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# Changes in expressions of miR-22-3p and MMP-9 in rats with thoracic aortic aneurysm and their significance

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**Abstract. – OBJECTIVE: The purpose of this study was to explore the changes in the expressions of micro ribonucleic acid (miR)-22-3p and matrix metalloproteinase-9 (MMP-9) in rats with thoracic aortic aneurysm (TAA) and their significance.**

**MATERIALS AND METHODS: A total of 16 specific pathogen-free Sprague-Dawley female rats were randomly divided into normal group (n=8) and angiotensin II (Ang II) group (n=8). Ang II was perfused using the micro pump in Ang II group, while the same amount of normal saline was perfused in the normal group. After continuous intervention, the tumor formation rate in the thoracic aorta was observed, and the expression of miR-22-3p was detected *via* Reverse Transcription-Polymerase Chain Reaction (RT-PCR) in both groups. Other 16 rats were selected and randomly divided into agomiR-22-3p group (n=8) and control group (n=8). In the agomiR-22-3p group, agomiR-22 and Ang II were continuously injected *via* angular vein. In the control group, agomiR negative control was injected, and Ang II was continuously perfused. After intervention for 4 weeks, the tumor formation rate in the thoracic aorta was observed, and the expression of MMP-9 was determined *via* immunofluorescence and immunohistochemistry in both groups.**

**RESULTS: After intervention for 4 weeks, the expression of miR-22-3p in Ang II group was significantly lower than that in normal group ($p<0.05$). After drug administration for 4 weeks, agomiR-22-3p group had a lower tumor formation rate ($p<0.05$) and a lower expression of MMP-9 than the control group ($p<0.05$).**

**CONCLUSIONS: The expression of miR-22-3p declines in TAA rats, and miR-22-3p can inhibit the expression of MMP-9, thus suppressing the formation of TAA in rats.**

*Key Words:*
Thoracic aortic aneurysm, MiR-22-3p, Matrix metalloproteinase-9.

## Introduction

Thoracic aortic aneurysm (TAA) is a disease with an extremely low morbidity rate, which will cause aortic rupture, secondary aortic dissecting aneurysm in severe cases, and a series of complications once it occurs, thereby leading to a high risk of death[1]. Despite numerous studies on the etiology, pathogenesis, therapeutic principles, and methods of TAA, as well as great progress, made in the treatment of disease, its pathogenesis is still controversial. *In vitro* and *in vivo* studies have pointed out that the imbalance of extracellular matrix (ECM) plays an important role in the pathological reaction of TAA[2].

ECM, the main component of the aortic wall, has special significance for maintaining the homeostasis of artery vessels and inhibiting the occurrence of aortic aneurysm. Related signaling pathways and cytokines that cause the increase or decrease of various components of ECM and lead to dysfunction are involved in the occurrence of aortic aneurysm, among which matrix metalloproteinases (MMPs) are the most extensive and highly related molecules involved in TAA. MMPs are a family of key proteolytic enzymes existing in tissues of the body, which can basically decompose all the components of ECM. MMP-9 has a strong ability to decompose related elastin and macromolecular collagen in the aneurysm wall, and it occupies a decisive position in the occurrence and development of aortic aneurysm[3,4].

Micro ribonucleic acid (miR)-22-3p is a highly specific non-coding RNA, which has remarkable significance for the origin, occurrence, and development of tumors[5-7]. However, whether it can affect TAA by influencing the expression of MMP-9 has not been reported in the literature. Therefore, the purpose of this study was to observe the effect of miR-22-3p on

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the MMP-9 expression, and further clarify its specific mechanism in the pathogenesis of TAA.

## Materials and Methods

### Laboratory Animals and Grouping

A total of 16 specific pathogen-free (SPF) Sprague-Dawley (SD) female rats aged 8 weeks old and weighing (25±5) g were randomly divided into normal group (n=8) and angiotensin II (Ang II) group (n=8). Other 16 SPF SD female rats aged 8 weeks old and weighing (25±5) g were selected and randomly divided into agomiR-22-3p group (n=8) and control group (n=8). The rats were fed with adequate sterile feed and water every day in the SPF animal experiment center. This study was approved by the Animal Ethics Committee of Jilin University Animal Center.

