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Up-regulation of matrix metalloproteinase-2 and membrane-type 1-matrix metalloproteinase were coupled with that of type I procollagen in granulation tissue response after the onset of aortic dissection

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Abstract The pathophysiological significance of matrix metalloproteinases (MMPs) in aortic dissection remains poorly understood. The purpose of the present study is to clarify the significance of MMPs in aortic dissection. The activities and distributions of MMP-2, membrane-type 1-MMP (MT1-MMP), and MMP-9 were evaluated by gelatin zymography, immunohistochemistry, and in situ hybridization in 29 patients and seven autopsy cases. To assess if these MMPs are related to a tissue remodeling process, we compared the expression of these MMPs with that of type I procollagen and platelet-derived growth factor receptor β chain (PDGF R β). Patients were divided into three groups based on histological findings: acute, intermediate, and healed groups. The most remarkable changes were observed in the intermediate group, in which MMP-2 activity peaked and tissue expression of mRNAs for MMP-2 and MT1-MMP were observed in spindle-shaped cells in the neointima, organizing thrombus, and the adventitia.

These expression patterns were essentially coupled with those of type I procollagen mRNA and PDGF-R β protein. The association of MMP-2, MT1-MMP, type I procollagen, and PDGF-R β suggests that MMP-2 and MT1-MMP could be involved not only in the degradation of aortic tissue but also in tissue remodeling, which may be associated with the healing process.

Keywords Cardiovascular · Clin pathol · Extracell matrix · Immunohistochem · In situ hybrid

Introduction

Aortic dissection is a dynamic disease that may involve any portion of the aorta. Despite significant advances in diagnostic and therapeutic techniques, morbidity and mortality rates remain unsatisfactory. Conversely, the spontaneous closure of the false lumen has been documented in some patients and this event is a favorable prognostic factor [1, 25]. These clinical data suggest the significance of the healing and repair processes in dissections of the aorta.

Tissue remodeling is an essential part of all repair processes involving the degradation and synthesis of the extracellular matrix. Matrix metalloproteinases (MMPs) can degrade various components of the extracellular matrix. Most MMPs are secreted as zymogens that are most likely activated in the extracellular space by other proteases. Membrane-type 1-MMP (MT1-MMP) forms a complex with tissue inhibitor of metalloproteinase-2 (TIMP-2) serving as a cell surface receptor for pro-MMP-2 activation [22], and degrades collagen fibers [20]. Among the MMPs, MMP-2 and MMP-9 have been implicated in the pathogenesis of aortic aneurysms and atherosclerotic plaque [6, 14, 21]. MMP-1, MMP-2, and MMP-9 have been reported to play a role in the development and progression of aortic dissection [10, 12, 15, 23].

Considering the complex mechanisms involved with these matrix-degrading enzymes, we constructed the

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present study to examine the gelatinolytic activities of MMP-2 and MMP-9, and in situ distributions of MMP-2, MMP-9, and MT1-MMP, and analyzed their role in aortic dissection. We also evaluated the tissue remodeling processes by examining in situ distributions of type I procollagen, a representative matrix protein, and platelet-derived growth factor receptor beta chain (PDGF-R β), a marker of activated fibroblasts/myofibroblasts [11]. The results reveal the complex significances of MMPs, and we propose that they are not only involved in tissue degradation but also in tissue remodeling after the onset of dissection.

Materials and methods

Patients

Twenty-nine patients with aortic dissection who underwent surgery at Tohoku University Hospital were analyzed in the present study (Tables 1, 2, and 3). To analyze the significance of MMPs after the onset of dissection, the patients were divided into three groups based on the histological findings [26]: acute, intermediate, and healed as described in the “Results” section. A full-thickness specimen oriented in cross-section was obtained from the dissected site adjacent to the entry site (within 3 cm) during surgery and processed for gelatin zymography, immunohistochemistry, and in situ hybridization. Aortic sample sites are listed in Tables 1, 2, and 3. Control specimens of the ascending aorta were obtained 1 to 3 h after death from seven autopsy cases without significant aortic disease (Table 4). To analyze the significance of MMPs at the onset of dissection, specimens from six patients just after the onset of dissection (<24 h, a subgroup of the acute group) were compared with specimens from seven normal individuals obtained during autopsy. A specimen from a macroscopically confirmed nondissected site was also

obtained from the ascending aorta as “nondissected site (<24 h)” in five of six patients who received surgery within 24 h after the onset of a dissection.

The specimens were conventionally processed and stained with hematoxylin–eosin and elastica-Masson’s staining for evaluation by light microscopy. Patients with Marfan’s syndrome, a bicuspid aortic valve, or those undergoing reoperation were excluded from this study. Patients with a traumatic or iatrogenic aortic dissection were also excluded. This study was approved by the Ethical Committee of Tohoku University School of Medicine (#2000-119). Written informed consent was obtained from all patients.

Gelatin zymography

A fresh full-thickness specimen was snap-frozen in liquid nitrogen and stored at -80°C until use. The frozen tissue was homogenized in chilled 0.01 mol/l NaCl containing 0.01 mol/l CaCl_2 , 0.05% Tween 20, 2 mmol/l PMSF, 0.02% NaN_3 , 2% dimethylsulfoxide (DMSO), 2 mol/l urea, and 50 mol/l Tris–Cl at a pH of 7.5. The homogenate was centrifuged at 13,000 rpm for 15 min at 4°C . The supernatant was collected and applied to gelatin zymography. Gelatin zymography was performed as previously described, with minor modifications [13, 17]. Tissue extract containing 7.5 μg of protein was separated on a sodium dodecyl sulfate (SDS) polyacrylamide gel containing 0.8 mg/ml gelatin in a nonreducing condition. Purified MMP-2 and pro-MMP-9 (Yagai, Yamagata, Japan) were used as positive controls. After electrophoresis, the gel was washed three times for 15 min in a washing buffer containing 2.5% Tween 20, 10 mmol CaCl_2 , 1 μmol ZnCl_2 , and 50 mmol Tris–Cl (pH 7.5) to remove SDS. The gel was then incubated with an incubation buffer containing 1% Tween 20, 10 mmol CaCl_2 , 1 μmol ZnCl_2 , and 50 mmol Tris–Cl (pH 7.5) at 37°C for 18 h, and stained with

