Regulation of Matrix Metalloproteinase-9 (MMP-9) by Translational Efficiency in Murine Prostate Carcinoma Cells

Yong Jiang and Ruth J. Muschel

Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT

Expression of increased levels of matrix metalloproteinase-9 (MMP-9) has been implicated in tumor progression and angiogenesis. Much of our knowledge of the controls of MMP-9 levels has focused on transcription. Here we show that MMP-9 levels are also controlled by translational efficiency in murine prostate carcinoma cells. The murine prostate carcinoma cells 148–1,LMD and 148–1,PA were derived from a single mouse that had been implanted with urogenital sinus transformed by ras and myc. 148–1,PA secretes little MMP-9 yet has equivalent amounts of MMP-9 mRNA as the cell line 148–1,LMD that secretes substantially more. Infection with a retroviral vector for murine MMP-9 led to more expression of MMP-9 in both cases, but the differential remained. Human MMP-9 is equally expressed in both cells after infection with a vector for human MMP-9 indicating that the effect is species-specific. Pulse chase analysis revealed that MMP-9 was synthesized more rapidly in the 148–1,LMD cells than in the 148–1,PA cells. Markedly more MMP-9 mRNA was associated with polysomes in the cell line synthesizing more MMP-9. These results indicate regulation of MMP-9 synthesis at the level of translational efficiency.

INTRODUCTION

Expression of MMP-9 has been shown to affect metastasis, angiogenesis, and tumor progression (1). This enzyme is a MMP, a family of homologous proteases subdivided into four subgroups: collagenses, gelatinases, membrane-associated MMPs and stromelysins (2). MMP-9, like MMP-2 its closest homologue in the family, is classified as a gelatinase, because both show a high affinity for digestion of denatured collagen I. This property of MMP-9 explains its alternate name, gelatinase B. It can also cleave a variety of proteins including many components of the extracellular matrix such as collagen I, III, IV, and V, elastin, and entactin (3, 4). This property has led to it being termed the M9, 92 kDa Type IV collagenase, because the human MMP-9 protein is M9, 92,000 although the murine form is M9, 105,000 because of an insert of an additional 24 amino acids (5, 6). The activity of MMP-9 against the secreted serpin inhibitor of MMP-9. Hence, the proregion must be cleaved and dissociated to allow enzymatic activity. The mediators of this cleavage in vivo are not well established for MMP-9 unlike the case for MMP-2 in which the MMP MT1-MMP can be shown to be the physiological activator in many cases (12–15). After the release of MMP-9 from the cell, it can be found in the medium but additionally is associated with the cell surface by formation of complexes with CD44 or with a chain of type IV collagen (16–19). It may also have the capacity to bind to the extracellular matrix (7). Furthermore, it interacts with the low density lipoprotein receptor, an interaction that can result in internalization of MMP-9 (20). MMP-9 has natural inhibitors (21). Tissue inhibitor of matrix metalloproteinase-1 is secreted and often found complexed to MMP-9 (22). Tissue inhibitor of matrix metalloproteinase-3 associates with the extracellular matrix and may also significantly contribute to MMP-9 inhibition (23).

MMP-9 is frequently up-regulated in cancer cells and also in the adjacent host tissues (1). Its expression by tumor cells has been shown to contribute to metastasis, because induction of expression enhanced metastasis both in a sarcoma and a melanoma system (24, 25). Inhibition of its expression by a ribozyme led to inhibition of metastasis by both sarcoma and prostate cancer cell lines without affecting tumorigenesis (26, 27). During breast cancer tumor progression induced by a mouse mammary tumor virus polyoma middle T-antigen transgene, a reporter construct consisting of the MMP-9 promoter linked to lacZ, was activated only during the later stages of progression, coincident with the development of invasion (28). MMP-9 expression by host stromal cells has also been shown to be important in metastasis and in tumor progression. MMP-9 has been linked to tumor progression in transgenic model systems of tumor progression where the MMP-9 supplied by mast cells was critical to angiogenesis (30–32). Mice deficient in MMP-9 have a transient defect in the angiogenesis that accompanies the conversion of avascular cartilage in the growth plate into bone (33). Thus, the control of MMP-9 expression has considerable significance for regulation of tumor progression.

