Cellular activation of mesangial gelatinase A by cytochalasin D is accompanied by enhanced mRNA expression of both gelatinase A and its membrane-associated gelatinase A activator (MT-MMP)

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Activation of gelatinase A represents a crucial regulatory step in the control of its enzymic activity. Rat kidney mesangial cells secrete predominantly latent gelatinase A that can be activated following treatment with cytochalasin D. In the present paper we provide new evidence, using reverse transcription-PCR, that treatment of rat mesangial cells with cytochalasin D enhances the steady-state level of mRNA of the membrane-type matrix metalloproteinase (MT-MMP), as well as of gelatinase A, with no change in the level of tissue inhibitor of metalloproteinases-2 (TIMP-2) mRNA. Since the TIMP-2 protein level is reduced in conditioned medium from cytochalasin D-treated cells, the results of the present study are consistent with a model in which the action of cytochalasin D is to cause extracellular gelatinase A and TIMP-2 to be sequestered at the plasma membrane, forming a heterotrimeric complex with MT-MMP. In this manner, TIMP-2 may assume a bifunctional role causing: (i) inhibition of gelatinase A in the extracellular compartment; and (ii) guiding gelatinase A to activation through a membrane association with MT-MMP.

INTRODUCTION

The glomerular mesangium participates in formation and reabsorption of the extracellular matrix, in processing of circulating macromolecules, and contributes to the control of glomerular capillary flow and ultrafiltration [1]. Proteinases are among the many regulatory molecules secreted by mesangial cells with the potential to influence local structure and function. The predominant metalloproteinase secreted by cultured rat mesangial cells is the 72 kDa gelatinase A [2,3], which migrates on SDS/PAGE under non-reducing conditions as a 68 kDa species [3] and requires activation in order to exert its catalytic action. Regulation of this activation process is of critical importance in determining the overall impact of gelatinase A on glomerular function in health and disease.

Recently, a specific gelatinase A activator termed membrane-type matrix metalloproteinase (MT-MMP) [4] has been cloned. This protein is a new member of the metalloproteinase family and is expressed on the surface of various tumour cells [4,5]. Further, Strongin et al. [6] have demonstrated that MT-MMP binds tissue inhibitor of metalloproteinases-2 (TIMP-2), and that the resulting complex acts as a receptor for the C-terminus of gelatinase A, following which activation of gelatinase A occurs.

We have shown previously that cytochalasin D-induced cytoskeletal disruption in rat mesangial cells causes increased activation of gelatinase A, yielding two products with gelatolytic activities migrating at 59 and 57 kDa [3]. It is now recognized that the 59 kDa fragment is an intermediate and eventually is converted to the 57 kDa fully activated species [7]. This activation is accompanied by reduction of the TIMP-2 protein, the naturally occurring inhibitor of gelatinase A, and reduction of the latent form of gelatinase A [3]. But the molecular sequence of events by which cytochalasin D initiates the proteolytic events leading to cellular activation of gelatinase A is unknown. Elucidation of these processes would improve our understanding of the mechanisms leading to cell matrix accumulation in the renal glomerulus.

In the present report, we demonstrate by reverse transcription (RT)-PCR that mesangial cells express a form of MT-MMP and that disruption of mesangial cell cytoskeleton by cytochalasin D leads to an increase in the level of the 182 and 227 bp DNA fragments, corresponding to mesangial cell MT-MMP and gelatinase A respectively. But, at the same time, there is no change in the level of the 379 bp fragment corresponding to TIMP-2 mRNA. On the basis of this new information we propose a general scheme for cellular activation of gelatinase A, in the context of interacting MT-MMP, TIMP-2 and gelatinase A.

MATERIALS AND METHODS

All reagents used in this study were purchased from Sigma (St. Louis, MO, U.S.A.) unless otherwise indicated.

Mesangial cell culture

Rat mesangial cells were cultured under conditions previously described [3]. Briefly, cells were trypsinized and plated in 100 mm-diam. culture Petri dishes in minimal essential medium (MEM; Gibco, Grand Island, NY, U.S.A.) containing 15% fetal bovine serum. The next day, cells were washed twice in MEM, incubated for 30 min in MEM to reduce adsorbed serum and secreted proteins, and then incubated for a further 24 h in the presence or absence of 1 μg/ml cytochalasin D. Mesangial cell conditioned medium was then collected. Following addition of Brij 35 and azide to a final concentration of 0.02% each, the mixture was centrifuged to remove cell debris. Supernatants were dialysed against 3 mM NaCl and lyophilized.