Table 1 Patient profiles (acute group)

| Age and gender | Hypertension (Y/N) | Duration (days) | Stanford classification | FL (P/O) | Indications for surgery | Site of replacement | Sample site |
|----------------|--------------------|-----------------|-------------------------|----------|-------------------------|---------------------|------------------|
| 52, M | Y | 0 | A | P | Shock, patent FL in Asc | Asc, Arch | Asc ^a |
| 62, F | Y | 0 | A | P | Patent FL in Asc | Asc, Arch | Asc ^a |
| 64, F | Y | 0 | A | P | Patent FL in Asc | Asc, Arch | Asc ^a |
| 78, F | Y | 0 | A | P | Tamponade | Asc | Asc ^a |
| 41, M | Y | 0 | A | P | Patent FL in Asc, AR | Root, Asc, Arch | Asc ^a |
| 62, F | Y | 0 | A | P | Shock, Patent FL in Asc | Asc | Asc |
| 65, F | Y | 1 | A | P | Patent FL in Asc | Asc | Asc |
| 62, M | N | 2 | A | P | Patent FL in Asc | Asc | Asc |
| 66, F | Y | 3 | A | P | Patent FL in Asc | Asc | Asc |
| 77, F | Y | 4 | A | P | Patent FL in Asc | Asc | Asc |
| 52, M | Y | 4 | A | P | Patent FL in Asc | Asc, Arch | Asc |

^aSpecimens were obtained from the dissected and nondissected sites

FL false lumen, P patent, O occluded, Asc ascending aorta, Arch aortic arch, AR aortic regurgitation, Root aortic root

Table 2 Patient profiles (intermediate group)

| Age and gender | Hypertension (Y/N) | Duration | Stanford classification | FL (P/O) | Indications for surgery (maximal aortic diameter) | Site of replacement | Sample site |
|----------------|--------------------|----------|-------------------------|----------|---|---------------------|-------------|
| 81, F | Y | 13 days | A | P | Impending rupture (60 mm) | Arch, Des | Des |
| 67, M | N | 14 days | A | O | Impending rupture (60 mm) | Asc, Arch | Asc |
| 66, M | N | 21 days | B | P | Impending rupture (55 mm) | Arch | Arch |
| 50, F | Y | 1 months | B | O | Redissection (53 mm) | Asc, Arch | Asc |
| 76, F | N | 2 months | A | O | Redissection (55 mm) | Asc | Asc |
| 68, F | N | 3 months | A | O | ULP (48 mm) | Asc, Arch | Asc |
| 70, F | Y | 3 months | B | O | Impending rupture (65 mm) | Arch | Arch |
| 63, M | N | 3 months | B | P | Impending rupture (55 mm) | Des | Des |
| 65, M | Y | 4 months | B | O | ULP (40 mm) | Des | Des |

FL false lumen, P patent, O occluded, Asc ascending aorta, Arch aortic arch, ULP ulcer-like projection, Des thoracic descending aorta

Coomassie blue R250. The bands for total MMP-2, active MMP-2, and pro-MMP-9 were quantified by densitometry with Image Gauge (FUJI FILM, Tokyo, Japan). A reference sample in each gel was used to normalize scanned bands among the gels. All results are expressed as the average value obtained from repeating the analysis twice. The relative activities of MMPs were expressed in arbitrary units (AU). To determine if the gelatinolytic bands were derived from MMPs, an additional extraction was incubated with EDTA (20 mmol/l) added to the previously described buffer.

Immunohistochemistry

The methods employed during this study were described previously [2, 11]. We used frozen sections that were prefixed with periodate-lysine-4% paraformaldehyde for 16 h at 4°C. The primary antibodies employed were mouse

monoclonal antibodies against human pro-MMP-2 (clone CA4001, IgG1, concentration 4.8 µg/ml) [9], TIMP-2 (67-4H11, IgG1k, 1.3 µg/ml, Fuji Chemical, Takaoka, Japan), pro-MMP-9 (GE213, 20 µg/ml) [16], PDGF-Rβ (61520.11, IgG1, 5 µg/ml, Genzyme, Cambridge, USA), and type I procollagen (PC-5-5, IgG1, 1.3 µg/ml, Takara, Kyoto, Japan). Frozen sections, 6 µm-thick, were incubated with these primary antibodies overnight and processed with a Nichirei Histofine Kit (Nichirei, Tokyo, Japan). Mouse monoclonal antibodies against human MT1-MMP (114-6G6 and 113-5B7, Fuji Chemical, Takaoka, Japan) were also employed. However, these antibodies were unsuccessful in detecting its respective antigen in frozen sections. As a negative control, the primary antibodies were replaced by isotype-matched control antibodies of the same concentration (DAKO, Grostrup, Denmark). All sections were then counterstained with hematoxylin.

Table 3 Patient profiles (healed group)

| Age and gender | Hypertension (Y/N) | Duration (years) | Stanford classification | FL (P/O) | Indications for surgery (maximal aortic diameter) | Site of replacement | Sample site |
|----------------|--------------------|------------------|-------------------------|----------|---|---------------------|-------------|
| 55, M | Y | 2 | B | P | Impending rupture (59 mm) | Des | Des |
| 56, F | Y | 4 | B | P | Aneurysm (48 mm) | Thoracoabd | Des |
| 59, M | Y | 4 | B | P | Aneurysm (53 mm) | Des | Des |
| 56, F | Y | 5 | B | P | Aneurysm (75 mm) | Thoracoabd | Des |
| 78, F | Y | 6 | B | P | Impending rupture (50 mm) | Des | Des |
| 72, M | Y | 8 | B | O | Aneurysm (63 mm) | Des | Des |
| 75, M | Y | 10 | B | P | Aneurysm (70 mm) | Thoracoabd | Abd |
| 72, M | Y | 10 | B | P | Aneurysm (63 mm) | Thoracoabd | Des |
| 68, F | Y | 12 | B | P | Impending rupture (60 mm) | Des | Des |

FL false lumen, P patent, O occluded, Des thoracic descending aorta, Thoracoabd thoraco–abdominal aorta, Abd abdominal aorta

