

# The expression of the BRM and MMP2 genes in thoracic aortic aneurysm and aortic dissection

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**Abstract.** – **OBJECTIVE:** To study the expression, roles, and clinical significance of Brahma (BRM) and matrix metalloproteinase 2 (MMP2) in the thoracic aortic aneurysm and aortic dissection.

**PATIENTS AND METHODS:** Arterial specimens from 20 cases of thoracic aortic dissection and 38 cases of thoracic aortic aneurysm, as well as normal tissue were collected, paraffin-embedded, sectioned, and stained with anti-BRM and MMP2 monoclonal antibodies. Sections were analyzed by immunofluorescence, and the distribution and expression of BRM and MMP2 in the aortic wall were determined. Reverse transcriptase-polymerase chain reaction (RT-PCR) was used to measure the expression of BRM and MMP2 mRNA in the thoracic aortic aneurysm, thoracic aortic dissection, and normal tissues. The expression of MMP2 and BRM protein in these tissues was analyzed by Western blot. SPSS 17.0 statistical software was used for data analysis.

**RESULTS:** MMP2 and BRM (mRNA and protein) were expressed in arterial tissue from thoracic aortic aneurysms and aortic dissections. Immunofluorescence also showed that BRM and MMP2 were expressed in the thoracic aortic aneurysm and aortic dissection tissue. The expression was very high in thoracic aortic aneurysm tissue. The differences in expression of BRM and MMP2 in the different arterial tissues were statistically significant ( $p < 0.01$ ).

**CONCLUSIONS:** Expression of BRM and MMP2 in the thoracic aortic aneurysm and aortic dissection is very high, indicating that BRM and MMP2 may play important roles in the occurrence and development of thoracic aortic aneurysm and aortic dissection. They may represent potential targets for the treatment of thoracic aortic aneurysm and aortic dissection and provide a new basis for clinical diagnosis.

Key Words:

BRM, MMP2, Thoracic aortic aneurysm, Thoracic aortic dissection.

## Introduction

Thoracic aortic aneurysms and thoracic aortic dissections are severe and extremely dangerous cardiovascular diseases<sup>1,2</sup>. In recent years, there have been significant breakthroughs and progress related to the diagnosis and treatment of thoracic aortic aneurysms and thoracic aortic dissections. However, at present, the specific pathogenesis of these diseases remains unclear<sup>3</sup>. In the present study, we applied immunofluorescence to measure the expression of Brahma (BRM) and matrix metalloproteinase 2 (MMP2) in arterial tissue harvested from patients with thoracic aortic aneurysm and thoracic aortic dissection, and from normal tissue. RT-PCR and Western blot were used to measure the levels of BRM and MMP2 mRNA and protein, respectively, in thoracic aortic aneurysm tissue, thoracic aortic dissection tissue, and normal tissue. The differences in the expression of BRM and MMP2 in these tissues were compared, and the relationship between changes in expression of BRM and MMP2 in the diseased tissue and clinicopathological features is discussed.

## Patients and Methods

### Sample Collection

Aortic tissues removed during surgery for thoracic aortic dissection or thoracic aortic aneurysm in our hospital were collected. There were 20 cases of thoracic aortic dissection, 38 cases of thoracic aortic aneurysm, and 12 cases

**Table 1.** RT-PCR primer sequences for BRM and MMP2.

Gene name	Primer sequences
BRM	5'-3' GATCAGGAATAAGTACGAATGTGAG 3'-5' GCCACCAATCCACACAGAGT
MMP2	5'-3' TGATCTTGACCAGAATACCATCGA 3'-5' GGCTTGCGAGGGAAGAAGTT
$\beta$ -actin	5'-3' GAGCCGGGAAATCGTGCGT 3'-5' GGAAGGAAGGCTGGAAGATG

with normal tissue. All specimens were fixed in 10% neutral formalin for over 48 h and sectioned with a thickness of 5  $\mu$ m.

### Reagents

Trizol reagent (Tiangen, Beijing, China); reverse transcription kit (Tiangen, Beijing, China); anti- $\beta$ -actin, anti-BRM, and anti-MMP2 monoclonal antibodies, and fluorescent secondary antibody (CST, Boston, MA, USA); protein quantification kit (Beyotime, Shanghai, China).