Abbreviations used: G_3PDH, glyceraldehyde-3-phosphate dehydrogenase; MEM, minimal essential medium; MT-MMP, membrane-type matrix metalloproteinase; RT, reverse transcription; TIMP-2, tissue inhibitor of metalloproteinases-2.

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Table 1 Primers used in PCR reactions

Number	Gene	Sequence	Amplified product (bp)	EMBL accession number
1	Gelatinase A	5′ primer: 5′-CAATACCTGAACACCTT-3′ 3′ primer: 5′-CTGTATGTGATCTGGTT-3′	227	J05471
2	MT-MMP	5′ primer: 5′-ATTGATGCTGCTCTCTTCTGG-3′ 3′ primer: 5′-GTGAAGACTTCATCGCTGCC-3′	182	D26512
3	TIMP-2	5′ primer: 5′-AGCGGTCAGTGAGAA-3′ 3′ primer: 5′-CCAGAGGCACTCGTC-3′	379	X54533
4	G_3PDH	5′ primer: 5′-ACCACAGTCCATGCCATCAC-3′ 3′ primer: 5′-TCCCACCACCCTGTTGCTGTA-3′	453	X02231

SDS/PAGE zymography

Gelatinase A activity was measured by SDS/PAGE zymography as previously described [3,8,9]. Briefly, samples were diluted with non-reducing buffer and applied to SDS/PAGE (10% gels co-polymerized with 0.2% (w/v) gelatin). Proteins were resolved overnight with 7.5 mA/gel. Gels were washed with 2.5% Triton X-100 for 2 h, rinsed three times with distilled water, and incubated overnight in 50 mM Tris, pH 8.0, containing 5 mM $CaCl_2$. Gels were then stained with 0.1% Coomassie Blue R-250 in water/methanol/acetic acid (6:3:1 by vol.).

RT-PCR

Total RNA was extracted from mesangial cells grown in culture dishes using the acid guanidine–phenol method [10]. RNA quality was assessed by resolving on denatured 1% agarose gels and measuring absorbance ratios at 260/280 nm, and quantified using absorbance at 260 nm [11]. First-strand cDNA was synthesized by RT. In a typical 20 μl reaction, 1 μg of total RNA/0.5 μg of oligo d(T) 12-18 (Gibco/BRL, Gaithersburg, MD, U.S.A.)/diethylpyrocarbonate-treated water was heated to 70 °C for 10 min and then chilled on ice. Reaction buffer (5×; 4 μl)/4 μl of dNTP (0.5 mM final concentration each; Pharmacia, Baie d'Urfe, Quebec, Canada), 20 units of RNase inhibitor (Boehringer-Mannheim, Laval, Quebec, Canada) and 1 μl of AMV reverse transcriptase (8 units final concentration; Promega, Madison, WI, U.S.A.) was added and incubated at 42 °C for 2 h, followed by heating for 5 min at 95 °C.

Primers used for PCR reactions were custom synthesized (Pharmacia/HSC, Toronto, Ontario, Canada) and are summarized in Table 1. The G_3PDH (glyceraldehyde-3-phosphate dehydrogenase) primers (Clontech, Palo Alto, CA, U.S.A.) will amplify a 453 bp fragment from both reverse transcribed and genomic DNA rat preparations (manufacturer's information). Primers used for amplification of gelatinase A and TIMP-2 were derived from human sequences and will amplify 227 and 379 bp products, respectively, from rat cDNA (EMBL accession nos. X71466 and L31884). Rat MT-MMP sequence was not available when this study was performed. Therefore primers for amplification of MT-MMP were based on human sequence, and will amplify a 182 bp fragment from human, mouse and rat genes (EMBL accession nos. D26512, X83536 and X83537 respectively).