Table 4 Patient profiles (autopsy group)

| Age and gender | Hypertension (Y/N) | Diagnosis at autopsy | Sample site |
|----------------|--------------------|--------------------------|-------------|
| 53, M | N | Diastolic cardiomyopathy | Asc |
| 55, M | Y | Gastric cancer | Asc |
| 74, F | Y | Uterine cancer | Asc |
| 81, M | Y | Gastric cancer | Asc |
| 60, M | Y | Pulmonary embolism | Asc |
| 63, F | Y | Colon cancer | Asc |
| 60, M | Y | Pneumonia | Asc |

All specimens were obtained from ascending aorta

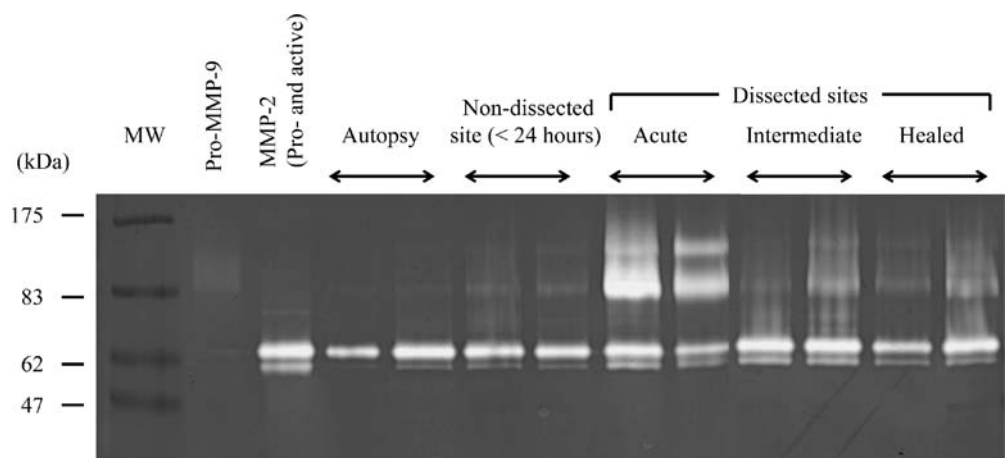
In situ hybridization

The methods employed during this study were described previously [18]. We used the following cDNAs: (1) 2,654 bp *HindIII*-*Xba I* fragment of MMP-2 cDNA [9]; (2) 1,242 bp *EcoRI*-*HindIII* fragment of MT1-MMP cDNA [22]; (3) 1,098 bp *EcoRI*-*EcoRI* fragment of type I procollagen cDNA [5]. Digoxigenin-labeled cRNAs were transcribed in vitro as described in the manufacturer's manual (DIG RNA Labeling Kit; Roche Diagnostics, Basel, Switzerland). For in situ hybridization, the specimen was fixed in 4% paraformaldehyde and 0.5% glutaraldehyde overnight, and then embedded in paraffin. The deparaffinized section was treated with 20 µg/ml proteinase-K for 30 min. The hybridization mixture containing 1 µg/ml antisense or sense probes was applied for 16 h at 50°C. After rinsing, alkaline-phosphatase-conjugated anti-digoxigenin antibody was added at a concentration of 1:500 overnight. The chromogen was nitroblue tetrahydrochloride. All sections were then counterstained with methyl green.

Statistical methods

Statistical analysis for the results of gelatin zymography was performed with SPSS 11.5.1J (SPSS Japan, Tokyo, Japan). Differences among groups greater than three were

Fig. 1 An example of gelatin zymography. *Autopsy*, the cadaveric specimen from autopsy case; *nondissected site* (<24 h) the nondissected site just after the onset (<24 h); *acute* the dissected site in the acute group; *intermediate* the dissected site in the intermediate group; *healed* the dissected site in the healed group. The positions of molecular weight standards (MW) are indicated on the left. Purified pro-MMP-9 and MMP-2 commercially available were used as positive controls



analyzed by the Kruskal–Wallis rank test and Scheffe's *F* test. All tests were considered to be significant at $P < 0.05$.

Two observers (MA and HO, with the latter being blind to the clinical data) independently performed semiquantitative analysis for immunohistochemistry and in situ hybridization. The expression of MMPs, PDGF-R β , and type I procollagen were semiquantified as follows: (+), abundant; (\pm), moderate; (-), minimal or absent. If the observers differed in their opinions, the two observers evaluated the microscopic fields and discuss the findings to reach a consensus.

Results

The clinical profiles of 29 patients and seven autopsy cases were shown in Tables 1, 2, 3, and 4. Twenty-nine patients were divided into three groups: acute ($n=11$, day 0–4, fibrin deposition, neutrophil infiltration, and minimal tissue response), intermediate ($n=9$, 13 days–4 months, abundant stromal cell-response and matrix synthesis), and healed ($n=9$, >2 years, sparse stromal cells and dense deposition of matrix) groups.

The mean age (year) \pm standard deviations of patients in acute, intermediate, and healed groups and that of autopsy cases were 61.9 \pm 9.7, 61.9 \pm 12.7, 65.7 \pm 9.2, and 63.7 \pm 10.2, respectively. There was no significant difference with regard to age among these four groups. As for five patients from whom the nondissected site (<24 h) was obtained, their mean age (year) \pm standard deviations were 56.2 \pm 9.7, which is also not different with that of autopsy cases.

Hypertension was present in 10 (91%), four (44%), and nine (100%) patients in the acute, intermediate, and healed groups, respectively. As for patients from whom the nondissected site (<24 h) was obtained, hypertension was present in all patients. Six of the autopsy cases (86%) had a history of hypertension.

Gelatin zymography

Figure 1 shows an example of gelatin zymography. First, we compared MMP levels among the three groups using all

cases. Active MMP-2 was most abundant in the intermediate group (Fig. 2a), while pro-MMP-9 was higher in the acute group (Fig. 2b). The total MMP-2 activity showed no significant differences among different groups. Next, we compared MMP levels between the dissected and non-dissected site just after the onset of dissection (<24 h; a subgroup of the acute group). As shown in Fig. 3a,b, the levels of active MMP-2 and pro-MMP-9 from the dissected sites were higher than the levels from the nondissected sites in those patients who received surgery within 24 h. The MMP levels in the nondissected sites (<24 h) did not differ from the levels found in the cadaveric specimens.

