelm, S. Visco, D. and Martel, P. (1993) Coordinate synthesis of sromelysin, IL-1 and oncogene protein in expremental osteoarthritis. *Am. J. Patho*. 146: 95-105

Quinones, S. Saus, J. Otani, Y. Harris, E.D. and Kurkinen, M.(1989) Transcriptional regulation of human stromelysin. *J. Biol. Chem*. 264: 8339-8334

Rigby, P. Dieckmann, M. Rhodes, C. and Berg, P. (1977) Labeling deoxyribonucleic acid to high specific *in vitro* by nick-translation with DNA polymerase. *J. Mol. Biol.* 113: 237-251

Saarialho-Kere, U. K. Pentland, A. P. Birkedal-Hansen, H. Parks, W. C. and Welgus, H. G. (1993) Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. *J. Clin. Invest.* 94: 79-88

Sambrook, J. Maniatis, T. and Fritsch, E. F. (1989) Molecular cloning In *Laboratory Mannual*, 2nd Edn., pp. 8.1-8.13, Cold Spring Harbor Labo-ratory, Cold Spring Harbor

Saus, J. Quinones, S. Otani, Nagase, H. Harris, E.D. Jr. and Kurkinen, M. (1988) The complete primary structure of human matrix metallo-proteinase-3. Identity with stromelysin. *J. Biol. Chem.* 263: 6742-6745

Sen, G. (1992) The interferon system: A bird's eye view of its biochemistry. *J. Biol. Chem*. 267: 5017-5020

Shaprio, S. Campbell, E. Kobayashi, D. and Welgus, H. (1990) Immune modulation of metalloproteinase production in human macrophage: selective pretranslational suppression of intersitial collagenase and stromelysin biosynthesis by interferon-γ. *J. Clin. Invest*. 86: 1204-1210

Tamai, K. Ishikawa, H. Mauviel, A. and Uitto, J.(1995) Interferon-γ coordiantely upregulate matrix metalloproteinase (MMP)-1 and MMP-3, but not tissue inhibitor of metalloproteinase (TIMP), expression in cultured keratinocytes. *J. Invest. Dermatol*. 104: 384-390

Tamai K, Li K, and Uitto J (1994) Identification of a DNA-binding protein (keratinocyte transcriptitonal protein-1) recognizing a keratinocyte-specific regulatory element in the 230-kDa bullous pemphigoid antigen gene. *J. Biol. Chem.* 269: 493-502

Thomas, P. (1980) Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Sci. USA* 77: 5201-5205

Unemori, E. Bair, M. Bauer, E. and Amento, E. (1991) Stromenysin expression regulates collagenase activation in human fibriblasts. *J. Biol. Chem*. 266: 23477-23482

Whal, G. Starn, M. and Starck, G. (1979) Efficient transfer of large DNA fragments from agarose gel to diabenzylloxymethyl paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Sci. USA* 76: 3683-3687

Woessner, J. F. Jr. (1990) Matrix metalloproteinases and their inhibitors in connective remodelling. *FASEB J.* 5: 2145-2154