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Sestrin2 increases in aortas and plasma from aortic dissection patients and alleviates angiotensin II-induced smooth muscle cell apoptosis via the Nrf2 pathway☆

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ABSTRACT

Background: Previous studies have demonstrated that oxidative stress is closely related to aortic dissection (AD). Sestrin2 (Sesn2) is an important antioxidant protein, and this study aimed to investigate whether Sesn2 participates in AD and the possible mechanisms.

Methods: Sesn2 expression was detected in aortas collected from AD patients and normal donors. In addition, blood samples were collected from AD patients and non-AD (NAD) patients, and the plasma Sesn2 levels were measured. Furthermore, the effects of Sesn2 on angiotensin (Ang) II-induced smooth muscle cell (SMC) apoptosis were investigated in vitro.

Results: Compared with the aortas from normal donors, aortas from AD patients had significantly increased Sesn2. Sesn2 was mainly secreted by macrophages, and low levels were secreted by CD4+ T lymphocytes, but not SMCs. Plasma Sesn2 levels were also increased in AD patients compared with NAD patients. Sesn2 levels were negatively corrected with superoxide dismutase (SOD) levels but positively corrected with malondialdehyde (MDA) levels in AD patients. In co-cultures of macrophages and SMCs, Sesn2 overexpression in macrophages significantly reduced Ang II-induced SMC apoptosis, and this effect could be reversed by Nrf2 silencing.

Conclusions: Sesn2 is increased in both aortas and plasma from AD patients. Sesn2 may alleviate Ang II-induced SMC apoptosis and participate in AD via the Nrf2 pathway. Sesn2 may be a new target in the treatment and prevention of AD.

1. Introduction

Aortic dissection (AD) is a rare but extremely high-risk clinical syndrome. AD can cause a variety of serious clinical complications, and without treatment, this condition can result in mortality in a short period of time [1]. A variety of inflammatory cells were observed at various stages, and the inflammatory response is considered one of the most important mechanisms of AD. Recently, growing evidence from animal studies has demonstrated that oxidative stress also participates in the occurrence and development of AD [2,3].

Sestrin2 (Sesn2) is a member of the Sestrin family and is mainly expressed in mammals. Sesn2 can be secreted by a variety of immune cells and non-immune cells, such as macrophages, T lymphocytes, endothelial cells, and cardiac fibroblasts [4–7], especially macrophages [4,8]. A variety of environmental stressors could promote Sesn2 secretion, including oxidative stress, DNA damage, and hypoxia; among

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them, oxidative stress has the most important role [9,10]. Similar to the other 2 family members Sesn1 and Sesn3, Sesn2 protects against cell apoptosis and toxicity through, various mechanisms by maintaining the oxidative balance [11–13].

Accumulating studies have also demonstrated that Sesn2 plays a critical role in the presence and development of cardiovascular disease. In an earlier study, knockdown of Sesn2 by siRNA elevated blood pressure in a dopamine D2 receptor deficiency-induced mouse hypertension model [12]. In another study, deletion of Sesn2 significantly reduced the activation of liver kinase B1 (LKB1), a major AMPK upstream kinase, and increased the area of the myocardial infarction in a mouse ischemia reperfusion model [14]. After treatment with irradiation for 4 weeks, Sesn2 knockout aggravated cardiac fibrosis [15]. In human heart samples and left atrial cells, Sesn2 expression was observed in the left atrium of a patient with atrial fibrillation and reduced the collagen expression in vitro [16]. In addition, increased Sesn2 levels were observed in mouse and human failing hearts and plasma from coronary artery disease patients [7,17]. However, the role of Sesn2 in AD remains unknown, and we aimed to detect Sesn2 expression in human AD and explore the possible mechanisms.