Immunohistochemistry and in situ hybridization

To clarify the in situ distribution, we performed immunohistochemistry and in situ hybridization to detect proteins and mRNAs, respectively. The semiquantitative results of these studies are shown in Table 5.

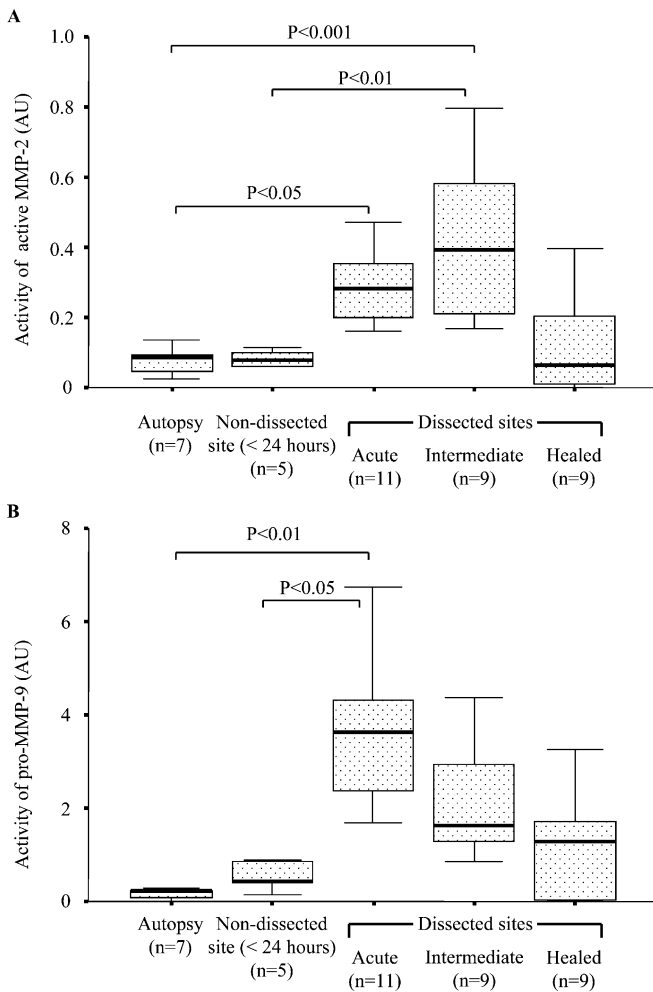


Fig. 2 Comparison of the activities of MMP-2 (a) and MMP-9 (b) from the dissected sites in different groups (acute, intermediate, and healed groups) by Box-Whisker plots. MMPs-2 and 9 peaked in the intermediate and the acute groups, respectively. The relative activities of MMPs were expressed in arbitrary units (AU)

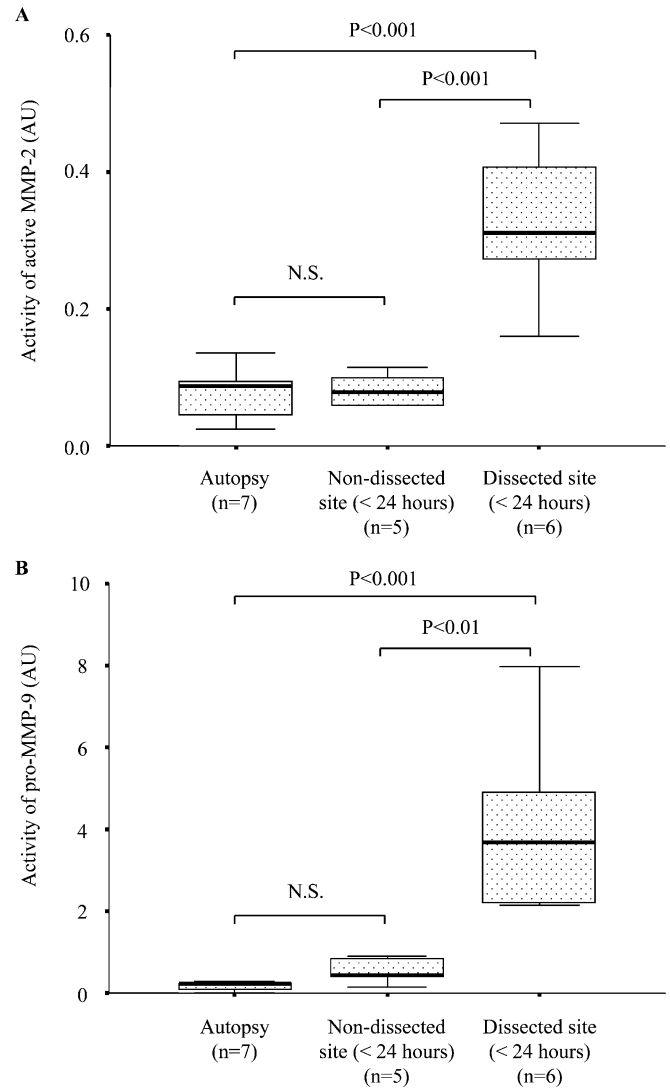


Fig. 3 Comparison of the activities of MMP-2 (a) and pro-MMP-9 (b) in the patients just after the onset (<24 h) and in the autopsy cases by Box-Whisker plots. The activities in the dissected sites were higher than those in the nondissected sites and in the cadaveric specimens. The relative activities of MMPs were expressed in arbitrary units (AU)

Dissected site in the acute group (>day 4, 11 patients)

As observed with light microscopy, a thin-layered thrombus was attached to the dissected surface (Fig. 4a), where it was infiltrated by neutrophils. These neutrophils abundantly expressed pro-MMP-9 protein (Fig. 4g). Neutrophils infiltrating in the adventitia also expressed pro-MMP-9. Medial spindle-shaped cells constitutively expressed pro-MMP-2 and TIMP-2 proteins (Fig. 4b,e) and moderately expressed mRNAs for MMP-2 and MT1-MMP (Fig. 4c,d). Type I procollagen mRNA was not observed (Fig. 4f).

