Matrix Metalloproteinase (MMP)-7 Activates MMP-8 But Not MMP-13

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Abstract: Matrix Metalloproteinases (MMPs) are a class of zinc-dependent enzymes that degrade extracellular matrix components, particularly collagen. MMPs have been implicated in a diverse list of pathological processes, including cancer and cardiovascular disease. Recent efforts to bring MMP inhibitors to clinical trials, however, have proved disappointing. These failures are attributed, in part, to the non-selective nature of current inhibitors. The possibility also exists, however, that inhibition of a particular MMP type will lead to feedback accumulation of parallel MMP members. MMP-7, also known as matrilysin, has a broad list of substrates, including denatured collagen and other MMPs involved in the collagenolytic pathway, namely MMP-1, MMP-2, and MMP-9. Whether the additional collagenases, MMP-8 and MMP-13, are also activated by MMP-7 has not been explored. We show here that recombinant active MMP-7 was able to process MMP-8 to its active form *in vitro*, but did not activate MMP-13. In the left ventricles of mice lacking the MMP-7 gene, MMP-8 levels increased while MMP-13 levels decreased *in vivo*. The switch in MMP profile was not accompanied by a change in left ventricular dimensions or wall thickness. Together, these data suggest that MMP-8 is an *in vivo* substrate of MMP-7, and that the accumulation of pro-MMP-8 in the absence of MMP-7 downregulates pro-MMP-13 levels in order to maintain baseline collagenolytic function. The interplay between MMP-8 and MMP-13 suggest that these MMPs may play reciprocal roles. The design of selective MMP inhibitors, therefore, must take into consideration changes in parallel MMP types as a potential compensatory mechanism.

Key Words: Matrix metalloproteinase, MMP inhibition strategies, MMP-7, MMP-8, MMP-13, collagenase.

INTRODUCTION

Matrix Metalloproteinases (MMP) are a family of >25 zinc dependent enzymes that regulate matrix turnover during remodeling processes in both normal and disease states [1]. MMPs play important roles in normal physiological events such as wound healing and development [2, 3], as well as during pathophysiological events such as cancer and cardiovascular disease [4, 5]. Although MMPs are collectively capable of degrading all the components of the extracellular matrix, each MMP is individually substrate specific. Matrilysin (MMP-7), the smallest of all the MMPs (28 kD pro form and 19 kD active form), is known to cleave a number of substrates including denatured collagen, elastin, and laminin [6]. Additionally, MMP-7 can activate other MMPs, including MMP-1, MMP-2, and MMP-9 [6-8]. Whether MMP-7 can activate other members of the collagenase subgroup has not been determined. This is particularly relevant in murine models, as adult mice do not express the MMP-1 gene [9]. This report provides the first *in vitro* and *in vivo* evidence that MMP-7 can activate MMP-8, but not MMP-13.

MATERIALS AND METHODS

MMP-8 Activation *In Vitro*

In order to determine whether MMP-7 could activate pro-MMP-8 or pro-MMP-13, we added increasing amounts of active MMP-7 with pro-MMP-8 and –13. For the *in vitro* activation assay, 40 ng of human pro-MMP-8 (Chemicon) or 8 μL of human MMP-13 positive control (Oncogene) in 1X developing buffer (12.1g Tris base, 63g Tris-HCl, 117g NaCl, 7.4g $CaCl_2$, 2 mL Brij 35; and water to 1L final volume) was incubated with increasing amounts of human active MMP-7 (0, 0.5, 1 and 2 units; Chemicon). Pro-MMP-8 and pro-MMP-13 were used to excess to ensure that substrate was not rate-limiting. After a 3-hour incubation period, equal amounts of the sample (20μL) were run on a 12 well precast 10-20% polyacrylamide gel (Bio-Rad). The gel was then stained with commassie blue and image analysis was used to quantify the densitometry of the bands.