### Immunofluorescence

Arterial tissues from a thoracic aortic aneurysm and thoracic aortic dissection were fixed with 10% neutral formalin for over 48 h and sectioned with a thickness of 5  $\mu$ m. Paraffin sections underwent repeated dewaxing with xylene, and dehydration with an ethanol gradient. Antigen retrieval was conducted, and tissue sections were washed three times in phosphate buffered saline (PBS) (pH 7.4) (5 min/wash). Tissue sections were then blocked with 10% BSA in a humidified chamber for 30 min (37°C). After an additional wash in PBS, excess BSA around tissues was removed, and fluorescently-labeled antibodies (1:70 dilution) were added drop wise to tissue sections. The sections were placed in a humidified chamber and incubated overnight at 4°C. The following day, sections were washed three times in PBS, and fluorescent secondary antibody was added drop wise while sections were shielded from light. Next, sections were placed in a humidified chamber at 37°C to incubate for 2 h. Slides were mounted with buffered glycerol and observed under a fluorescence microscope.

### RT-PCR

Roughly 50 mg of arterial tissue from patients with thoracic aortic aneurysm and thoracic aortic dissection were harvested and immediately placed in 1 mL Trizol. Tissue samples were ho-

mogenized by grinding. After centrifugation for 5 min at 12,000  $\times$  g and 4°C, supernatants were carefully removed. Chloroform was added to supernatants and mixed well. After standing at room temperature for 5 min and centrifugation for 15 min at 12,000  $\times$  g and 4°C, supernatants were removed carefully. Next, equal volumes of isopropanol were added, and samples were left to stand at room temperature for 10 min. RNA was precipitated by centrifugation for 10 min at 12,000  $\times$  g and 4°C. The RNA precipitate was then washed and mixed well with 75% ethyl alcohol. RNase-free water was then added to fully dissolve RNA. The OD<sub>260</sub>/OD<sub>280</sub> ratio and RNA concentration were then determined. Finally, gradual amplification was conducted according to instructions. The primer sequences are shown in Table I.

### Western blot

About 30 mg of thoracic aortic aneurysm tissues and thoracic aortic dissection tissues were washed in ice-cold physiological saline. The protocol was conducted according to the instructions of the total protein extraction kit. Lysis buffer was added. The tissues were placed on ice for homogenization by grinding. Tissue homogenates were then centrifuged twice for 10 min at 12,000  $\times$  g and 4°C, and the supernatants were collected. After quantification of protein in the supernatant according to the instructions of the protein kit, equal amounts of total protein were added to appropriate wells for SDS-PAGE under constant voltage (150V). Electrophoresis was stopped when bromophenol blue reached the bottom of the gel. The gel was cut according to the molecular weight of the target proteins. Separated protein was transferred to PVDF membranes. The PVDF membranes with bound protein were blocked in 5% skim milk powder on a shaker at room temperature for 2 h and then incubated with the corresponding primary antibodies (1:500) overnight at 4°C. The following

day, after membranes were fully washed with TBST (three washes, 10 min/wash), secondary antibody (1:2000) was added for incubation at room temperature for 1 h. After three additional washes for 10 min with TBST, ECL color-substrate solution was added drop wise for coloration and to acquire pictures.

### Statistical Analysis

SPSS 17.0 statistical software was used for data analysis. Experimental data are presented as mean  $\pm$  standard deviation (mean  $\pm$  SD). The *t*-test, *p*-test, and One-Way analysis of variance (ANOVA) followed by Post Hoc test (Least Significant Difference) were applied.  $p < 0.05$  was considered statistically significant.

## Results

### Histopathology

As shown in Figure 1, the histopathological characteristics of thoracic aortic aneurysm tissue specimens included different degrees of medial degeneration of the artery, laminar necrosis accompanied by depigmentation of smooth muscle cells, and collagens deposition. The specimens from thoracic aortic dissection were also characterized by medial arterial degeneration of different degrees, sparse elastic fibers, and depigmentation. Compared with the thoracic aortic aneurysm group, the dissection group had endometrial hyperplasia, thickening, and mild lymphocyte infiltration. More specifically, the inflammatory reaction was characterized by monocyte or plasmocyte infiltration.

### Immunofluorescence Analysis of BRM and MMP2 in Thoracic Aortic Aneurysm tissue, Thoracic Aortic Dissection Tissue, and Normal Tissue

As shown in Figure 2, BRM and MMP2 were expressed in arterial tissues from patients with thoracic aortic aneurysm and thoracic aortic dissection, while they were weakly expressed in normal tissues. These results suggest an important role for BRM and MMP2 in the occurrence and development of thoracic aortic aneurysm and thoracic aortic dissection.