Examination of the mechanisms regulating expression of MMP-9 have mainly focused on transcription regulation. A minimal promoter that responds to induction by 12-O-tetradecanoylphorbol-13-acetate or tumor necrosis factor-α was cloned by Sato et al. (34, 35). This promoter contains an AP-1-binding consensus site upstream from the start site that is required for transcriptional induction in most settings (for an exception see Farina et al., Ref. 36). Farther upstream is a cluster of regulatory elements including another AP-1 binding site, an AP-2 site, an ets consensus binding site and an nuclear factor α binding site, all of which have been shown to contribute to both constitutive expression and expression induced by various cytokines and growth factors (35, 37–40). In addition to transcriptional regulation, several reports have described instances of post-transcriptional regulation. Sehgal and Thompson (41) noted that transforming growth factor β led to elevation of mRNA levels for MMP-9 through prolonged message stability. Expression of the gene RECK reduced the amount of MMP-9 released by HT1080 cells, although mRNA levels were unchanged implying a post-transcriptional mechanism that was not additionally delineated (42). Liu et al. (43) also found that a post-transcriptional mechanism accounted for inhibited secretion of...
MMP-9 through the ras-mitogen-activated protein /extracellular signal-regulated kinase kinase pathway.

Here we describe a system in which translational efficiency can be shown to affect the levels of secreted MMP-9. Murine prostate carcinomas can be induced by ras and myc retroviral infection of the urogenital sinus followed by reimplantation of the tissue (44). The resultant carcinomas bear murine prostate-specific antigens and historically have the morphology characteristic of prostate cancer. When derived from p53 wild-type mice, these tumors rarely give rise to metastases, but when derived from p53-deficient mice some but not all of the resultant tumors now form metastases (45). Sehgal and Thompson (41) showed that cells isolated from metastatic prostate tumors tended to secrete MMP-9, whereas those from tumors that remained localized did not. We have explored the mechanisms controlling secretion in these two types of cell lines using the 148–1, LMD (derived from a metastasis) and 148–1, PA (the primary tumor in the same mouse) as cell lines for study. In this case differing translational efficiency for MMP-9 between the cell types was found to be an important factor regulating the amount of MMP-9 secreted. This is the first demonstration to our knowledge of MMP-9 regulation through translational efficiency.

MATERIALS AND METHODS

Cells. Murine prostate carcinoma cells 148–1, PA and 148–1, LMD were generated as described in Thompson et al. (45). Both were derived from tumors in the same mouse, 148–1, PA from a primary tumor and 148–1, LMD from a metastasis. Cells were routinely cultured in DMEM supplemented with 10% heat-inactivated fetal bovine serum, penicillin, and streptomycin.

DNA Constructs and Retroviral Infection. Retroviral expression vectors for the murine and human MMP-9 were constructed as follows. The full length murine MMP-9 cDNA (m105) cloned into pSK– (a generous gift of Dr. Hidekazu Tanaka, Shionogi & Co., Ltd., Osaka, Japan; Ref. 5), was removed by cleavage with restriction enzymes EcoRI and NotI, and subcloned into the retrovirus expression vector pMIGR1L, which is a derivative of pMIGR1 with a polylinker (pMIGR1L/m105). The full-length human MMP-9 cDNA cloned in pSK, (a gift of Dr. Gregory I. Goldberg, Washington University, School of Medicine, St. Louis, MO; Ref. 46), was similarly subcloned into pMIGR1L (pMIGR1L/MMP-9; Ref. 47). The expression vector pMIGR1L contains the internal ribosome entry site. The expression cassette contains a single promoter that, with the internal ribosome entry site, permits the translation of two open reading frames from one mRNA. The gene of interest and GFP protein are translated from the same mRNA. After infection (48 h), GFP-positive cells were sorted by flow cytometry, because the GFP-positive cells also express the gene of interest. Packing cell line 293T was cotransfected with the retroviral gene of interest. Packing cell line 293T was cotransfected with the retroviral