### Main Laboratory Reagents and Instruments

The main reagents used were: Ang II (Sigma-Aldrich, St. Louis, MO, USA), agomiR (RiboBio, Guangzhou, China), MMP-9 antibody (Boster, Wuhan, China), immunohistochemistry kit (ProteinTech, Wuhan, China), animal ultrasound imaging system (Visual Sonics Inc., Atlanta, GA, USA), and Polymerase Chain Reaction (PCR) Instrument (ABI 7500, Applied Biosystems, Foster City, CA, USA).

### Preparation and Treatment of Animal Model

The TAA model was established *via* perfusion of Ang II using the micro pump. Specifically, after intraperitoneal anesthesia with 7% chloral hydrate, the chest hair was shaved off, and the skin was disinfected with 75% ethanol and cut open. Subcutaneous tissues were bluntly separated, and the Ang II micro pump was buried. Ang II was continuously perfused (1200 ng/min/kg) using the micro pump for 4 weeks in Ang II group, while the same amount of normal saline was perfused in the normal group. In the agomiR-22-3p group, Ang II was continuously perfused (1200 ng/min/kg), and agomiR-22 was injected once every 4 days. In the control group, Ang II was also continuously perfused (1200 ng/min/kg), and agomiR negative control was injected once every 4 days.

### Echocardiography

After intervention for 4 weeks, the cardiac function in Ang II group and normal group was detected using the small animal imaging system.

### Immunohistochemistry and Immunofluorescence

Immunohistochemistry: after intervention, the heart was perfused with paraformaldehyde (Sigma-Aldrich, St. Louis, MO, USA). The samples were taken, fixed with paraformaldehyde, dehydrated with gradient alcohol, soaked in paraffin, prepared into paraffin-embedded tissues, and sliced into 5 μm-thick paraffin sections using a microtome. After deparaffinization with xylene and dehydration with alcohol, the sections were subjected to antigen retrieval *via* water bath, sealed with serum at room temperature, and incubated with primary and secondary antibodies, followed by image development, dehydration, sealing, and observation. Immunofluorescence: The early steps were the same as those in immunohistochemistry. The sections were sealed with serum and incubated with primary and secondary antibodies in the dark, followed by image development, dehydration, and sealing.

### RT-PCR

After sampling, the tissues were ground using a grinding rod and liquid nitrogen, and the RNA was extracted and reversely transcribed into complementary deoxyribose nucleic acid (cDNA) (TaKaRa, Otsu, Shiga, Japan). Conditions: reaction at 55°C for 5 min, denaturation at 95°C for 10 min (maintained for 10 s), and annealing at 60°C for 40 s. The primer sequences are as follows in Table I.

### Statistical Analysis

The Statistical Product and Service Solutions (SPSS) 22.0 software (IBM Corp., Armonk, NY, USA) was used for statistical analysis. The *t*-test was used for the data in line with normal distribution and homogeneity of variance, corrected *t*-test for the data in line with normal distribution and heterogeneity of variance, and the non-parametric test for the data not in line with normal distribution and homogeneity of variance. In addition, the rank sum test was adopted for ranked data, and the chi-square test for the comparison between two groups.

**Table I.** Primer sequences.

| Gene | Primer sequence |
|---|---|
| MiR-22-3p | F: 5'-GGTTAAGCTGCCAGTTGAA-3'<br>R: 5'-CAGTGCGTGTCGTGGAGT-3' |
| U6 | F: 5'-CTCGCTTCGGCAGCACA-3'<br>R: 5'-AACGCTTCACGAATTTGCGT-3' |