2. Methods and materials

2.1. Collection of aortic and blood samples

Normal aortas (n = 9) were obtained from donors who were diagnosed with brain death and had no significant cardiovascular diseases. Aortic samples (n = 12) were donated from patients who suffered from acute AD and received aortic replacement surgery. All aortic samples were collected by experienced cardiologists. Consecutive patients (n = 190) who were hospitalized because they suffered sudden chest pain from February 2018 to May 2018 were enrolled in this experiment. Blood samples were collected by nurses after the computed tomography angiography (CTA) was performed, and the patients returned to the inpatient wards without treatment. Of these 190 patients, 30 patients with a history of significant diseases that affect Sesn2 secretion, including 15 patients with atherosclerosis or coronary artery disease, 5 patients with chronic heart failure, 4 patients with acute left heart failure, 3 patients with connective tissue disease, and 3 patients with tumors, were excluded from this experiment. According to the clinical symptoms and the results of CTA, the remaining 160 patients were divided into the NAD (n = 40) group and the AD (n = 120) group, and the AD group was further divided into the Stanford A (n = 70) and Stanford B groups (n = 50). The aortic samples and blood samples were collected from Longhua Central Hospital Affiliated Guangdong Medical University, Beijing Anzhen Hospital of Capital Medical University, and Renmin Hospital of Wuhan University, and the present study was approved by the Institutional Review Board at these three institutions. Patients themselves or their families provided informed consent before the blood samples and aortic samples were collected.

2.2. Western blot analysis

After the aortic samples were lysed, the total protein of each sample was collected and quantified by a BCA Protein Assay Kit (Thermo Fisher Scientific). A total of 20 μg of protein was added to 10% SDS polyacrylamide gels for electrophoresis, and then transferred to Immobilon-FL PVDF membranes (Millipore). The membranes were blocked with 5% non-fat milk and further incubated with primary antibodies (including anti-Sesn2 antibody and anti-GADPH antibody, both from Abcam) at 4 °C overnight. Then, the membranes were incubated with secondary antibody at room temperature for 1 h. The blots were scanned using an Odyssey system.

2.3. Histological analysis

Aortic samples were fixed in 4% neutral paraformaldehyde, embedded in paraffin, cut into 5–6 μm slices and mounted onto slides. Immunofluorescence staining with Sesn2 antibody was performed to measure the Sesn2 expression in each sample. Double immunofluorescence staining with anti-CD68 antibody and anti-Sesn2 antibody, anti-CD4 antibody and anti-Sesn2 antibody, and anti-α-SMA antibody and anti-Sesn2 antibody was used to label the source of Sesn2 in macrophages, CD4+ T lymphocytes and smooth muscle cells (SMCs).

2.4. Determination of plasma superoxide dismutase (SOD), malondialdehyde (MDA) and Sesn2 levels

Blood samples were centrifuged for 20 min at 3000*g*, and the supernatant of each sample was collected and stored at −80 °C until the beginning of the experiments. The plasma SOD, MDA (both from Sigma) and Sesn2 (Abcam) levels were measured according to the manufacturer's instructions.

2.5. Cell isolation and culture

Bone marrow-derived macrophages and SMCs were isolated from male C57BL/6 mice as previously described [18–20]. First, the macrophages were treated with different doses of Ang II (25 mM, 50 mM, and 100 mM, Sigma) or SOD (0.3 U/ml) for 24 h [21], and treatment with saline was used as a control. Then, the total RNA in macrophages was collected for Sesn2 mRNA analysis.

In addition, complementary DNA (cDNA) targeting Sesn2 (cDNA-Sesn2) were used to up-regulate Sesn2 expression and were generated by Biochrom (German). The macrophages were transfected with cDNA-Sesn2 or a scrambled cDNA according to the manufacturer's instructions. The cDNA could greatest up-regulate the Sesn2 mRNA level about 2.5-fold in preliminary study and was used for the further study (Supplemental Fig. 1A). To down-regulate Nrf2 mRNA in SMCs, three siRNA were generated (RiboBio, Guangzhou, China) and the siRNA resulting in the strongest down-regulation of Nrf2 mRNA level (decreased 0.65-fold) was used for the further study (Supplemental Fig. 1B). The macrophages (Mø) and MSCs were divided into the following groups: 1.SMCs; 2.SMCs + Mø; 3.SMCs + Mø + cDNA-Sesn2; 4.SMCs + Mø + cDNA-Sesn2 + SiNrf2; 5.SMCs + Ang II (100 mM); 6.SMCs + Mø + Ang II; 7.SMCs + Mø + cDNA-Sesn2 + Ang II; 8. SMCs + Mø + cDNA-Sesn2 + SiNrf2 + Ang II. After treatment for 24 h, the total RNA in SMCs was collected for Bax, and Bcl2 mRNA analysis.