Mice

All animal procedures were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, National Academy Press, Washington, DC, 1996) and were approved by the Institutional Animal Care and Use Committee at the Medical University of South Carolina. Mice with targeted deletion of the MMP-7 gene (MMP-7 null) were generated in the laboratory of Dr. Lynn M. Matrisian using homologous recombination technology in embryonic stem cells and backcrossed >10 generations into the C57BL/6 strain, as previously described [10]. Genotypes were confirmed by tail DNA examination. For this study, the left ventricle from 9 wild type (n=4 males and 5 females) and 6 MMP-7 null (n=3 male and 3 female) young adult mice, 8 to 12 weeks old, were harvested. Mice were anesthetized with 5% inhalational isoflurane and euthanized by injecting cardioplegic solution [11] into the left ventricular cavity. After flushing the coronary vasculature,

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the heart was removed and the left ventricle was separated, weighed, and snap frozen.

Echocardiography

Mice were anesthetized with 1-2% isoflurane and heart rates maintained at ≥400 beats per minute to ensure physiologic conditions [12]. Echocardiographic acquisition and analysis were performed using the Sonos 5500 ultrasound (Agilent Technologies) with a high band linear 15.7 MHz transducer to obtain short axis views at the mid-papillary level and M-mode images. Dimensions and wall thickness were obtained as previously described [13].

MMP-8 and MMP-13 Immunoblotting of Left Ventricle Extracts

Frozen tissue was homogenized and immunoblotting was performed as described previously [13, 14]. The antibodies for MMP-8 (Oncogene) and MMP-13 (Chemicon) were used at 0.1 μg/mL, and the blots were visualized by chemiluminescence. Image analysis was used to quantify the densitometry of the bands.

Statistical Analysis

Data are presented as mean±standard error of the mean. Statistical analyses were performed using Intercooled Stata 8.0 for Windows (Stata Corporation, College Station, TX). Activation of MMP-8 by MMP-7 was evaluated by regression analysis to determine linearity. Comparisons between groups were made by unpaired t-test.

RESULTS

MMP-7 is Able to Activate MMP-8 But Not MMP-13 In Vitro

The ability of MMP-7 to activate either pro-MMP-8 and/or pro-MMP-13 was determined by *in vitro* substrate analysis using recombinant active MMP-7 and recombinant pro-MMP-8 and pro-MMP-13. MMP-7 induced a concentration dependent increase in active MMP-8 levels following 3 h incubation, shown in Fig. (**1**). The amount of MMP-8 produced correlated directly and significantly with the amount of active MMP-7 added (R^2= 0.97; p=0.015), shown in Fig. (**2**). In contrast, MMP-7 generated no active MMP-13 bands (Fig (**1**)).

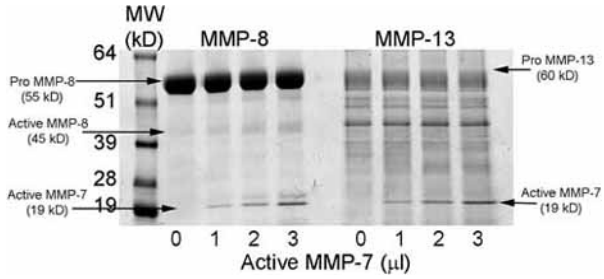

Fig. (1). MMP-7 activates pro-MMP-8. SDS-PAGE analysis of recombinant pro-MMP-8 (40 ng) incubated with increasing amounts of recombinant active MMP-7 (0, 1, 2, and 3 μL, which corresponds to 0, 0.5, 1, and 2 enzyme units). A molecular weight (MW) marker is shown on the left.