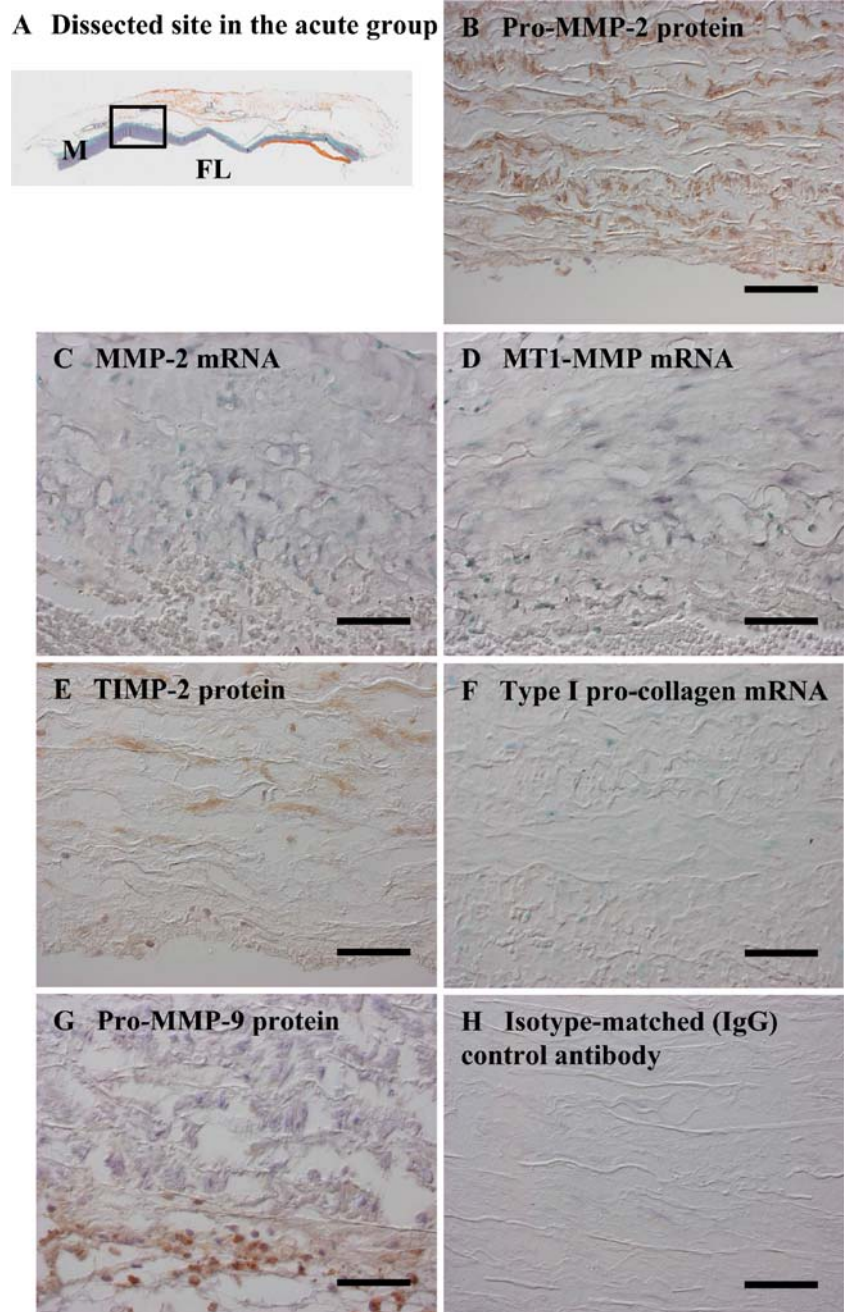
In the nondissected site (<24 h, five patients), however, no case revealed expression of pro-MMP-9 protein or mRNAs for MMP-2, MT1-MMP, and type I procollagen. Medial spindle-shaped cells constitutively expressed pro-

Table 5 Summary for the results of immunohistochemistry and in situ hybridization

| | Nondissected site, <24 h | | Dissected site, acute | | Dissected site, intermediate | | | Dissected site, healed | | |
|----------------------------|--------------------------|----|-----------------------|----|------------------------------|---|----|------------------------|---|----|
| | M | Ad | M | Ad | FL | M | Ad | FL | M | Ad |
| MMP-2 mRNA | - | - | ± | ± | + | - | + | - | - | - |
| MT1-MMP mRNA | - | - | ± | ± | + | - | + | - | - | - |
| Type I procollagen mRNA | - | - | - | - | + | - | + | - | - | - |
| Pro-MMP-2 protein | + | + | + | + | + | + | + | - | ± | - |
| TIMP-2 protein | + | + | + | + | + | + | + | - | ± | - |
| Type I procollagen protein | - | - | - | - | + | - | + | - | - | - |
| PDGF-Rβ protein | - | - | - | - | + | - | + | - | - | - |
| Pro-MMP-9 protein | - | - | + | + | + | - | ± | - | - | - |

M media, *Ad* adventitia, *FL* false lumen (neointima or organized thrombus), +, abundant; ±, moderate; -, minimal or absent

Fig. 4 Morphological results in the acute group (day 2). **a** A panoramic view. *FL* false lumen; *M* media. Higher magnifications of boxed area in **a**, showing the expression of pro-MMP-2 protein (**b**, brown, abundantly), MMP-2 mRNA (**c**, dark blue, moderately), MT1-MMP mRNA (**d**, dark blue, moderately), and TIMP-2 protein (**e**, brown, moderately) by the spindle-shaped cells in the media. Type I procollagen mRNA (**f**) was not detected. Pro-MMP-9 protein (**g**, brown) is abundantly positive in neutrophils. **h** Isotype-matched control antibody for pro-MMP-2 is not stained. Scale bar=50 μm



MMP-2 and TIMP-2 proteins as in dissected sites (data not shown). As shown in Fig. 4h, IgG isotype-matched control antibodies showed no specific stains in all patients, confirming the specificity of our results.

*Dissected site in the intermediate group
(day 13–4 months, nine patients)*

The adventitia was markedly thickened (Fig. 5a). Neointima developed on the dissected surface in patients with patent false lumen. In patients with an occluded false lumen, the thrombus in the false lumen was partially organized, showing a proliferation of spindle-shaped cells and deposition of collagen fibers in the peripheral areas (Fig. 6a). Messenger RNAs for MMP-2 (Figs. 5b and 6b) and MT1-MMP (Figs. 5c and 6d), and pro-MMP-2 (Fig. 6c) and TIMP-2 (Figs. 5h and 6e) proteins were abundantly expressed by the spindle-shaped cells in the neointima, organizing thrombus, and adventitia, which were closely coupled with the expression of type I procollagen mRNA (Figs. 5d and 6f) and protein (Figs. 5i and 6g) in the same areas. The spindle-shaped cells in those areas further expressed PDGF-R β protein

(Figs. 5j and 6h). The number of neutrophils expressing pro-MMP-9 protein decreased, and macrophages and multinucleated giant cells in the organizing thrombus were positive for pro-MMP-9 protein (Fig. 6i). As shown in Figs. 5e–g, the sense probes for mRNAs for MMP-2, MT1-MMP, and type I procollagen showed no specific signals in all patients, confirming the specificity of our results.