2.6. Quantitative polymerase chain reaction (RT-qPCR)

After lysed by TRIzol reagent W, total RNA in macrophages and SMCs was collected. A total of 2 μg of total mRNA was used to synthesize cDNA using a reverse transcription kit according to the manufacturer's instructions. LightCycler 480 SYBR Green Master Mix was used to perform the PCR amplifications. The relative Sesn2, Bax, and Bcl2 mRNA levels were measured, and the results were normalized to the expression levels of GAPDH. All the reagents in this section were purchased from Roche, and the RT-qPCR primer sequences are shown in Table 1.

2.7. Statistical analysis

The data in the clinical study were first analyzed to determine if they conformed to a normal distribution. Data with a normal distribution were expressed as the mean ± standard deviation (SD) and compared using Student's *t*-tests. The median (lower quartile to upper quartile) was used to express the data with an abnormal distribution, and Mann-Whitney *U* test was performed to compare the differences

Table 1
RT-qPCR primers used.

Gene	Forward primer	Reverse primer
Sesn2	GGCGGTGGTGATGGGTCTAC	GACGACCCGGAAGTGGCCC
Bax	TTGCTGATGGCAACTTCAAC	GATCAGCTCGGGCACTTTAG
Bcl2	CAGAAGATCATGCCGTCCTT	CTTTCTGCTTTTTATTTCATGAGG
GAPDH	CGGCAAATTCAACGGCACAG	GTTTTCCCAGTAGTAGAGGCGG

between different groups. Counts (percentages) and chi-square tests were performed to express and compare the categorical variables. The correlations between SOD, MDA and Sesn2 levels were analyzed by Spearman's correlation analysis. Simple linear regression analyses and subsequent binary logistic regression analyses were performed to identify whether Sesn2 was an independent predictor of the onset of AD. The data in the cell culture were expressed as the mean ± SD and compared with one -way ANOVA, followed by Tukey's multiple comparisons test. All the data were analyzed using SPSS 22.0 software, and a p value < 0.05 was considered statistically significant and was the threshold used to reject the null hypothesis.

3. Results

3.1. Basic clinical characteristics of patients who provided aortic samples and blood samples

In the patients who provided aortic samples, higher D-dimer, white blood cells (WBCs), and C-reactive protein were observed in the AD group when compared with the normal group. No differences in male gender, age, poor blood pressure control (PBPC), smoking, fast glucose (Glu), total cholesterol (TC), triglycerides (TGs), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and creatinine (CREA) levels were found between the two groups. Some of the patients with uneven vital signs in the normal group were treated with vasoactive drugs; therefore, the real blood pressure and heart rate (HR) could be determined. The clinical characteristics are listed in Table 2.

Among the patients who provided blood samples, AD patients exhibited higher PBPC, Glu, WBC, HR, CRP, and D-dimer levels than the NAD patients. No differences in other clinical characteristics were observed, including male gender, age, smoking, systolic blood pressure (SBP), diastolic blood pressure (DBP), lipids, CREA and time intervals between chest pain onset and collection of blood samples (times). In addition, no differences in the clinical characteristics of the Stanford A and Stanford B groups were found. The clinical characteristics are listed in Table 3.

Table 2
Clinical characteristics in patients who provide aortic samples.

