Extracellular Matrix Metalloproteinase Inducer (EMMPRIN) Is Increased in Human Left Ventricle after Acute Myocardial Infarction

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Introduction
Infarct expansion is a major cause of early left ventricular dilation after acute myocardial infarction (AMI), which may lead to congestive heart failure, aneurysm formation, and myocardial rupture (1). Past studies have demonstrated that the degradation of the myocardial extracellular matrix (ECM) is a recognized prerequisite for the occurrence of dilation. There are >20 known endogenous matrix metalloproteinases (MMPs) that have been identified in the heart, and each has specificity for one or more ECM components (2). Experimental and clinical studies have demonstrated that MMP activation is involved in the development and progression of myocardial ECM remodeling and infarct expansion after AMI (3–5). Among the MMP species, MMP-2 and MMP-9 are activated within myocardial tissues, and targeted deletion of MMP-2 (MMP-9) or MMP inhibitors can ameliorate left ventricular...
myocardial remodeling after AMI in mice models (6–10). These data indicate that MMP-2 and MMP-9 may play an important role in LV remodeling after AMI (11,12). To date, there are no effective methods to control the expression of MMPs.

EMMPRIN was first identified on the surface of tumor cells and stimulates adjacent fibroblasts, endothelial cells or tumor cells to produce MMPs, facilitating the invasion of cancer cells (13). Recently, studies have demonstrated that EMMPRIN is expressed on cardiac myocytes (14,15), and its expression is significantly elevated in association with MMP expression in the myocardium of patients with dilated cardiomyopathy (14). Upregulation of EMMPRIN on monocytes was also reported in humans after AMI (16). However, there is a paucity of data in humans evaluating EMMPRIN expression on cardiac myocytes and its potential relevance for MMP-2 (MMP-9) upregulation in myocardium after AMI. The present study was undertaken to analyze the expression of EMMPRIN and MMPs in human myocardium after AMI. We analyzed the amounts of MMPs and EMMPRIN in a unique set of samples obtained from the border zone of human infarcts and compared these with myocardial samples from healthy accident victims. Moreover, we investigated whether changes in EMMPRIN expression are related to MMP expression in human left ventricle after AMI.

Materials and Methods

Study Subjects

Tissues from the human left ventricular myocardium were obtained from the surrounding ventricular infarction area during autopsy (n = 10, age 34–70 years; eight males, two females); the span of AMI was from 7–15 days. The infarct area (identified by nitroblue tetrazolium staining) was present at different anatomic locations as indicated in Table 1. Normal left ventricular samples (n = 5, age 16–40 years; four males and one female) were obtained from donor hearts of healthy accident victims. There was no significant difference in the time of autopsy between the two groups (AMI: 24.0 ± 6.2 h; control group: 20.8 ± 3.8 h). Patient data are shown in Table 1. Each specimen was obtained at autopsy, snap-frozen in liquid nitrogen, and stored at −80°C until further use.

Sections of these tissues were fixed with 10% buffered formalin to maintain morphologic integrity. Then, each specimen was embedded in paraffin, cut into 4-μm sections and stained with hematoxylin-eosin (H&E). These same sections were also used for immunohistochemical analysis. Consent was obtained from the families of all patients for collecting the myocardial samples used in the study, and protocols were approved by the Medical School of Sun Yat-sen University Institutional Review Board for Human Research.

Immunohistochemical Staining

Goat polyclonal antibodies against human EMMPRIN, MMP-2 and MMP-9 (Santa Cruz Biotechnology, Santa Cruz, CA) were used as the primary antibodies for immunohistochemistry. Peroxidase-conjugated secondary antibodies (rabbit anti-goat IgG) were used with these primary antibodies.