Dissected site in the healed group (>2 years, nine patients)

Spindle-shaped cells were sparse with dense deposition of collagen fibers in the neointima, organized false lumen, and adventitia (Fig. 7a). The number of cells positive for pro-MMP-2 (Fig. 7b), TIMP-2, type I procollagen (Fig. 7f), and PDGF-R β (Fig. 7g) proteins markedly decreased, and there were few cells expressing pro-MMP-9 protein (Fig. 7h). The cells positive for these substances were primarily localized around vasa vasorum in the adventitia. The number of cells positive for mRNAs for MMP-2 (Fig. 7c), MT1-MMP (Fig. 7d), and type I procollagen (Fig. 7e) also markedly decreased in those areas.

Fig. 5 Morphological results of the dissected site with a thickened adventitia from the intermediate group (day 14). **a** A panoramic view. *M* media; *FL* false lumen; *Ad* adventitia. Higher magnifications of boxed area in **a**, showing abundant expression of MMP-2 mRNA (**b**, dark blue), MT1-MMP mRNA (**c**, dark blue), and type I procollagen mRNA (**d**, dark blue) by the spindle-shaped cells in the adventitia. Note a clear similarity of the expression pattern of MMP-2, MT1-MMP, and type I procollagen mRNAs. Negative controls are shown in sense probe for MMP-2 mRNA (**e**), sense probe for MT1-MMP mRNA (**f**), and sense probe for type I procollagen mRNA (**g**). TIMP-2 protein (**h**, brown), type I procollagen protein (**i**, brown), and PDGF-R β protein (**j**, brown) are abundantly expressed by the spindle-shaped cells in the adventitia were shown. Scale bar=50 μ m

A Adventitia in the intermediate group

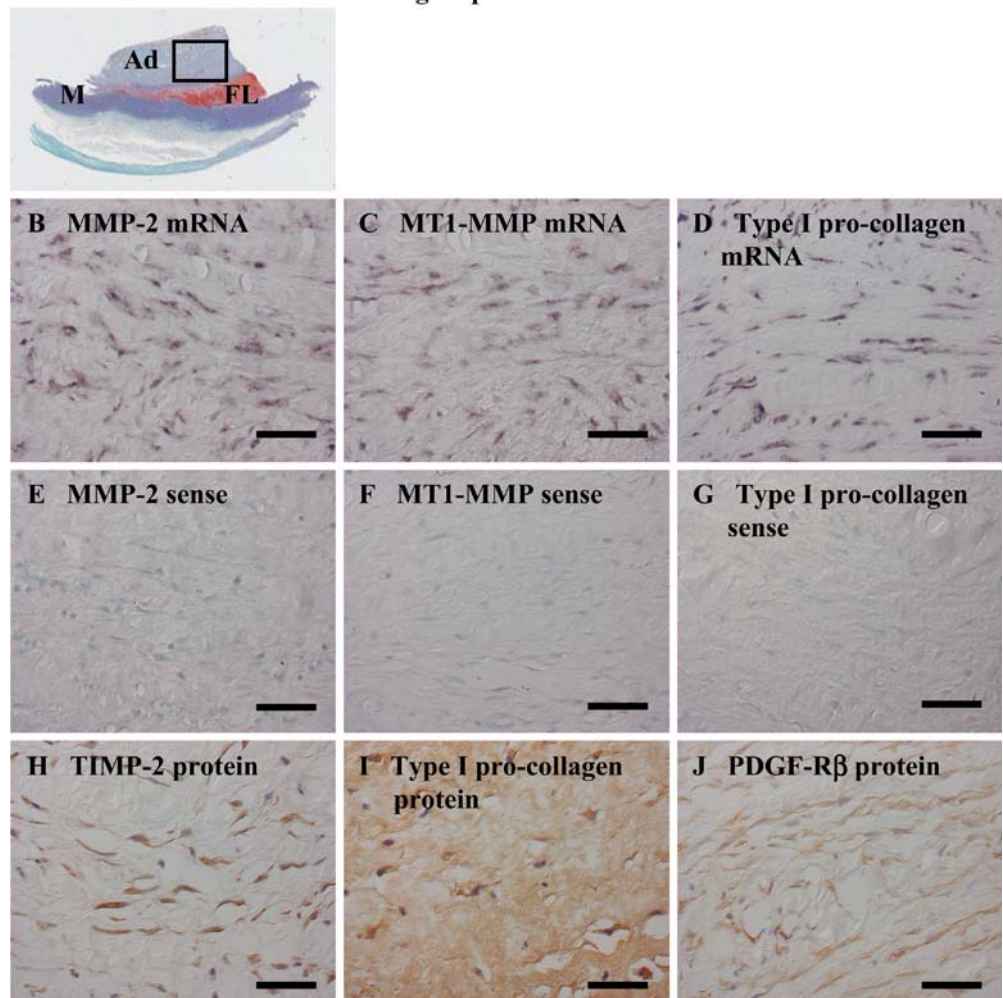
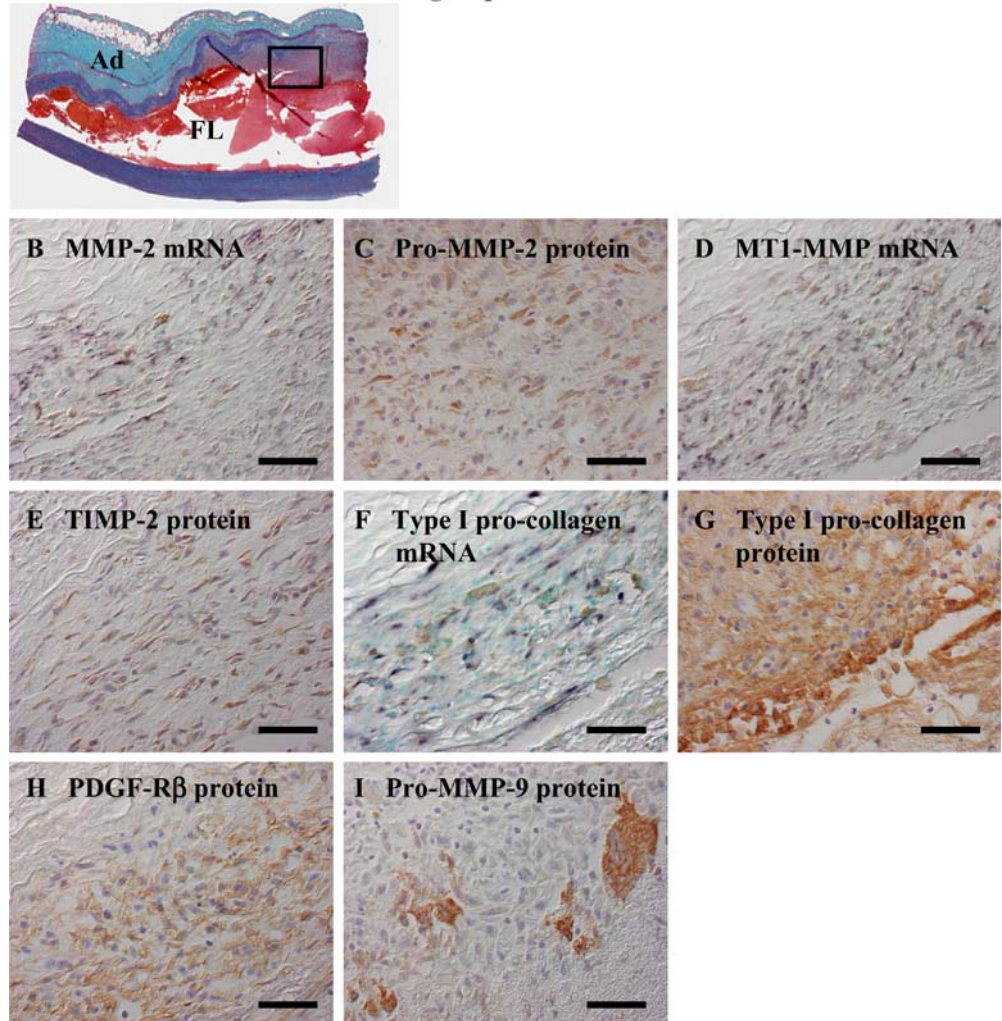