Characteristic	Control	TAD	P value
Male (n, %)	5 (55.6%)	10 (83.3%)	0.331
Age (years)	49 ± 12	53 ± 13	0.651
PBPC (n, %)	4 (44.4%)	8 (66.7%)	0.396
Smoking (n, %)	3 (33.3%)	8 (66.7%)	0.198
Glu (mmol/L)	6.1 ± 1.0	6.0 ± 1.2	0.602
SBP (mmHg)	–	151 ± 24	–
DBP (mmHg)	–	81 ± 15	–
TC (mmol/L)	4.9 (4.6, 5.6)	4.5 (3.9, 5.2)	0.193
TG (mmol/L)	1.1 (0.8, 1.9)	1.3 (1.0, 1.7)	0.554
HDL-C (mmol/L)	1.6 (1.0, 1.7)	1.3 (0.9, 1.6)	0.382
LDL-C (mmol/L)	2.5 (1.9, 2.6)	2.2 (1.3, 3.1)	0.651
HR (bpm)	–	72 ± 12	–
CREA (μmol/L)	92 ± 19	93 ± 26	0.972
D-dimer (μg/ml)	1.1 (1.6, 2.0)	4.7 (1.9, 7.2)	0.006
WBC ($\times 10^9$/L)	5.6 (4.8, 7.1)	10.1 (8.6, 14.3)	< 0.001
CRP (mg/L)	0.7 (0.3, 1.7)	10.0 (2.1, 21.8)	0.001

PBPC: poor blood pressure control; Glu: fasting glucose; SBP: systolic blood pressure; DBP: diastolic blood pressure; TC: total cholesterol; TG: triglyceride; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; HR: heart rate; CREA: creatinine; WBC: while blood cell; CRP: C-Reactive protein.

3.2. Expression of aortic Sesn2 in AD patients

The aortic Sesn2 expression was measured by both western blot and histologic analysis. The western blot results showed that the aortic Sesn2 in the AD group increased approximately about 0.5-fold compared to that in the normal group (Fig. 1A). Similar trends were observed in the results of the histologic analysis (Fig. 1B). In addition, double immunofluorescence staining showed that both the aortic macrophages and CD4+ T lymphocytes, especially macrophages, rather than SMCs, were the source of Sesn2 (Fig. 1C).

3.3. Plasma Sesn2, SOD and MDA levels in AD patients

Plasma Sesn2 and MDA levels were increased, while SOD levels were reduced in the AD groups compared with the NAD group (Fig. 2A–C). In addition, no differences in Sesn2, SOD and MDA levels were found between the Stanford A and Stanford B groups (Fig. 2A–C). Furthermore, the Sesn2 levels were negatively corrected with the SOD levels in AD patients (Fig. 2D), but positively corrected with MDA levels (Fig. 2E). Plasma Sesn2, SOD and MDA levels in each group are listed in Table 4.

3.4. Univariate analysis and multivariate linear regression analysis

Sesn2, SOD, MDA, TC, LDL-C, and the difference clinical characteristics were used to perform univariate analysis. Some variables that showed a trend in the univariate analysis were used to further perform multivariate linear regression analysis. The results showed that the presence of AD was independently associated with plasmaSesn2 levels (β = −0162; 95%CI −260 to −0.055; P = 0.003), SOD levels (β = −0192; 95%CI −299 to −0.086; P < 0.001), and MDA levels (β = 0.578; 95%CI 0.454 to 0.703; P < 0.001). The β value, 95% CI of β, and P values are listed in Table 5.

3.5. Effect of Sesn2 on Ang II-induced SMC apoptosis

After treatment with Ang II, the Sesn2 mRNA levels in macrophages were detected and the results showed that Ang II treatment dose-dependently increased Sesn2 mRNA levels (Fig. 3A). In addition, SOD treatment significantly reduced Sesn2 mRNA levels in macrophages (Fig. 3A). The Sesn2 mRNA in macrophages was increased 2.5-fold by cDNA-Sesn2, while the Nrf2 mRNA levels in SMCs were reduced by SiNrf2 (Supplementary material). Co-culture with macrophages significantly enhanced the Ang II-induced increases in Bax mRNA levels in SMCs, and up-regulation of Sesn2 decreased the Bax mRNA levels in SMCs co-cultured with macrophages. These effects were reversed by Nrf2 down-regulation (Fig. 3B). While co-culture with macrophages decreased Ang II-induced Bcl2 mRNA levels, these effects could be reserved by up-regulation of Sesn2, and down-regulation of Nrf2 alleviated the Bcl2 mRNA increase mediated by cDNA-Sesn2 (Fig. 3B).