The PCR mixture was heated to 94 °C for 5 min and then run for 20 (G_3PDH) or 30 (gelatinase A or MT-MMP) cycles at 94 °C for 30 s, 55 °C for 1 min and 72 °C for 1 min, followed by 10 min elongation at 72 °C. Under these conditions, the three gene products were found to be at the exponential phase of the amplification. TIMP-2 product was amplified under lower stringency conditions using 45 °C for annealing and 30 cycles. Equal addition of RT products was verified by serial double dilutions of the RT reaction mixture and by running the PCR with G_3PDH primers for 20 cycles. A typical 25 μl reaction mixture contained water, $MgCl_2$ (1.5 mM), dNTP (250 μM each) and primers (1 μM each; except for 0.25 μM for G_3PDH). *Taq* DNA polymerase (5000 units/ml; Sangon, Scarborough, Ontario, Canada) was preincubated with TaqStart antibody (1.1 μg/ml; Clontech) for 5 min at room temperature, and 0.5 μl of this mixture was added to the reaction mixture along with adjusted amounts of cDNA, in a volume of 0.5 μl. The G_3PDH primers used will also amplify a 453 bp fragment from genomic DNA. Therefore to verify absence of contamination with genomic DNA, the RNA preparation was first run in a PCR using G_3PDH primers, before carrying out the RT. PCR products were resolved on 1.5% agarose gels containing 0.5 μg/ml ethidium bromide.

Verification of MT-MMP PCR product

In addition to identifying the PCR product of MT-MMP on agarose gels according to molecular size, restriction analysis and DNA sequencing of the product was also performed. According to the rat sequence, a *Sca*I site exists at position 62 of the amplified 182 bp product. A PCR aliquot (4μl) was incubated with 1.5 units of *Sca*I (Pharmacia) in a final volume of 20 μl for 1 h at 37 °C, and resolved on a 1.8% agarose gel. The PCR product of MT-MMP was also subjected to DNA sequencing with the dideoxynucleotide chain-termination technique [12] (Pharmacia/HSC, Toronto, Ontario, Canada) using SequiTherm™ Long-Read™ automated sequencing (Epicentre Technologies, Madison, WI, U.S.A.), for 35 cycles. Fluorescent 5′-labelled MT-MMP sense primer (Table 1) was used for sequencing. Two rounds of PCR amplification were carried out to yield sufficient material for sequencing. The first round of amplification utilized *Taq* polymerase, TaqStart and 40 cycles under the conditions described above. An aliquot (0.5 μl) from this reaction served as template for a second round of PCR amplification utilizing *Pfu* polymerase (Stratagene Cloning Systems, La Jolla, CA, U.S.A.) under the same conditions used for the first amplification.

PCR amplification of mesangial cell genomic DNA

Genomic DNA was amplified with primer pairs for the G_3PDH and MT-MMP genes using GeneReleaser reagent for lysis and quenching endogenous inhibitory reagents (BioVentures, Murfreesboro, TN, U.S.A.). After trypsinization, cells were resuspended in 15% fetal bovine serum in MEM to inhibit traces of trypsin, to yield a final density of 2.25×10^6 cells/ml. An aliquot (40 μl) of the suspension containing 9×10^4 cells was transferred to a 0.2 ml PCR tube, spun for 2 min at 360 ***g*** and the

supernatant was discarded. The GeneReleaser reagent (20 μl) was added directly to the tube, and cells were lysed in the PCR apparatus according to the manufacturer's recommendation (except for the last step where the tube was brought to 21 °C) using the following program: 65 °C for 30 s, 8 °C for 30 s, 65 °C for 90 s, 97 °C for 180 s, 8 °C for 60 s, 65 °C for 180 s, 97 °C for 60 s, 65 °C for 60 s and 21 °C hold. A PCR cocktail containing the appropriate primers was added to a final volume of 100 μl, and PCR was performed as described in RT-PCR section above.

RESULTS

In the presence of cytochalasin D, mesangial cell gelatinase A is converted upon activation to a predominant 57 kDa species ([3] and Figure 1 insert). This cellular activation is associated with reduced latent gelatinase A in the conditioned medium, as revealed by detection on immunoblots [3]. However, it is not clear whether the observed decrease in latent gelatinase A protein is a consequence of lowered expression or a consequence of conversion of latent to active enzyme. To test which of these possibilities is correct, we used RT-PCR to enhance the level of a 227 bp fragment of the gelatinase A gene. Following treatment with cytochalasin D, we observed an increase in the level of the amplified 227 bp fragment of gelatinase A with no change in the level of the 453 bp fragment of housekeeping gene G_3PDH (Figure 1).