Gelatin Zymography. Gelatin-substrate gel electrophoresis was accomplished as described previously (48). To prepare conditioned medium, cells were washed with serum-free medium and resupplied with fresh serum-free medium. After 24 h, the conditioned medium was harvested, spun at 1500 × g to remove cellular debris, and concentrated five times. The cells were counted. The amount of medium loaded was normalized according to cell number. Concentrated medium was electrophoresed under nonreducing conditions and without heating through a 7% SDS-PAGE containing 0.1% gelatin. Gels were washed in 0.05 M Tris (pH 7.4) 2% TritonX-100, 0.2 M of NaCl, and 0.02% NaN 3, and were stained in 0.2% Coomassie Blue for 1 h and destained in 20% (V/V) methanol and 10% (V/V) acetic acid. The clear bands represent gelatinase activity.

Western Blotting. Denatured protein samples were separated by 7% SDS-PAGE. Proteins were transferred to nitrocellulose using a XcellIII Blot Module (Invitrogen) apparatus according to the manufacturer’s instructions. Blots were blocked in PBS buffer containing 3% dried milk and 0.1% Tween 20 for 1 h at room temperature, followed by incubation with primary antibody (anti-MMP-9, M-17; Santa Cruz Biotechnology; 1:500 dilution) for additional 1 h. After washing with PBS three times, blots were incubated with secondary antibody (horseradish peroxidase-conjugated goat, Santa Cruz Biotechnology; 1:2000 dilution) for 1 h at room temperature.

Northern Blotting. Total cellular RNA (20 μg) was electrophoresed in a 0.9% formaldehyde agarose gel and transferred to a Hybond-N+ membrane in 10 × SSC. The filters were prehybridized in PerfectHyb plus buffer (Sigma) for 3 h at 65° C. RNA blots were hybridized using 32P-labeled probe derived from the mi105 cDNA or from the cDNA for fibroblast protein rpl32 as internal standard by random primer labeling (Stratagene).

Pulse Chase. Cells were grown to 80% confluence in 100-mm dishes. The medium was aspirated, and the cell monolayers were washed twice with PBS followed by a 1-h incubation with 3 ml/dish pulse medium (DMEM without methionine and cysteine supplemented with 5% dialyzed FCS) The cells were then pulsed with 500 μCi/ml [35S]methionine and cysteine (NEC, Boston, MA) in pulse medium for 1 h at 37° C. The medium was aspirated, and the cells were washed twice with PBS before adding 2 ml/dish of chase medium (DMEM with 5 μM of methionine and 5 μM of cysteine). At the end of the chase period, the medium was collected and debris removed by centrifugation (5 m; 12,000 × g). The cell monolayer was washed twice with cold PBS and lysed in 0.8 ml/dish of radioimmunoprecipitation assay lysis buffer. The lysates were then clarified by centrifugation at 10,000 × g for 20 min at 4° C.

For immunoprecipitation, the medium or cell lysates were incubated overnight at 4° C with anti-MMP-9 antibody (GE213; Neomarkers) in 2 μg/mg protein or medium followed by the addition of 30 μl of protein A agarose for an additional 3-h incubation at 4° C. After a brief spin, the beads were washed five times and resuspended in 15 μl of Laemmli sample buffer (24). After boiling for 5 min, the samples were separated by 6% SDS-PAGE. Detection of radiolabeled protein was performed by autoradiography.