The 4-μm, paraffin-embedded sections were dewaxed in xylene, rehydrated in alcohol, and immersed in 3% hydrogen peroxide for 10 min to suppress endogenous peroxidase activity. Antigen retrieval was performed by heating (100°C) each section for 30 min in 0.01 mol/L sodium citrate buffer (pH 6.0). After three rinses (5 min each) in phosphate-buffered saline (PBS, pH 7.2–7.4), sections were incubated for 2 h at room temperature with 1:100 diluted EMMPRIN, MMP-2 and MMP-9 primary antibodies, and the sections were incubated in a moist chamber for 1 h. After washing and bathing for 5 min in PBS, the biotinylated secondary antisera cocktail including rabbit anti-goat IgG diluted 1:400 was incubated on the slides for 15 min at room temperature in a moist chamber. The sections were then processed by using the MaxVision HRP-Polymer Anti-Rabbit IHC Kit and DAB solution (both from Maxim, Fuzhou, China). Sections were then counterstained with Mayer’s hematoxylin.

Western Blot Analysis

Proteins were subjected to 11% gradient SDS-PAGE gel and transferred to Immobilon P membrane (Millipore, Bedford, MA) at 12 V for 1 h. Proteins were detected using a 1:2000 diluted rabbit polyclonal antibody to human MMP-2 (Abcam, Cambridge, MA), a 1:4000 rabbit polyclonal antibody to human MMP-9 (Abcam), a 1:200 diluted monoclonal antibody to human EMMPRIN (Pharmingen, Mabtech, Sweden), and incubated with HRP-labeled secondary antibody. Bands were visualized using enhanced chemiluminescence (ECL). Western blots are shown in Figure 1.

Table 1. Overview of patient data

<table>
<thead>
<tr>
<th>M/F</th>
<th>Age (years)</th>
<th>Sample locations</th>
<th>Hypertension</th>
<th>Diabetes</th>
<th>IV thrombolysis</th>
<th>PCI</th>
<th>Previous MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMI</td>
<td>8/2 (58 ± 11)</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
<td>1/10</td>
</tr>
<tr>
<td>Control group</td>
<td>4/1 (29 ± 10)</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1/5</td>
<td>0</td>
<td>–</td>
</tr>
</tbody>
</table>

PCI, percutaneous coronary intervention; MI, myocardial infarction.
San Diego, CA), a 1:4000 diluted polyclonal antibody against glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Abcam); a secondary antibody (anti-human rabbit IgG, horseradish peroxidase conjugated, Amersham, UK) was used at 1:200 dilution. Signals were detected with an ECL kit (Amersham). The relative intensities of the protein bands were analyzed by Bandscan (v. 4.3, Glyko, Novato, CA).

**Statistical Analysis**

Data are shown as the mean ± standard deviation. Each experiment was repeated three times. Statistical analyses were performed with an independent-sample t-test, and the relationship between EMMPRIN and MMPs was determined by linear regression analysis using SPSS v.13.0 software (SPSS, Chicago, IL). A p value <0.05 was considered statistically significant.

**Results**

**Immunohistochemical Staining**

Myocardial infarction was confirmed by pathology diagnosis in all 10 subjects in the AMI group. In the normal group, the myocardial nuclei were clear, fibers were integral and arranged in an orderly manner (Figure 1B), and a trace of immunoreactivities for MMP-2, MMP-9 and EMMPRIN was detected in the myocardial fibers. However, in the AMI group, myocardial fibers were fractured and disordered, and a massive influx of inflammatory cells was also observed in the ruptured infarcts (Figure 1, panel A). EMMPRIN stained as dark brown in the myocardial fibers and was located on the cell membranes. The expression of EMMPRIN was strongly increased in the AMI group (Figure 1G) compared with the normal group (Figure 1H). In addition, MMP-2 (Figure 1, panel C), MMP-9 (Figure 1, panel E) expression was also increased in the AMI group. Furthermore, EMMPRIN was co-localized with MMP-2 and MMP-9 immunoreactivities.