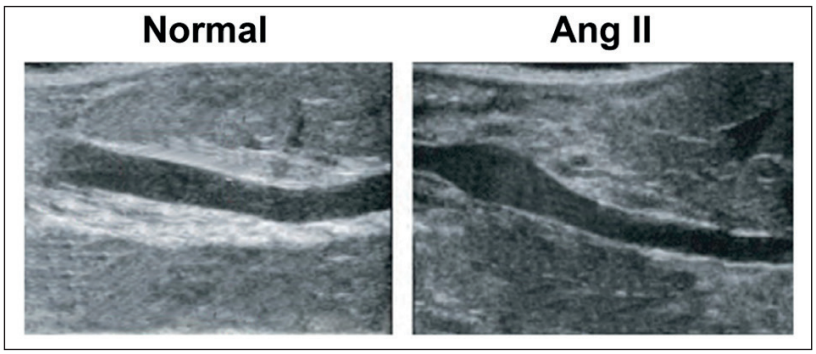


**Figure 1.** Tumor formation in thoracic aorta in normal group and Ang II group detected via echocardiography.

## Results

### *Echocardiography*

It was clearly found *via* echocardiography that blood vessels had local dilatation and hypertrophy in Ang II group, suggesting the tumor formation, while the vascular wall was normal in normal group (Figure 1).

### *RT-PCR*

The results of RT-PCR revealed that the expression of miR-22-3p in Ang II group was significantly lower than that in the normal group, and the difference was statistically significant ($p<0.05$) (Figure 2).

### *Sampling and Immunohistochemistry Results*

Notably, the tumor formation rate was 0 after injection of agomiR-22 ($p<0.05$), but the incidence rate of TAA was higher after injection of agomiR negative control ($p<0.05$) (Figures 3 and 4). The results of immunohistochemistry showed that agomiR-22-3p group had a lower expression of MMP-9 than the control group ($p<0.05$) (Figures 5 and 6).

### *Immunofluorescence*

According to immunofluorescence results, agomiR-22-3p group had an evidently lower expression of MMP-9 than the control group ($p<0.05$) (Figures 7 and 8).

## Discussion

TAA is a highly risky disease. The daily life, inheritance, age, high blood pressure, and special infection may cause the decline in strength of aortic wall and abnormalities in structure and function, in which case, under the massive impact of blood, the vascular wall will suffer from bulging and even tumor-like dilatation in severe cases.

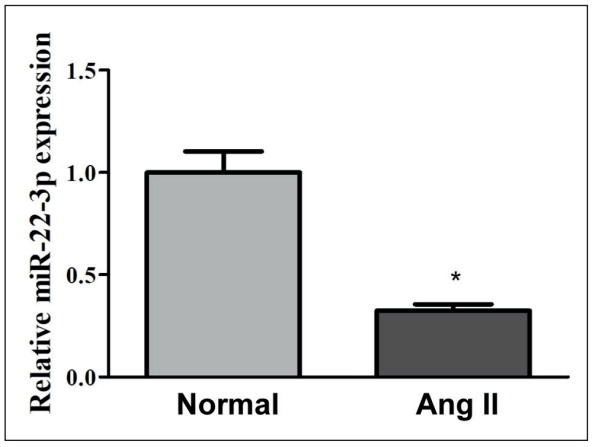


**Figure 2.** Expression of miR-22-3p in thoracic aorta in normal group and Ang II group detected using RT-PCR. Note: *$p<0.05$ *vs.* normal group.