Polyosomes Gradients. Cytoplasmic extracts from 5 × 107 cells were loaded on the top of linear 15–40% sucrose gradients containing 0.5 mg/ml heparin, 150 μg/ml cycloheximide, and 20 mM of DTT. The samples were centrifuged at 4° C for 2 h at 38,000 rpm in a SW41 swinging bucket rotor. Immediately after centrifugation, gradients were separated into 11 fractions by pipetting 0.5-ml fractions from the top of gradient. After deproteinization and ethanol precipitation, each pellet was resuspended in 50 μl of diethyl procatecholate-treated water. From each fraction, 5 μl was loaded on Hybond-N membranes in a MINIFOLD apparatus (Schleicher & Schuell Inc.) with gentle vacuum. Probe labeling and hybridization was as described for Northern blotting (49).

RESULTS

The MMP-9 mRNA levels in the 148–1, LMD and 148–1, PA cells were compared by RNA blot analysis and were found to be equivalent in both cell lines (Fig. 1). Nonetheless, these cell lines released widely differing amounts of secreted MMP-9. Fig. 2A used gelatin zymography to examine the difference in MMP-9 in conditioned medium from the cell lines. The medium from 148–1, LMD cells contained higher levels of gelatinolytic activity at M, 105,000 than the 148–1, PA cells. Fig. 2B confirmed these results with immunoblotting. We asked whether this difference would be maintained if mRNA

![Fig. 1. RNA blot analysis of MMP-9 mRNA in murine prostate carcinoma cell lines. Total RNA (20 μg) was blotted using a probe for murine MMP-9 mRNA and rpl32 as a loading control. Lane A shows RNA from 148–1, LMD and B from 148–1, PA.](cancerres.aacrjournals.org)
levels were elevated using an expression vector for MMP-9. Infection with the retrovirus pMIGR1L bearing the murine MMP-9 cDNA led to higher amounts of secreted MMP-9 in the conditioned medium for both cell types. Still the levels of MMP-9 found in conditioned medium from the infected 148–1,LMD cell line were higher than those from the 148–1,PA cell line. The mRNA levels directed by the retroviral vector expressing the murine MMP-9 cDNA were similar in both infected cell lines (Fig. 3). Thus, the differences between amounts of MMP-9 released by the cell lines could not be attributed to transcriptional differences for MMP-9.

A retroviral vector was constructed from the cDNA for the human MMP-9 and used to infect the cells. The amount of human MMP-9 released from the 148–1,LMD cell line and the 148–1,PA cell line were the same (Fig. 2). The gelatinolytic activity from the human MMP-9 was seen at Mr 92,000 as expected, clearly differentiating it from the murine form. Infection resulted in relatively high levels of expression, but these were similar between both cell lines. These results indicate that the differences seen after infection with the murine MMP-9 retrovirus were not an artifact particular to the retrovirus virus, but reflected a difference in response of the cells to murine MMP-9. The RNA was separated by electrophoresis and hybridized to probes for murine MMP-9. Total RNA was isolated from 148–1,LMD (A) or 148–1,PA (B) cells after infection with the retroviral vector pMIGR1L/m105 coding for the expression of murine MMP-9. The RNA was separated by electrophoresis and hybridized to probes for murine MMP-9 and the loading control rpL32. The top band (m105 + GFP) corresponds to the fusion message for MMP-9 and GFP. The band indicated as m105 is the mRNA predicted to code for authentic MMP-9, and the band labeled rpL32 is a loading control.

DISCUSSION

In this report we describe studies examining the mechanisms regulating differential secretion of MMP-9 between two prostate carci-

Fig. 2. Detection of MMP-9 in conditioned medium, endogenous expression and expression after infection with retroviral vectors for MMP-9. 148–1,LMD, the medium conditioned by 148–1 LMD cells and 148–1,PA, lanes with medium from 148–1,PA cells. M indicates conditioned medium from parental cells. H indicates conditioned medium from cells infected with a retrovirus coding for human MMP-9. M indicates conditioned medium from cells infected with a retrovirus encoding murine MMP-9.

Conditioned medium was concentrated and subjected to gelatin gel electrophoresis (A) or subjected to immunoblotting using an anti-MMP-9 antibody (B) as detailed in “Materials and Methods.”