**Western Blot Analysis**

The total amounts of MMP-2, MMP-9 and EMMPRIN protein in samples from ruptured infarcts were compared to samples from the normal group by Western blotting. The total amount of MMP-2 protein was higher in the infarcted hearts (0.25 ± 0.03 vs. 0.64 ± 0.11, p <0.001) (Figure 2). MMP-9 levels were also significantly elevated in infarct tissue compared with normal tissue obtained from the control group (0.16 ± 0.03 vs. 0.39 ± 0.07, p <0.001) (Figure 2). Furthermore, EMMPRIN levels demonstrated a more than 3-fold increase in AMI patients compared with the normal group (1.10 ± 0.17 vs. 0.25 ± 0.04, p <0.001) (Figure 2).

**Association Between EMMPRIN Expression and MMP Levels After AMI**

To further examine the proximity of EMMPRIN expression to MMP-2 and MMP-9 upregulation, we determined whether EMMPRIN was co-expressed with MMP-2 and MMP-9 by analyzing the Western blot data. Figure 3 demonstrates that enhanced EMMPRIN expression not only correlated positively with elevated MMP-2 levels (Figure 3A, r² = 0.80, p <0.001) but also correlated with MMP-9 upregulation (Figure 3B, r² = 0.424, p = 0.041). These data show for the first time that enhanced expression of EMMPRIN may induce MMP in human left ventricle after AMI.

**Discussion**

This study revealed that EMMPRIN, MMP-2 and MMP-9 expression increased in the area surrounding the infarct zone in human AMI. EMMPRIN was predominantly expressed by myocardial cells but was not observed in cardiac fibroblasts. In addition, our data show that EMMPRIN upregulation was correlated with elevated MMP levels in the infarct border zone.

Pathological remodeling of the left ventricle is a recognized risk factor contributing to the pathogenesis of heart failure after AMI, and the development of ventricular dilation is a fundamental component of this structural remodeling (17). Many studies have shown that MMPs are able to degrade ECM and affect ventricular remodeling during the healing process after AMI (3–5). Our study showed that the expression of MMP-2 and MMP-9 increased significantly in the areas surrounding the infarct zone, which suggests that MMPs play a critical role in ventricular remodeling after AMI in humans.

EMMPRIN, also known as Basigen, collagenase stimulatory factor, and CD147, is a novel 58-kDa transmembrane glycoprotein of the Ig superfamily (13). EMMPRIN has been most extensively investigated with respect to tumor invasion and metastasis and has been implicated as a factor contributing to the induction of MMP expression (13,18). It has been shown that EMMPRIN is expressed on cardiac myocytes (14,15), forms a complex with α3β1 integrin and plays a role in outside-in signaling and induction of MMP expression (14). Our study showed that the expression of EMMPRIN was increased on the membranes of cardiac myocytes but not on cardiac fibroblasts. This suggests that EMMPRIN may provide not only an autocrine mechanism for regulation of myocyte-derived MMPs, but also a paracrine mechanism for fibroblast MMP production (15).