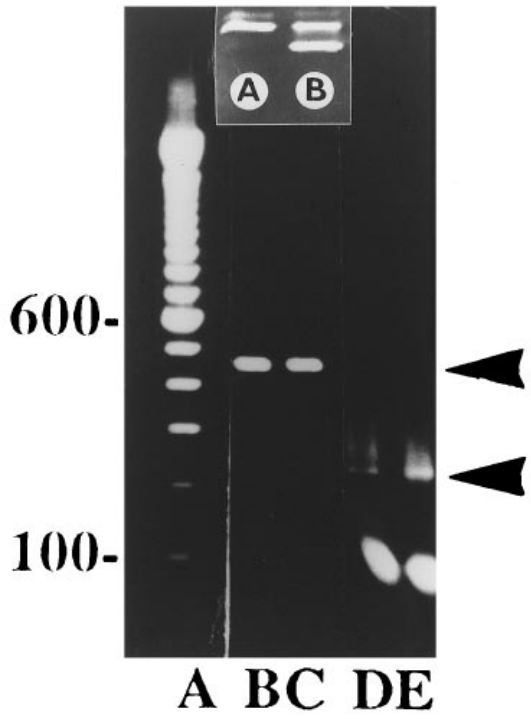

Figure 1 Changes in gelatinase A caused by cytochalasin D-induced disruption of rat mesangial cell actin filaments

Mesangial cells were treated with 1 μg/ml cytochalasin D overnight. Gelatinase A activity was monitored by zymography in the conditioned medium (insert) and gelatinase A mRNA levels were assessed using RT-PCR. Latent 68 kDa gelatinase A is the predominant enzyme secreted into the condition medium (insert, lane A) and is converted to a lower predominant 57 kDa active species after treatment with cytochalasin D (insert, lane B). Treatment with cytochalasin D (main panel, lanes C and E) enhances amplification of the 227 bp PCR product of the gelatinase A gene (main panel, lane E, lower arrow) but not the 453 bp product of G_3PDH (main panel, lane C, upper arrow), compared with the respective non-treated controls (main panel, lanes B and D). Bands under 100 bp represent primers used for PCR. PCR products were run on 1.5% agarose gels. Lane A, 100 bp ladder.

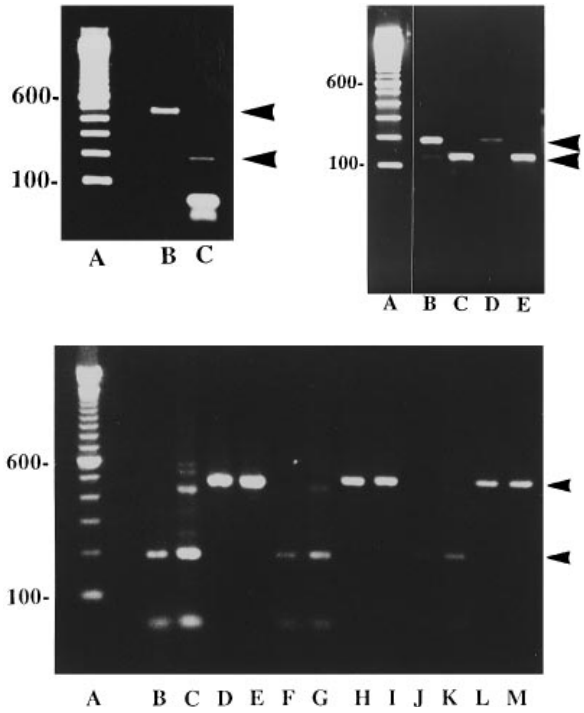

Figure 2 Amplification of G_3PDH and MT-MMP from rat mesangial cell genomic DNA (upper left panel) and influence of disruption of mesangial cell actin filaments with cytochalasin D on the expression of these genes (lower panel)

(Upper left panel) Genomic DNA was derived from cells using GeneReleaser reagent (see the Materials and methods section) and served as template for PCR amplification. As expected, G_3PDH primers amplify a 453 bp product from mesangial cells and MT-MMP primers amplify a 182 bp product. (Lower panel) Total RNA was reverse transcribed following PCR amplification with primers for MT-MMP (lanes B, C, F, G, J and K) or G_3PDH (lanes D, E, H, I, L and M). Cytochalasin D enhanced the 182 bp amplification product of MT-MMP (lanes C, G and K) but not the 453 bp product of G_3PDH (lanes E, I and M) compared with the respective non-treated control (MT-MMP, lanes B, F and J; G_3PDH, lanes D, H and L). RT products depicted in the lower panel were run in serial double dilutions (B–E, F–I and J–M) and resolved on 1.5% agarose gels. (Upper right panel) PCR product was digested with 1.5 units of *Sca*I for 1 h at 37 °C. Aliquots of 5 μl (lanes B and C) or 2 μl (lanes D and E) of the reaction mixture before (lanes B and D) or after (lanes C and E) digestion were resolved on a 1.8% agarose gel. A predominant expected 121 bp band is evident after *Sca*I treatment (lower arrow), compared with the non-digested 182 bp MT-MMP band (upper arrow). Bands under 100 bp represent primers used for PCR. Lane A, 100 bp ladder.