Fig. 3. mRNA levels after infection with a retrovirus expression vector for murine MMP-9. Total RNA was isolated from 148–1,LMD (A) or 148–1,PA (B) cells after infection with the retroviral vector pMIGR1L/m105 coding for the expression of murine MMP-9. The RNA was separated by electrophoresis and hybridized to probes for murine MMP-9 and the loading control rpL32. The top band (m105 + GFP) corresponds to the fusion message for MMP-9 and GFP. The band indicated as m105 is the mRNA predicted to code for authentic MMP-9, and the band labeled rpL32 is a loading control.

Fig. 4. Pulse chase analysis for MMP-9 in prostate carcinoma cells. 148–1,LMD and 148–1,PA cells were depleted of methionine, pulsed with [35S]methionine and cysteine and then chased with cold methionine/cysteine. The RNA was separated by electrophoresis and hybridized to probes for murine MMP-9 and the loading control rpL32. The top band (m105 + GFP) corresponds to the fusion message for MMP-9 and GFP. The band indicated as m105 is the mRNA predicted to code for authentic MMP-9, and the band labeled rpL32 is a loading control.

Fig. 5. mRNA levels in the polysome fractions 7–11 was markedly higher in the 148–1,LMD cells than in the 148–1,PA cells. This result confirms that the translation efficiency for MMP-9 is greater in the 148–1,LMD cells than the 148–1,PA cells, because more mRNA is associated with the polysomes, although the levels of the mRNA are the same (Fig. 5). Similar results were obtained for these cells without retroviral infection using the endogenous MMP-9 mRNA message (data not shown).
MMP-9 mRNA levels in murine prostate carcinoma cell lines. RNA from 148-1 LMD and 148-PA cells after infection with the retrovirus pMIGR1I1m105 was centrifuged on sucrose gradients. The fractions from each gradient were electrophoresed on 0.9% formaldehyde agarose gels and stained with ethidium bromide. The ribosomes can be seen in fractions 3–11 shown in A. B shows a dot blot of equal amounts of each fraction shown in A hybridized with a murine MMP-9 probe. The right panel labeled loaded RNA shows the dot blot of the cytoplasmic RNA before loading on the gradient.

The recruitment of mRNAs to polysomes can be an indicator of translational efficiency and can be an important factor in the regulation of MMP-9 secretion. Graff et al. reported a correlation between reduction in eIF-4E levels and decreased MMP-9 translation. In many cases MMP-9 regulation has been reported to be a component of translational efficiency regulating a difference in MMP-9 secretion. Graff et al. document a significant correlation between reduction in eIF-4E levels and decreased MMP-9 translation.

The recruitment of mRNAs to polysomes can be an indicator of translational efficiency and can be a component of translational efficiency regulating a difference in MMP-9 secretion. Graff et al. reported a correlation between reduction in eIF-4E levels and decreased MMP-9 translation. In many cases MMP-9 regulation has been reported to be a component of translational efficiency regulating a difference in MMP-9 secretion. Graff et al. document a significant correlation between reduction in eIF-4E levels and decreased MMP-9 translation.
MMP-9 regulation by translational efficiency


Brew, K., Dinakapandian, D., and Nagase, H. Tissue inhibitors of metalloprotei-


Goldberg, G. I., Strongin, A., Collier, I. E., Gerrič, L. T., and Marmer, B. L. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metallopro-


Pavloff, N., Staikou, P. W., Kushnani, N. S., and Hawkes, S. P. A new inhibitor of metalloprotei-


Thompson, T. C., Southgate, J., Kičhener, G., and Land, H. Multistage carcinogene-


Regulation of Matrix Metalloproteinase-9 (MMP-9) by Translational Efficiency in Murine Prostate Carcinoma Cells

Yong Jiang and Ruth J. Muschel


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/62/6/1910

Cited articles
This article cites 58 articles, 28 of which you can access for free at:
http://cancerres.aacrjournals.org/content/62/6/1910.full.html#ref-list-1

Citing articles
This article has been cited by 14 HighWire-hosted articles. Access the articles at:
/content/62/6/1910.full.html#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.