Our data show that EMMPRIN upregulation was correlated with elevated MMP-2 and MMP-9 level in the areas surrounding the infarct. It has been shown that EMMPRIN is upregulated with the increased expression of MT1-MMP on monocytes as well as plasma MMP-9 activity in human
Figure 1. H&E staining of the ventricular myocardium reveals increased infiltration of neutrophils and that myocardial fibers were fractured and disordered in patients with acute myocardial infarction (AMI) (A, ×200). In contrast, myocardial fibers were integral and arranged in an orderly manner in the normal group (B, ×200). MMP-2 (C, ×200), MMP-9 (E, ×200) and EMMPRIN (G, ×200) were enhanced in human myocytes after AMI compared with the normal group (panel D, F and G, ×200). EMMPRIN was co-localized with MMP-2 and MMP-9 immunoreactivities. Solid arrow: MMP-2 (−9) in the cardiac myocytes or EMMPRIN expression on the membrane of these cells. Hollow arrow: the absence of EMMPRIN localization in cardiac fibroblasts (×400). Color version of this figure available online at www.arcmedres.com.
AMI (16). β-adrenergic receptor stimulation and H₂O₂ can increase MMP activity and MMP-2 expression as well as increase EMMPRIN expression in cultured cardiac myocytes (15). EMMPRIN upregulation associated with the elevated MMP-2 levels was found in the left ventricle of patients with aortic stenosis (19). Recently, Zavadzkas et al. (20) reported that persistent cardiac restricted overexpression of EMMPRIN caused increased levels of both MMP-2 and MT1-MMP. Our study showed that the expression of EMMPRIN was increased and correlated with local MMPs in AMI. As a key upregulator of local MMP expression, the increased expression of EMMPRIN in the area surrounding the infarct zone might play an important role in ventricular remodeling in AMI patients. Therefore, inhibiting the expression of EMMPRIN may be effective in preventing MMP expression and may decrease the extent of ventricular remodeling after AMI.

In summary, this is the first study to demonstrate that EMMPRIN expression is significantly increased in human AMI myocardial tissue. Our data show that EMMPRIN upregulation correlated with enhanced MMP-2 and MMP-9 levels. This study underscores the potential of EMMPRIN as a novel target for AMI ventricular remodeling. Further studies should be undertaken to prove that inhibition of EMMPRIN can suppress ischemia-induced MMP production and ventricular remodeling.

Figure 2. Western blotting of MMP-2, MMP-9 and EMMPRIN protein in myocytes from AMI group and the control group. The expression of MMP-2, MMP-9 and EMMPRIN protein in AMI group was higher than in the control group. Glyceraldehyde phosphate dehydrogenase (GAPDH) was used to confirm equal loading. Values (ratio of the density = EMMPRIN/GAPDH) are the mean ± standard deviation. *p < 0.001 (AMI group vs. control group).

Figure 3. Relationship between MMP levels and EMMPRIN expression. Scatter graph shows the correlation of MMP-2 levels (A), MMP-9 levels (B) and EMMPRIN expression after AMI. The relationship between MMP-2 levels, MMP-9 levels and EMMPRIN expression was analyzed using simple regression ($r^2 = 0.82, p < 0.001$, $r^2 = 0.424, p = 0.041$, respectively).
Study Limitations

Tissue inhibitors of metalloproteinases (TIMPs) are well-recognized suppressors of MMP activities. Obviously, we cannot fully exclude that the increase in MMP activity and content may be due to decreased TIMP activity in human AMI. However, previous studies showed that TIMP-1 and TIMP-2 mRNA remain markedly elevated on days 3 and 21 at the site of myocardial infarction in both mice and rats (24–26). In rabbits, TIMP-1 protein expression was at basal levels on days 7 and 14 after AMI (27). TIMP-1 levels were also observed significantly upregulated in human ruptured infarcts (28). These findings show that the expression of TIMPs increases in the early stage of AMI. Therefore, we are convinced that elevated levels of MMPs are at least primarily caused by EMMPRIN rather than TIMP downregulation in early human AMI.

In the present study, there were three patients who received specific therapy such as ACE inhibitors (ACEI) and statins, and 1/3 of these patients received percutaneous coronary intervention (PCI). Another seven patients had sudden death without medical records. It has been well documented that many medications such as ACEI and statins, as well as PCI, will decrease MMP levels (29–34). However, we observed that both EMMPRIN and MMPs were upregulated in the AMI group compared with the normal group. This suggests that the upregulation of EMMPRIN and MMPs was mainly caused by AMI rather than medications or PCI.

Another important limitation is that our samples were not collected immediately after AMI but about 24 h after death. Because of degradation of proteins and proteases after death and most likely greater degradation from infarcted tissue, we therefore cannot completely exclude this effect on our results.

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References

metalloproteinase-9 expression after acute myocardial infarction. 