To assess the role of MT-MMP in the mesangial cell gelatinase A activation process, a primer pair that amplifies a 182 bp fragment was designed based on the DNA sequence of MT-MMP cloned from human placenta cDNA (Table 1). To test whether these primers would amplify the rat MT-MMP gene, we employed genomic DNA as template for PCR amplification and revealed a 182 bp fragment for MT-MMP, as well as the expected 453 bp fragment for G_3PDH (Figure 2, upper left panel). Using RT-PCR, a 182 bp fragment was amplified from mesangial cells, suggesting that these cells express MT-MMP under resting conditions (Figure 2, lower panel). The level of this fragment was further enhanced after treatment of mesangial cells with cytochalasin D, but no change was noted in the 453 bp amplified fragment of the housekeeping gene G_3PDH (Figure 2, lower panel). To verify that the 182 bp amplified fragment indeed represented MT-MMP, the DNA fragment was subjected to restriction analysis. As depicted in Figure 2 (upper right panel), treatment of the PCR product with *Sca*I generated an expected

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ATTGATGCAG CTCTCTTCTG GATGCCCAAC GGGAAGACCT ACTTCTTCCG      50
          T                          T   A

GGGAAATAAG TACTACCGCT TCAATGAGGA GTTCAGGGCA GTGGACAGCG     100
T    C          T    C  A   C              T

AATACCCCAA AAACATCAAA GTCTGGGAAG GAATCCCTGA GTCTCCCAGA     150
 G      G                  G

GGCTCATTCA TGGGTAGCGA TGAAGTCTTC AC                        182
  G          C
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Figure 3 Partial nucleotide sequence of rat mesangial cell MT-MMP cDNA amplified by PCR

The cDNA was sequenced in one direction using dideoxy methodology. The second line denotes different nucleotides in the human sequence (EMBL accession no. D26512). The underlined nucleotides represent the primers used based on the human sequence. The lines above the sequence denote regions that could not be sequenced, presumably due to DNA structure; the sequence is taken from a rat-skin-wound-healing library (EMBL accession no. X83537). There is 100% identity between the rat mesangial cell fragment sequenced and that of the rat-skin-wound healing cDNA. One nucleotide in the 3′ primer, depicted in bold type, was derived from the rat skin sequence, although the human nucleotide incorporated in the primer was in effect sequenced. Arrowhead denotes the cut site of *Sca*I employed in the restriction analysis.

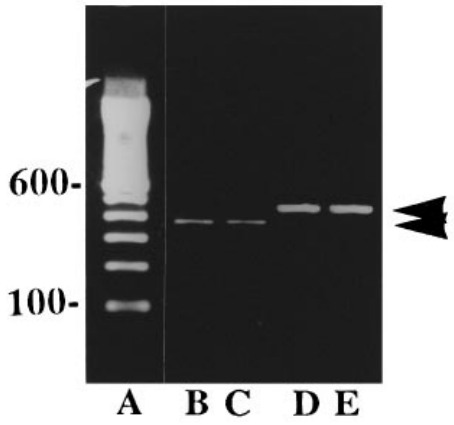

Figure 4 Influence of cytochalasin D treatment of rat mesangial cells on mRNA levels of TIMP-2

Mesangial cells were treated with 1 μg/ml cytochalasin D overnight. Total RNA extracted from mesangial cells was used for RT-PCR. No apparent change in the amplified 379 bp band corresponding to TIMP-2 (lower arrow) was noted after cell treatment with cytochalasin D (lane C) compared with the non-treated control (lane B). The amplification products were normalized to the amplification of the 453 bp bands of G_3PDH (upper arrow) without (lane D) or with (lane E) treatment of cytochalasin D. PCR products were run on 1.5% agarose gels. Lane A, 100 bp ladder.

predominant band migrating at 121 bp (see also Figure 3). The PCR product was also subjected to sequencing, which confirmed that the amplified product was MT-MMP (Figure 3), with 100% identity to rat cDNA derived from a skin-wound-healing library and 92% identity to the corresponding human cDNA fragment.