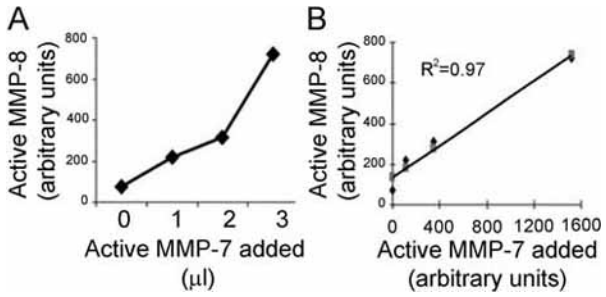

Fig. (2). MMP-8 activation is dependent on the concentration of MMP-7. A) The amount of activated MMP-8 (y axis; arbitrary densitometry units) increases as a function of the amount of active MMP-7 (x axis; enzyme units) added. B) The density of activated MMP-8 (arbitrary densitometry units) is linearly proportional to the density of active MMP-7 (arbitrary densitometry units) added. The correlation coefficient (R^2) of the relationship was 0.97 (p=0.015). The triangles are the actual data points; the squares are the predicted points.

In the Absence of MMP-7, MMP-8 Accumulates and MMP-13 Levels Decrease in the Left Ventricle

Because MMP-7 was able to activate MMP-8, we evaluated whether MMP-8 levels were altered in the left ventricle of MMP-7 null mice. We selected the left ventricle for analysis because past and future studies from our laboratory are focused on MMP regulation in the myocardium. Pro-MMP-8 levels were elevated in the left ventricle of MMP-7 null mice, shown in Fig. (**3**). Surprisingly, MMP-13 levels were significantly lower in the MMP-7 null mice, suggesting

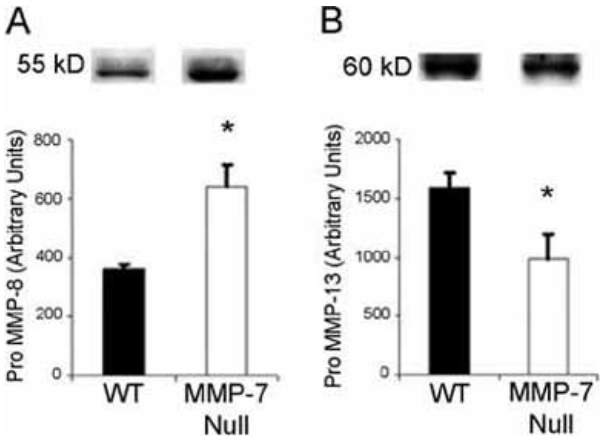

Fig. (3). MMP-8 levels increase and MMP-13 levels decrease in the left ventricle of MMP-7 null mice. A- top) A representative immunoblot band for MMP-8 in left ventricle extracts from wild type (WT) and MMP-7 null (Null) mice. bottom) Densitometric analysis of the MMP-8 immunoblot for WT (n=9) and Null (n=6) left ventricle extracts demonstrated that MMP-8 was significantly elevated in Null mice (p=0.03). B- top) A representative immunoblot band for MMP-13 in left ventricle extracts from WT and Null mice. bottom) Densitometric analysis of the MMP-13 immunoblot for WT (n=9) and Null (n=6) left ventricle extracts demonstrated that MMP-13 was significantly decreased in Null mice (p=0.02).

that a reciprocated decrease in MMP-13 occurred when pro-MMP-8 accumulated. Given that MMP-7 was not able to activate MMP-13, this data suggests that MMP-7 can indirectly regulate MMP-13 levels *via* regulation of MMP-8, illustrated in Fig. (**4**). We did not evaluate MMP-1 levels, as adult mice do not produce the MMP-1 protein [9].