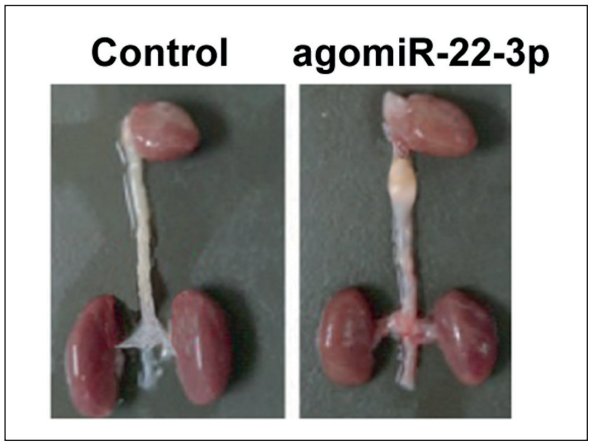


**Figure 3.** Tumor formation in thoracic aorta in control group and agomiR-22-3p group.

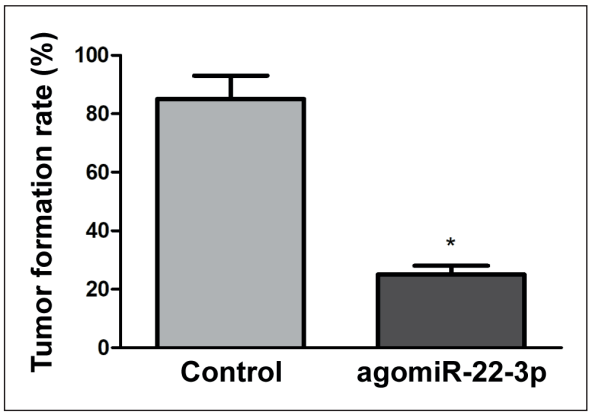


**Figure 4.** Tumor formation rate in thoracic aorta in control group and agomiR-22-3p group. Note: *$p$<0.05 *vs.* control group.

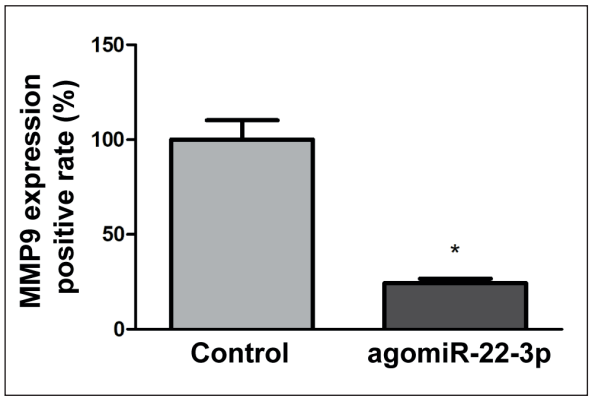


**Figure 6.** Statistical results of MMP9 expression in thoracic aorta in control group and agomiR-22-3p group. Note: *$p$<0.05 *vs.* control group.

The incidence rate of TAA is high and continuously rises with the gradual aging of population, and changes in dietary and living habits in China, attracting more concern and attention of researchers. Currently, the major clinical treatment means for TAA are special vascular intervention and surgery, but there is no definitive conclusion about the drugs for preventing, alleviating or reversing the occurrence of aneurysm due to the specificity, complexity, and variability of the disease, which has long been a research hotspot[8-11]. Therefore, studying the occurrence, development, and patho-

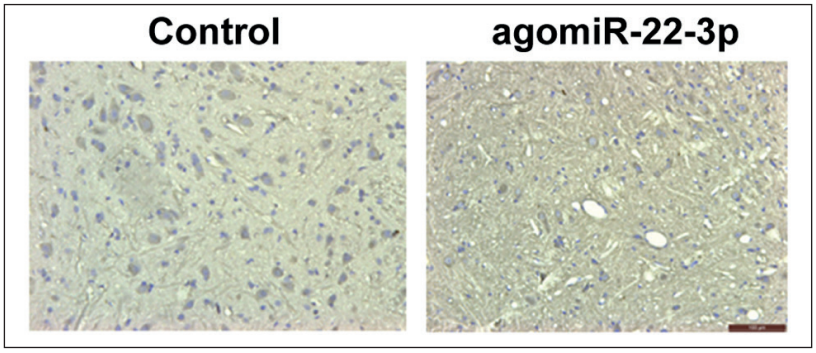


**Figure 5.** MMP9 expression in thoracic aorta in control group and agomiR-22-3p group determined using immunohistochemistry (magnification: 200×).

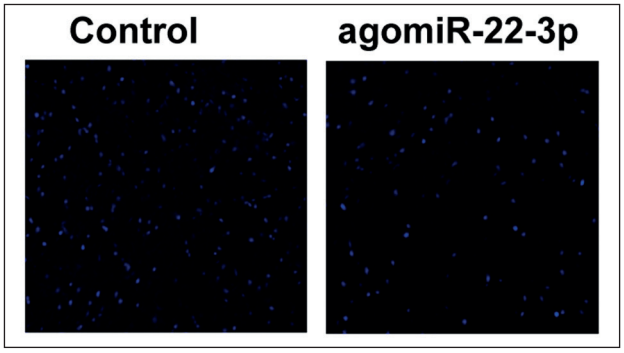


**Figure 7.** MMP9 expression in thoracic aorta in control group and agomiR-22-3p group determined using immunofluorescence (magnification: 200×).