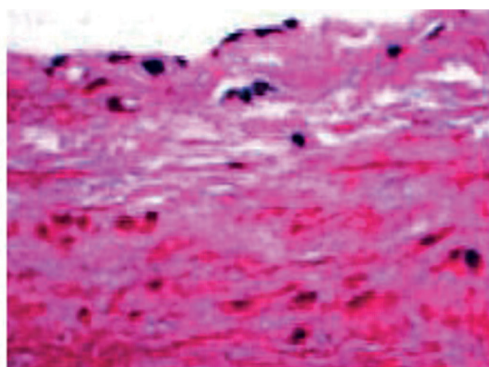
### RT-PCR Results

Total RNA was extracted from thoracic aortic aneurysm tissues, thoracic aortic dissection tissues, and normal tissues. Through RT-PCR, it was found that the expression of BRM and MMP2 in the thoracic aortic aneurysm tissue and thoracic aortic dissection tissue was significantly higher than in normal tissue ( $p < 0.05$ ). These results indicate that all cells in the thoracic aortic aneurysm and thoracic aortic dissection tissue expressed BRM mRNA and MMP2 mRNA (Figure 3).

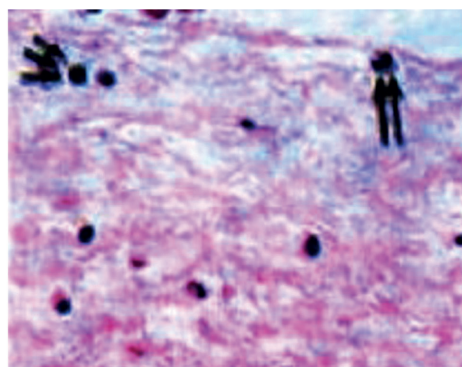
### Expression of BRM and MMP2 in Thoracic Aortic Aneurysm Tissue, Thoracic Aortic Dissection tissue, and Normal Tissue

The results from Western blot analysis showed that BRM and MMP2 protein were highly expressed in thoracic aortic aneurysm tissue and thoracic aortic dissection tissue, while BRM and MMP2 protein were weakly expressed in normal tissue. Moreover, as shown in Figure 4, the expression of BRM and MMP2 in thoracic aortic aneurysm tissue was higher than in thoracic aortic dissection tissue.

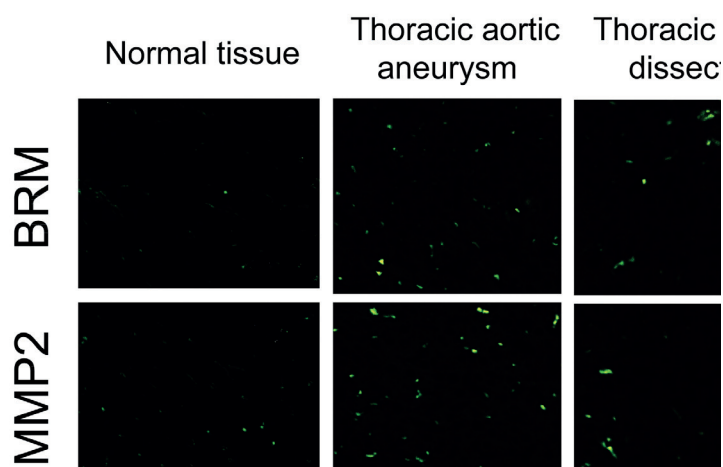
Thoracic aortic aneurysm



Thoracic aortic dissection



**Figure 1.** Representative histopathology of arterial tissue from thoracic aortic aneurysm and thoracic aortic dissection.



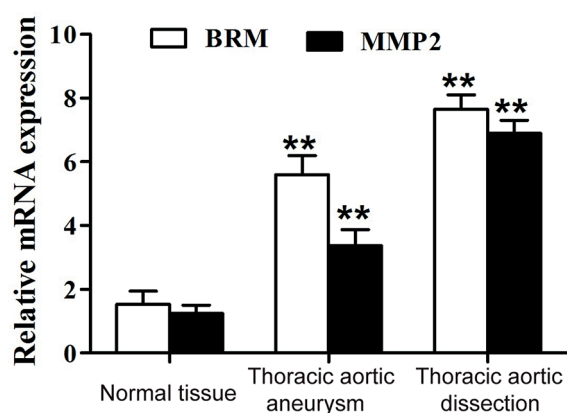
**Figure 2.** Expression of BRM and MMP2 in normal tissue, thoracic aortic aneurysm tissue, and thoracic aortic dissection tissue ( $\times 400$ ).