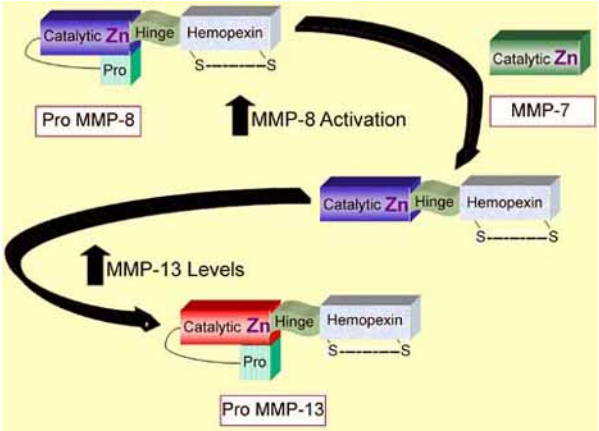

Fig. (4). A schematic representation of the interplay between MMP-7, MMP-8, and MMP-13. The activation of MMP-8 by MMP-7 serves as a regulatory mechanism to increase levels of MMP-13. The net effect, therefore, is a higher overall collagenolytic potential. This would be relevant in models of inflammation and remodeling, for example. In MMP-7 null mice, the lack of MMP-7 leads to substrate (pro-MMP-8) accumulation and decreased MMP-13 levels. This would serve to maintain a basal collagenolytic potential at a particular set point.

Left Ventricular Dimensions and Wall Thickness are Preserved in the MMP-7 Null Mice

MMP-8 is expressed in several cell types, including macrophages [15]. To rule out the possibility that MMP-7 deletion resulted in an inflammatory response, which would induce left ventricular dilation and also explain the increase in MMP-8 levels, left ventricular dimensions and wall thickness were measured by echocardiographic analysis (Table **1**). None of the parameters measured differed between wild type and MMP-7 null mice, suggesting that the increase in MMP-8 levels was not due to an increase in macrophage accumulation within the left ventricle.

DISCUSSION

The results presented here demonstrate that MMP-7 is an *in vitro* activator of MMP-8 and that substrate accumulation of MMP-8 and subsequent downregulation of MMP-13 occurs *in vivo* in the absence of MMP-7. There are two main implications of this study. First, MMP-7 serves as an important regulator of collagenase levels under basal conditions in the left ventricle. In addition to MMP-2 and MMP-9, MMP-7 can also degrade collagen that has been initially cleaved by collagenases [16]. Crabbe and colleagues previously demonstrated that activated MMP-2 could activate MMP-1 and stimulate collagen degradation [17]. A similar mechanism of MMP activation, which served to regulate overall collagenolytic capabilities, was also demonstrated in this study for MMP-7 and MMP-8. Second, this study provides insight into the reciprocal nature of MMPs that can be used for rational MMP drug design.

The co-expression of MMP-7 and MMP-8 is relevant in several cardiovascular and inflammatory processes, including atherosclerosis and gingivitis. Both MMP-7 and MMP-8 are present in atherosclerotic plaques [15, 18, 19]. Active MMP-7 and active MMP-8 co-localize in peri-implant sulcular fluid from patients with gingivitis, and the levels of each correlate significantly with the other [20]. This coupling suggests a relationship between these specific MMPs in several models of tissue injury. The fact that left ventricular dimensions were not altered between wild type and MMP-7 null mice indicates that the shift in MMP types likely did not influence structure and function in the baseline state. Homeostatic perturbation, such as would occur during an infection or inflammatory response, may unmask functional differences in expressing different MMP types.

In conclusion, we have shown that MMP-7 is able to activate pro-MMP-8 *in vitro*. The accumulation of pro-MMP-8 in the absence of MMP-7 *in vivo* is accompanied by a decrease in pro-MMP-13 levels, suggesting an interplay between MMP-7, MMP-8, and MMP-13 to regulate collagen turnover. The design of selective MMP inhibitors should take into consideration the cross regulation of MMP family members.

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Table 1. Echocardiographic Analysis of Wild Type and MMP-7 Null Mice

	Wild Type (n=51)	MMP-7 Null (n=30)	p
Heart Rate (beats per minute)	470±7	459±7	0.33
Left Ventricle Mass / Body Weight Ratio (mg/g)	4.0+0.1	4.0+0.1	0.96
End Diastolic Dimensions (mm)	4.05±0.05	3.93±0.06	0.11
End Systolic Dimensions (mm)	2.72±0.06	2.66±0.09	0.53
Posterior Wall Thickness (diastolic; mm)	0.62+0.01	0.60±0.02	0.42

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