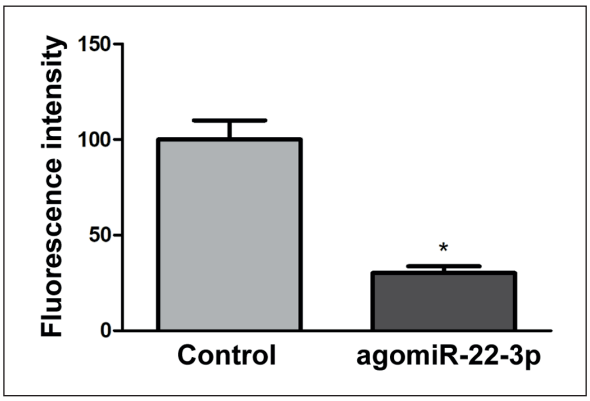


**Figure 8.** Statistical results of fluorescence intensity in thoracic aorta in control group and agomiR-22-3p group. Note: *$p$<0.05 *vs.* control group.

logical mechanism of TAA can effectively inhibit aortic aneurysm rupture and promote prognosis of disease. At present, the research mainly focuses on the ECM closely related to the pathogenesis of aortic aneurysm. The imbalance of ECM in mid-layer tissues of the aortic wall is highly likely to lead to aortic wall rupture and tumor formation. Moreover, studies have found that MMP-9 is an important factor for maintaining ECM balance, and plays a key role in preventing ECM imbalance. Therefore, many researchers have begun to focus on the pathological mechanism of ECM imbalance in aneurysm, so MMP-9 is increasingly studied. MMP-9 has a strong ability to decompose key components (elastin and collagen) in the aneurysm wall. Therefore, it has profound significance for the stability of arterial wall structure, integrity of related functions, formation, rapid expansion, and eventual rupture of aortic aneurysm.

MiRNAs are a class of highly conserved endogenous non-coding RNAs in eukaryotic cells, which can silence related genes, inhibit protein expression, and participate as important bio-regulators in the occurrence and development of various diseases[12-16] widely studied currently. MiR-22-3p is a common miRNA closely related to the development and migration of cancer cells and the tumor formation, and it plays an important role in preventing tumor formation and outcome[17-21].

The novelty of this study was that we found for the first time that miR-22-3p showed low expression in thoracic aortic aneurysms, and we tried to explain the mechanism of action of miR-22-3p. We revealed that miR-22-3p may inhibit the formation of thoracic aortic aneurysm by inhibiting the expression of MMP-9. In this study, the TAA model was established *via* continuous perfusion of Ang II using the micro pump. After 4 weeks, tumor was formed in the thoracic aorta, suggesting the successful modeling. The results of RT-PCR showed that the expression of miR-22-3p significantly declined in TAA rats, indicating that the expression of miR-22-3p declines during the formation of TAA. The tumor formation rate was 0 after injection of agomiR-22, while it was high after injection of agomiR negative control, suggesting that miR-22-3p can suppress the tumor formation. Besides, the immunohistochemistry and immunofluorescence results manifested that the expression of MMP-9 in rats injected with agomiR-22 was remarkably lower than that in rats injected with agomiR negative control, indicating that MMP-9 can promote the formation of TAA, and miR-22-3p can exert an inhibitory effect on MMP-9. To sum up, miR-22-3p can inhibit the expression of MMP-9, thus suppressing the formation of TAA in rats.

## Conclusions

Summarily, miR-22-3p can inhibit the formation of TAA in rats, whose mechanism is to inhibit the expression of MMP-9. However, the mechanism of action of miR-22-3p in inhibiting MMP-9 remains to be further studied.

### Conflict of Interests

The authors declare that they have no conflict of interest.

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