## Discussion

Thoracic aortic aneurysms are lesions that occur in the tunica media of the aorta. The structure of the external elastic membrane of the artery becomes severely damaged, and structural reconstruction occurs so that lesions occur in vascular smooth muscle accordingly<sup>4-7</sup>. A thoracic aortic dissection refers to serious damage and reconstruction of the artery. The vascular wall can begin to bleed in large amounts, which causes blood to enter between the medial membrane and external membrane of blood vessels, which can cause serious changes in angiostasis, and cause arteriectasis<sup>8-10</sup>. Since blood flow has a great impact force, dissection can cause blood

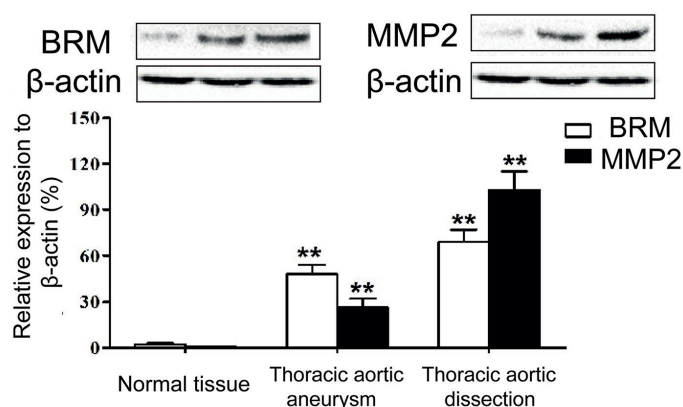
outflow to a large area and to break through the vascular wall and flow to unknown areas, which can cause severe complications and even death<sup>11-13</sup>. In general, there is a connection between the two diseases. However, identification of the specific connection requires further investigations.

The BRM gene expresses a component of the chromatin-remodeling complex that has DNA dependence and ATPase activity. Furthermore, BRM can affect gene expression by adjusting the structure of chromatin<sup>14</sup>. BRM can affect the alternative splicing of several genes. The effects of BRM in the occurrence of malignant tumors are likely related to the chromatin-remodeling complex. The chromatin remodeling complex can use the energy generated by ATP hydrolysis to change chromatin structure and control gene expression, thus regulating cell proliferation, cell differentiation, and the occurrence of tumors<sup>15,16</sup>. The chromatin-remodeling complex can interact with tumor suppressor genes. The deletion of expression of BRM can inhibit regulation and control of growth. MMPs are zinc-dependent endopeptidases. MMPs play an established role in the metastasis of many cancers<sup>17</sup>. Their proteolytic activities can directly promote the passing of invasive cancers through the extracellular matrix and tissue barriers, and can further activate chemokines and growth factors<sup>18</sup>. MMP2 is a key arterial enzyme that plays an essential role in the division and degradation of elastin, which is related to decreased elasticity of the arterial wall. The effects of MMP2 are mediat-



**Figure 3.** Expression of BRM mRNA and MMP2 mRNA in thoracic aortic aneurysm tissue, thoracic aortic dissection tissue, and normal tissue.





**Figure 4.** Expression of BRM and MMP2 protein in thoracic aortic aneurysm tissue, thoracic aortic dissection tissue, and normal tissue.

ed through degrading the extracellular matrix<sup>19</sup>. Extracellular matrix is an important substance that maintains the normal structure and strength of the aortic wall. Damage to the extracellular matrix inevitably leads to weakness of blood vessel walls. Furthermore, under the impact and pressure of blood flow, tumors can form. A large number of studies have shown that the expression of MMP2 in intimal smooth muscle cells of dissected vessels was significantly higher than in normal controls and abdominal aortas without dissection<sup>20,21</sup>.

### Conclusions

The results of the present study showed that the expression of BRM and MMP2 in the arterial tissue harvested from patients with thoracic aortic dissections and aortic aneurysms was very high. RT-PCR and Western blot analysis showed that the differences in the expression of BRM and MMP2 in normal tissue, thoracic aortic dissection tissue, and aortic aneurysm tissue were statistically significant ( $p < 0.05$ ). BRM and MMP2 may, therefore, play important roles in the occurrence and development of thoracic aortic dissection and aortic aneurysm. They may represent new targets for the treatment of thoracic aortic dissection and aortic aneurysm, and provide a new basis for clinical diagnosis and treatment.

### Conflict of interest

The authors declare no conflicts of interest.

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