4. Discussion

In the present study, we found for the first time that Sesn2 expression was increased in both aortas and plasma from AD patients. In addition, aortic macrophages were the main source of Sesn2, and plasma Sesn2 levels were negatively corrected with SOD levels but positively corrected with MDA levels. Sesn2 levels were also independently associated with the occurrence of AD. Macrophages increased Ang II-induced SMC apoptosis, and this effect could be reversed by Sesn2 up-regulation. The protective role of Sesn2 on SMC apoptosis

Table 3
Clinical Characteristics in patients who provide blood samples.

Characteristic	NAD	AD		
		Total	Stanford A	Stanford B
Male (n, %)	29 (72.5)	86 (71.7)	50 (71.4)	36 (72.0%)
Age (years)	60.1 ± 11.3	56.8 ± 10.1	56.6 ± 10.2	56.9 ± 10.2
Smoking (n, %)	15 (37.5)	43 (45.8)	32 (45.7)	23 (56.0%)
PBPC (n, %)	25 (62.5)	100 (83.3)*	62 (88.6%)*	38 (76.0%)
Glu (mmol/L)	6.1 ± 1.6	7.4 ± 1.6*	7.3 ± 1.2*	7.5 ± 1.8*
SBP (mmHg)	144 ± 27	150 ± 27	154 ± 24	147 ± 29
DBP (mmHg)	86 (76, 100)	90 (80, 100)	90 (80, 100)	89 (80, 101)
WBC ($\times 10^9$/L)	5.9 ± 1.1	11.1 ± 4.0*	10.5 ± 3.8*	11.5 ± 4.1*
TC (mmol/L)	3.9 ± 1.1	3.9 ± 0.8	4.0 ± 0.7	3.9 ± 0.9
TG (mmol/L)	1.0 (0.9, 1.3)	1.1 (0.9, 1.5)	1.2 (0.9, 1.7)	1.0 (0.9, 1.3)
HDL-C (mmol/L)	0.9 (0.8, 1.1)	1.0 (0.9, 1.3)	1.0 (0.9, 1.4)*	1.0 (0.8, 1.3)
LDL-C (mmol/L)	2.2 ± 0.7	2.1 ± 0.6	2.1 ± 0.6	2.1 ± 0.7
HR (bpm)	69 (66, 74)	75 (66, 84)*	80 (72, 89)*	73 (63, 80)*
CR (μmol/L)	75 (72, 83)	82 (68, 105)	85 (71, 106)	80 (65, 102)
CRP (mg/L)	0.6 (0.2, 1.2)	9.5 (3.4, 46.5)*	14.6 (3.3, 60.7)*	7.7 (3.3, 23.5)*
D-dimer (μg/ml)	0.6 (0.3, 1.0)	4.2 (2.0, 7.3)*	4.2 (2.0, 5.5)*	4.1 (1.5, 7.5)*
Time (hours)	8 (6, 12)	10 (6, 14)	11 (8, 15)	10 (6, 12)
Medications, n (%)				
ACEI/ARB	29 (72.5)	83 (69.2)	47 (67.1)	36 (72.0)
Beta-blockers	11 (27.5)	35 (29.2)	22 (31.4)	13 (26.0)
CCB	26 (65.0)	76 (63.3)	41 (58.6)	35 (70.0)
Diuretic	17 (42.5)	62 (51.7)	37 (52.9)	25 (50.0)

Time: time intervals between chest pain onset and collection of blood samples; ACEI: angiotensin-converting enzyme inhibitor; ARB: angiotensin receptor blocker; CCB: calcium channel blocker.
* $p < 0.05$ vs. the NAD group.