Fig. 6 Morphological results of the dissected site with organizing thrombus in the false lumen from the intermediate group (1 month). **a** A panoramic view. *FL* false lumen; *Ad* adventitia. Higher magnifications of *boxed area*, showing abundant expression of MMP-2 mRNA (**b**, *dark blue*), pro-MMP-2 protein (**c**, *brown*), MT1-MMP mRNA (**d**, *dark blue*), TIMP-2 protein (**e**, *brown*), type I procollagen mRNA (**f**, *dark blue*), type I procollagen protein (**g**, *brown*), and PDGF-R β protein (**h**, *brown*) by the spindle-shaped cells in the organizing thrombus. Pro-MMP-9 protein is detected in giant cells (**i**, *brown*). Scale bar=50 μ m

A False lumen in the intermediate group



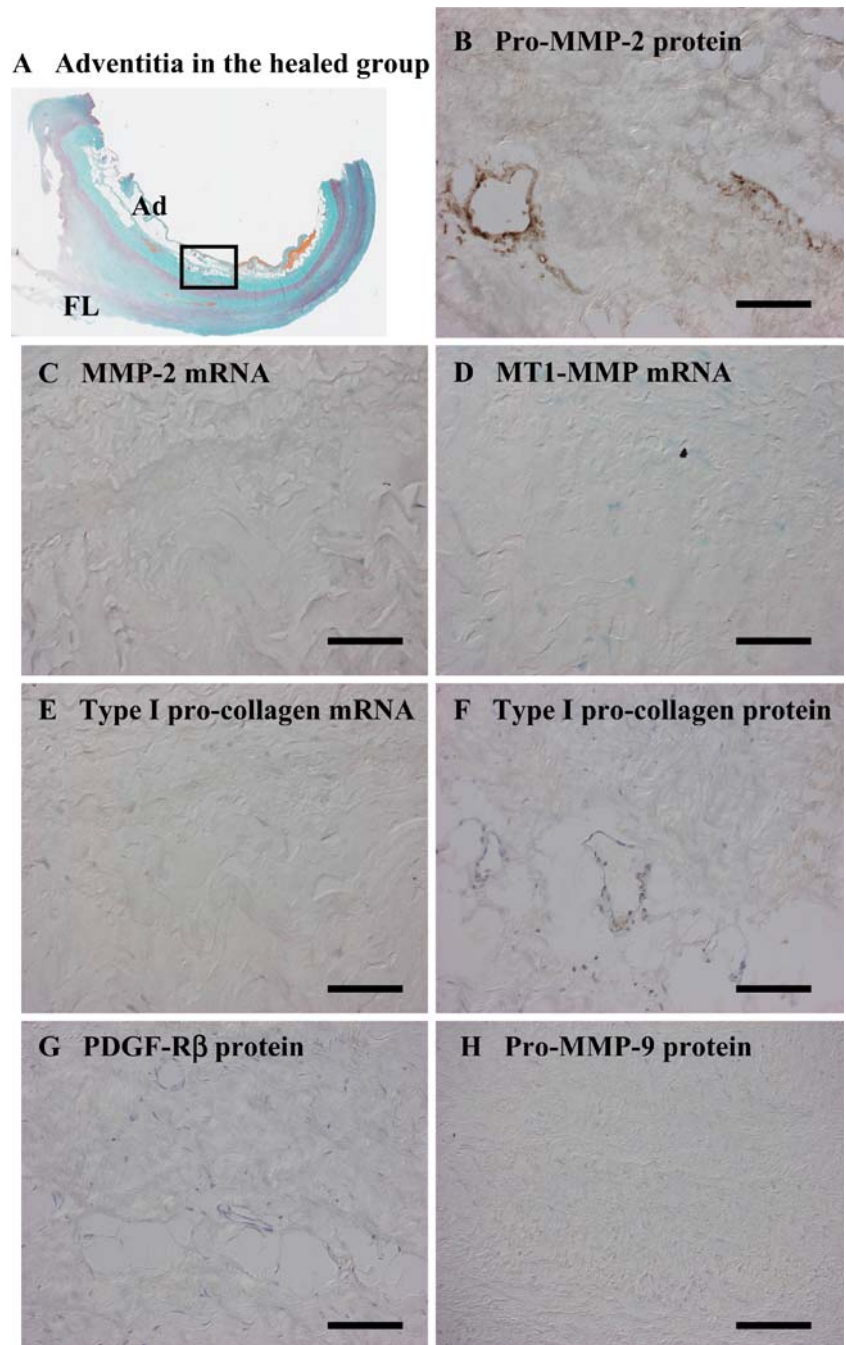
Discussion

The present study was designed to clarify the significance of MMP-2, MT1-MMP, and MMP-9 in aortic dissection. We have revealed two types of distinctive up-regulation of MMPs: MMP-9 in the acute group, and MMP-2 in the intermediate group, the latter coupled with MT1-MMP, TIMP-2, type I procollagen, and PDGF-R β . The gelatinolytic activities and the in situ distribution of these MMPs in these groups were significantly higher than those in the nondissected aorta (<24 h) or the control aorta obtained from autopsy cases.

It is well known that immunohistochemistry for MMP-2 shows constitutive expression of its protein in smooth muscle cells irrespective of its production status [6, 14]. Therefore, in situ hybridization was performed to evaluate the in situ production of MMP-2 as reported previously [18, 19]. To consider the significance of MMPs in various stages after the onset of dissection, we have divided patients into three groups based on the histopathological changes [26] because these changes were related to the time course.

The most pronounced signal of MMP-2 mRNA was observed in the cytoplasm of spindle-shaped cells in the organizing thrombus, neointima, and thickened adventitia in the intermediate group, in which the gelatinolytic activity of MMP-2 was highest. The spindle-shaped cells in the same areas also abundantly expressed MT1-MMP mRNA and TIMP-2 protein in the same pattern. Moreover, such abundant expression of MMP-2, TIMP-2, and MT1-MMP were coupled with those of type I procollagen and PDGF-R β . These quite notable findings required us to consider tissue remodeling because matrix degradation is not only important for tissue degradation, but is also required for deposition of new collagen fibers in the injured vessels [24]. Morphologic findings showed the formation of granulation tissue on the surface of the false lumen in the intermediate group, which later became fibrosis (scar) in the equivalent areas in the healed group. The same findings were also observed in the thickened adventitia. Briefly, matrix synthesis is stimulated and matrix accumulation actually occurs in these areas where MMP-2, TIMP-2, and MT1-MMP are up-regulated. If we focused only on tissue degradation, it would have been difficult to explain these morphological findings. When the simultaneous up-regu-

Fig. 7 Morphological results of the dissected site with scarring adventitia from the healed group (6 years). **a** A panoramic view. *FL* false lumen; *Ad* adventitia. Higher magnifications of *boxed area* in **a**, showing pro-MMP-2 protein (**b**, *brown*, scanty positive in spindle-shaped cells in vasa vasorum), MMP-2 mRNA (**c**, no signal), MT1-MMP mRNA (**d**, no signal), type I procollagen mRNA (**e**, no signal), type I procollagen protein (**f**, no staining), PDGF-R β protein (**g**, *brown*, no staining), and pro-MMP-9 protein (**h**, no staining). Scale bar=50 μ m