TIMP-2 is a specific inhibitor of gelatinase A activity [13] and also mediates binding of gelatinase A to a membrane-associated complex with MT-MMP [6]. Since we had previously found that treatment of mesangial cells with cytochalasin D leads to reduction in TIMP-2 protein levels in the conditioned medium [3], it was of interest to determine if mesangial cell exposure to cytochalasin D was also accompanied by reduction of TIMP-2 mRNA . But, as shown in Figure 4, TIMP-2 mRNA levels were unchanged after treatment with cytochalasin D. This suggests that the observed decrease of extracellular TIMP-2 in treated cells is brought about by increased membrane sequestration of TIMP-2 protein rather than by altered expression of TIMP-2.

DISCUSSION

Proteinases facilitate tumour dissemination and metastatic processes [14]. Their role as a pathogenetic factor in kidney disease is suggestive but not well established [15]. Given the potential damage that could result from uncontrolled metalloproteinase function, it is important to define the regulatory controls determining enzymic activity in the normal glomerulus.

Earlier studies suggested that gelatinase A exhibits cell-surface-associated binding [16] and that activation is restricted to the plasma membrane [4,6,17,18]. More direct evidence in support of this premise has emerged after the cloning of MT-MMP [4] and the demonstration that (i) TIMP-2 binds to MT-MMP and (ii) the resulting membrane complex acts as a receptor for gelatinase A [6].

In the present investigation we have demonstrated that mesangial cells express MT-MMP. The gene structure of MT-MMP is not yet known. However, amplification of a 182 bp fragment from genomic DNA (Figure 2, upper left panel) implies that this fragment is located on a single exon. The possibility that this fragment is derived from an intronless pseudogene, as appears to be the case for G_3PDH (Figure 2, upper left panel; manufacturer's information) cannot be excluded at this stage.

Previously, we found that cytochalasin D decreases the level of the latent form of extracellular gelatinase A [3,9]. In the present study, we provide additional data showing that under these same conditions there is also a marked increase in the level of the mRNAs for both gelatinase A and MT-MMP. Therefore the reduction in the level of extracellular gelatinase A is probably associated with the process of its conversion from latent to active state.

Treatment of human fibroblasts with concanavalin A induces morphological changes but affects only marginally the mRNA levels of gelatinase A [19]. Since concanavalin A and cytochalasin D treatment of mesangial cells produce similar effects on gelatinase A activation [3], it might have been anticipated that cytochalasin D would also have little or no effect on latent gelatinase A expression. The fact that this is not the case probably reflects varying responses for the two cell types due to different degrees of differentiation. Further evidence suggesting that this explanation is valid can be inferred from experiments showing that gelatinase A levels of human fibroblasts are not altered after treatment with phorbol esters [18], whereas rat mesangial cell gelatinase A is significantly increased by this drug [20].

It is well recognized that TIMP-2 in solution acts as a specific gelatinase A inhibitor. In addition, Strongin et al. [6] have recently reported that TIMP-2 serves as a mediator for gelatinase A binding to MT-MMP, thus participating in a heterotrimeric membrane-associated complex that ultimately results in activation of latent gelatinase A. The results of the present study provide new information that may help to clarify the role of TIMP-2 as a regulatory molecule in the activation of gelatinase A.

In the present study we have demonstrated that, during the process of cytochalasin D activation, the level of TIMP-2 mRNA remains unchanged. But in earlier experiments we established that the level of extracellular TIMP-2 protein is reduced under these same conditions [3]. One way to account for these obser-