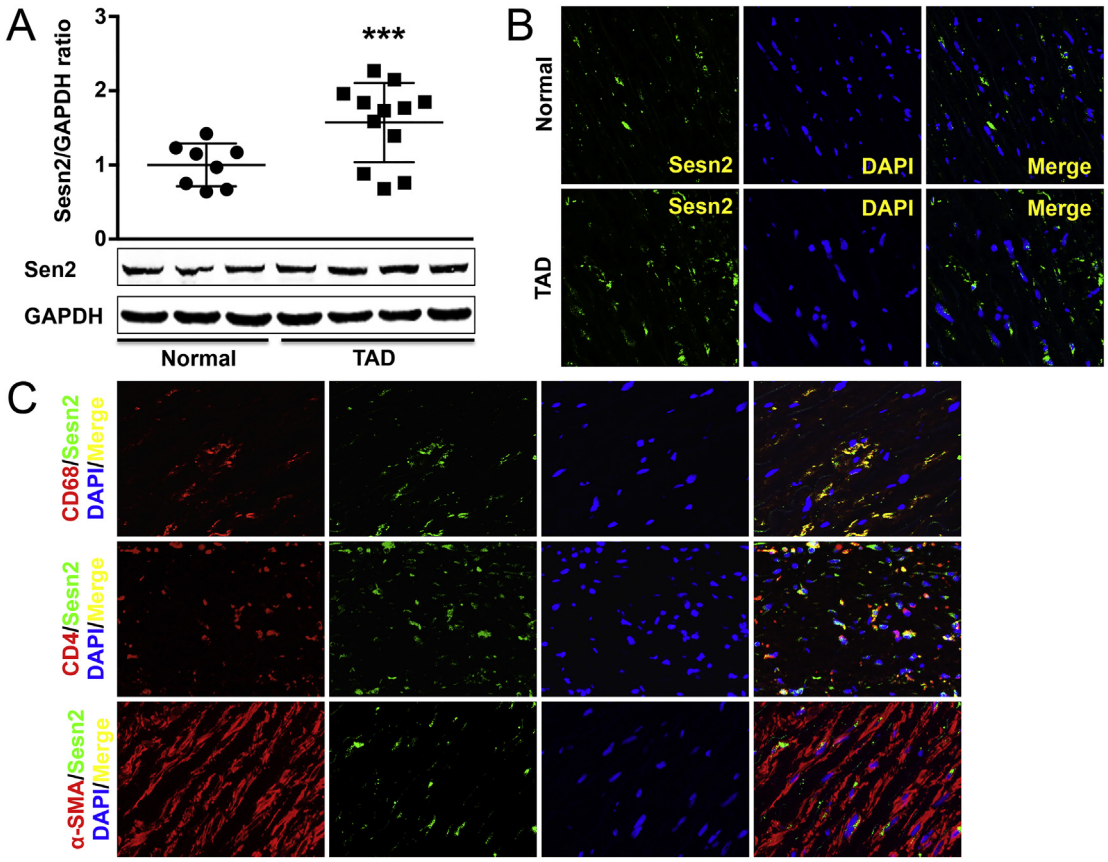

Fig. 1. Sesn2 expression in AD patients. (A). Sesn2 levels in the normal group and AD group were detected by western blot analyses. (B). Sesn2 expression in these two groups was measured by immunofluorescence staining (200×). (C). Double immunofluorescence staining with anti-CD68 antibody, anti-CD4 antibody, anti-α-SMA antibody and anti-Sesn2 antibody (200×).