lation of MMP-2, TIMP-2, MT1-MMP, type I procollagen, and PDGF-R β are compared with these morphologic changes described above, we have concluded that the up-regulation of these substances is most probably associated with the deposition of new collagen fibers. The spontaneous closure of false lumen is documented as a favorable prognostic factor [1, 25]. In this situation, the dissection may heal, obliterating the false lumen and leaving a linear scar in the outer media. Our data suggested that MMP-2, TIMP-2, and MT1-MMP in the intermediate group are related to the matrix regeneration process, which may be related to the healing process.

As for MMP-9, we observed its more pronounced expression in neutrophils in the acute group. Neutrophils play a role in tissue damage caused during the inflammatory process. The MMP-9 expressed by neutrophils is involved in the digestion of dead tissue that is a step in the tissue repair process [7]. We could consider two possibilities for this observation. First, from our data, it could be interpreted that MMP-9 may contribute to the degradation of the aortic wall in the early stage. This event may be one of the factors that worsen the prognosis of the disease. The other possibility is that MMP-9 activity may be significant in tissue remodeling. However, further study is required to elucidate the significance of MMP-9.

We compared the activities of MMP-2 and MMP-9 in the nondissected sites (<24 h), the dissected sites (<24 h), and the cadaveric specimens by gelatin zymography. The activities of these MMPs in the dissected sites (<24 h) were significantly higher than in the nondissected sites (<24 h) and in the cadaveric specimens. Our data suggested that the up-regulation of MMP-2 and MMP-9 have already been affected by the dissection itself, not indicating higher MMP-2 and MMP-9 before the dissection. Therefore, we have not obtained the data that supports the concept that MMP-2 and MMP-9 are involved in the onset of aortic dissection. However, the possibility remains that the actual MMP-2 and MMP-9 activities in the entry sites may be higher even before the aortic dissection because aortic dissection occurs at the points of presumed greatest hemodynamic stress [8], which can alter the MMPs activity [3, 4]. The present study revealed the distribution of mRNA and/or protein of MMP-2, MT1-MMP, TIMP-2, and type I procollagen. To evaluate the molecular events more precisely, we should explore in situ zymography or double/triple-labeling tissue staining in future studies. Also, our present study covered a rather limited number of patients, and therefore, future studies will require a larger patient cohort to more accurately compare MMP activities in various clinical conditions. In addition, the involvement of additional MMPs, such as MMP-1, MMP-8, and MMP-12 should be evaluated in the future studies.

In conclusion, the present study revealed the complex activities of MMPs and TIMPs, with a role not only in tissue degradation but also in tissue remodeling after the onset of an aortic dissection.

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