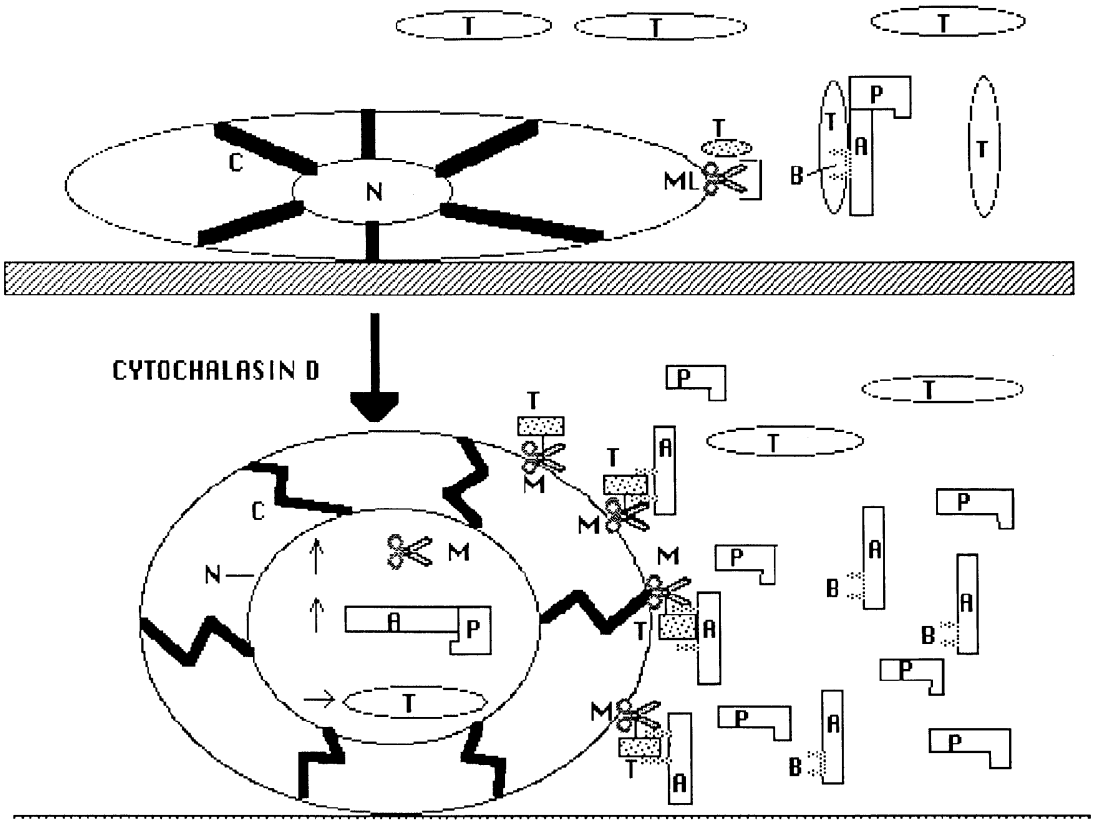

Figure 5 Schematic representation of a model for rat mesangial cell gelatinase A activation after cytoskeletal disruption

Under resting conditions (upper panel), cultured mesangial cells assume a flattened morphology due to cell–extracellular matrix attachment and the cytoskeletal (C) structure. The cell produces basal levels of membrane-associated latent MT-MMP (ML) and secretes latent gelatinase A composed of active (A) and pro segments (P). Latent gelatinase A is complexed to excess molecules of secreted TIMP-2 (T). Under the circumstances depicted in the upper panel, no proteolytic activity is evident, due to lack of active enzyme molecules. Upon addition of cytochalasin D, actin filaments are disrupted, and, through as yet unknown mechanisms, this leads to enhanced nuclear (N) expression of both MT-MMP and gelatinase A with no change in TIMP-2 expression. The increased ratio of gelatinase A to TIMP-2 yields free gelatinase A molecules. During this process, MT-MMP is activated and the affinity for TIMP-2 is increased. As a consequence, an increased proportion of TIMP-2 molecules bind to activated MT-MMP and serve in turn as receptors for free latent gelatinase A, yielding a three-molecule complex [6]. TIMP-2 guides gelatinase A to activation by catalytic cleavage mediated through a specific binding site (B) on the gelatinase A C-terminus [7,21]. See text for further details.

vations is that after exposure to cytochalasin D there is increased cellular clearance and/or degradation of extracellular TIMP-2.

Another equally plausible and perhaps more interesting explanation that could also reconcile results of the present study together with the findings of others is represented diagramatically in Figure 5. Briefly, we propose that cytochalasin D treatment of mesangial cells causes cytoskeletal disruption as a primary event, and that subsequently this leads to enhanced expression of both gelatinase A and its activator MT-MMP. We speculate that under basal conditions TIMP-2 affinity for latent MT-MMP is low, and consequently a majority of inhibitor molecules exist in soluble form, associated with extracellular gelatinase A. Upon treatment with cytochalasin D, MT-MMP is activated in an as yet unknown manner, increasing its affinity for TIMP-2, and causing TIMP-2, as well as gelatinase A, to relocate to the membrane to form a complex with activated MT-MMP. In effect, we are suggesting that TIMP-2 'guides' gelatinase A to activation by catalytic cleavage, through a specific binding site on the gelatinase A C-terminus [7,21]. The result is to render gelatinase A active and cause a net increase in proteolytic activity. The activation process is presumed to be cell-associated [4,6,17,18]. In this model, TIMP-2 assumes a bifunctional role as: (i) gelatinase A inhibitor and (ii) gelatinase A receptor, shuttling between a soluble and a membrane-associated locus, presumably as a result of enhanced affinity of TIMP-2 to activated MT-MMP. Since MT-MMP conforms to the general metalloproteinase structure that requires activation in order to exert catalytic activity, this scheme predicts that cytochalasin D treatment of mesangial cells will render the latent MT-MMP active.

Table 2 Summary of cytochalasin D-induced effects on gene expression in mesangial cells

Number	Gene	Effect of cytochalasin D on gene expression
1	Gelatinase A	Enhanced
2	TIMP-2	No change (shuttled ?)
3	MT-MMP	Enhanced
4	G_3PDH	No change

It appears that gelatinase A activation is a tightly co-ordinated, well-regulated event. A summary of the experimental findings obtained when mesangial cells are treated with cytochalasin D is given in Table 2. Our intention is to use the model presented in Figure 5 as a framework for further experiments in order to

improve our understanding of the molecular events involved in cellular activation of mesangial cell metalloproteinases.

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REFERENCES

1 Nath, K. A., Salahudeen, A. K. and Shah, S. V. (1993) in Current Nephrology (Gonick, H. C., ed.), vol. 16, pp. 221–256, Mosby Year Book, St. Louis
2 Davies, M., Thomas, G. J., Martin, J. and Lovett, D. H. (1988) Biochem. J. **251**, 419–425
3 Ailenberg, M., Weinstein, T., Li, I. and Silverman, M. (1994) J. Am. Soc. Physiol. **4**, 1760–1770
4 Sato, H., Takino, T., Okada, Y., Cao, J., Shinagawa, A., Yamamoto, E. and Seiki, M. (1994) Nature (London) **370**, 61–65
5 Okada, A., Bellocq, J.-P., Rouyer, N., Chenard, M.-P., Rio, M.-C., Chambon, P. and Basset, P. (1995) Proc. Natl. Acad. Sci. U.S.A. **92**, 2730–2734
6 Strongin, A. Y., Collier, I., Bannikov, G., Marmer, B. L., Gregory, A. and Goldberg, G. I. (1995) J. Biol. Chem. **270**, 5331–5338
7 Strongin, A. Y., Marmer, B. L., Grant, G. A. and Goldberg, G. I. (1993) J. Biol. Chem **268**, 14033–14039
8 Heussen, C. and Dowdle, E. B. (1980) Anal. Biochem. **102**, 196–202
9 Ailenberg, M., Stetler-Stevenson, W. G. and Fritz, I. B. (1991) Biochem. J. **279**, 75–80
10 Chomczynski, P. and Sacchi, N. (1987) Anal. Biochem. **162**, 156–159
11 Sambrook, J., Fritsch, E. F. and Maniatis, T. (1989) in Molecular Cloning: a Laboratory Manual, p. E5, Cold Spring Harbor Laboratory Press, Cold Spring Harbor
12 Sanger, F., Nicklen, S. and Coulson, A. R. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 5463–5467
13 Goldberg, G. I., Marmer, B. L., Grant, G. A., Eisen, A. Z., Wilhelm, S. and Chenshi, H. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 8207–8211
14 Liotta, L. A., Tryggvason, K., Garbisa, S., Hart, I., Foltz, C. M. and Shafie, S. (1980) Nature (London) **284**, 67–68
15 Baricos, W. H. and Shah, S. V. (1991) Kidney Int. **40**, 161–173
16 Emonard, H. P., Remacle, A. G., Noel, A. C., Grimaud, J.-A., Stetler-Stevenson, W. G. and Foidart, J.-M. (1992) Cancer Res. **52**, 5845–5848
17 Murphy, G., Willenbrock, F., Ward, R. V., Cockett, M. I., Eaton, D. and Docherty, A. J. (1992) **283**, 637–641
18 Brown, P. D., Kleiner, D. E., Unsworth, E. S. and Stetler-Stevenson, W. G. (1993) Kidney Int. **43**, 163–170
19 Overall, C. M. and Sodek, J. (1990) J. Biol. Chem. **265**, 21141–21151
20 Marti, H. S., Mcneil, L., Davies, M., Martin, J. and Lovett, D. H. (1993) Biochem J. **291**, 441–446
21 Ward, R. V., Atkinson, S. J., Slocombe, P. M., Docherty, A. J., Reynolds, J. J. and Murphy, G. (1991) Biochim. Biophys. Acta **1079**, 242–246

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