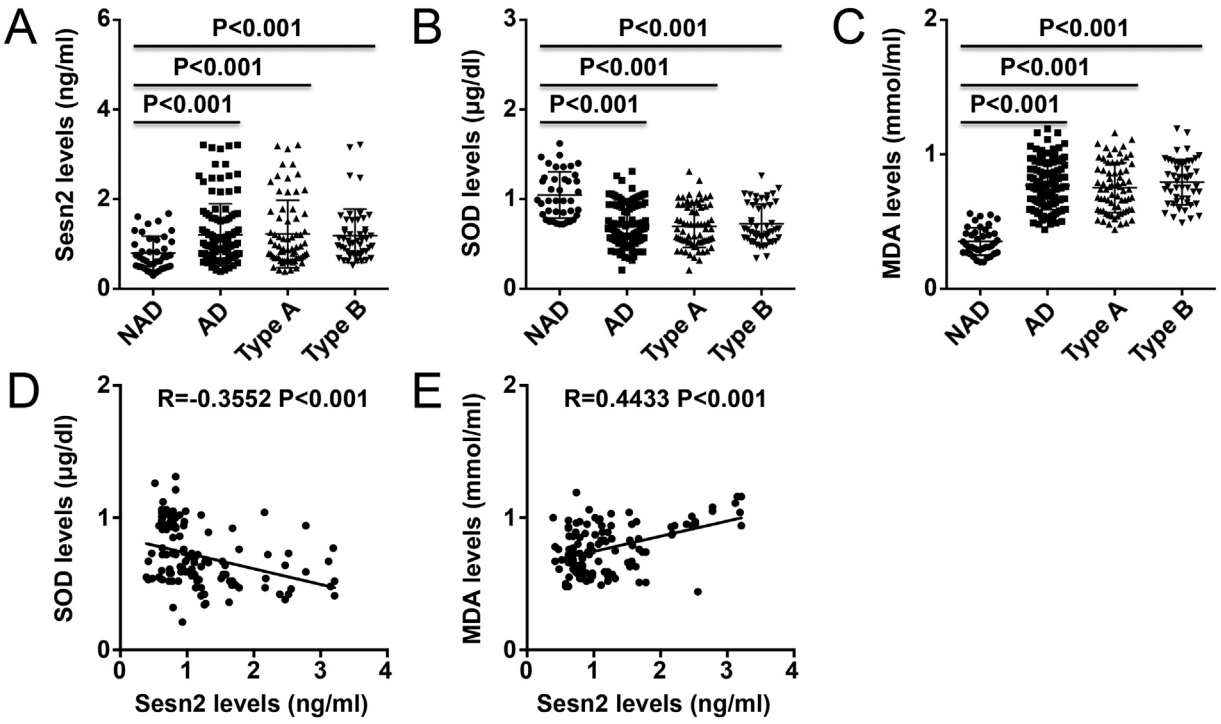

Fig. 2. Plasma superoxide dismutase (SOD), malondialdehyde (MDA) and Sesn2 levels in AD patients. The plasma Sesn2 (A), SOD (B), and MDA (C) levels in the NAD, AD, Stanford A, and Stanford B groups were measured. Correlation between Sesn2 levels and SOD levels (D), and MDA (E) levels in AD patients.

could be prevented by Nrf2 down-regulation.

In vivo and in vitro studies have demonstrated that Sesn2 levels are increased in a variety of diseases. Hu HJ et al. reported that treatment with oxidized low density lipoprotein time and dose-dependently increased Sesn2 mRNA levels in RAW264.7 cells [4]. In addition, the Sesn2 levels were increased by Ang II and related to the timing and concentration of the stimulus [6]. In an ischemic mouse model, Sesn2 was reported to be increased in heart and gradually decrease according to over time [14]. In addition, Sesn2 levels were observed to increase in patients with chronic heart failure, coronary artery diseases, and atrial fibrillation [7,16,17]. However, the Sesn2 levels in AD have not yet been reported. In this study, we measured Sesn2 levels in both aortas and plasma from AD patients and found that Sesn2 levels were increased in both aortas and plasma. These results suggest that Sesn2 participates in the occurrence of AD.

At various stages of AD, large numbers of macrophages and T lymphocytes were found to infiltrate the aortic wall. According to previous reports, both macrophages and CD4+ T lymphocytes are important sources of Sesn2 [4,5,8]. As SMCs are the main component of the aorta, we investigated whether macrophages, CD4+ T lymphocytes and SMCs were the source in AD using double immunofluorescence staining. The results showed that Sesn2 was mainly secreted by macrophages, and was secreted in small amounts by lymphocytes rather than by SMCs. Although macrophages and CD4+ T lymphocytes are considered inflammatory cells, previous studies also found that they could be involved in the regulation of oxidative stress [22,23]. Combined with the involvement of macrophages and CD4+ T lymphocytes in AD, our results further demonstrated that Sesn2 participated in the occurrence of AD.

Sesn2 is considered an antioxidant protein and participates in diseases via regulating oxidative stress levels. Hu HJ et al. reported that up regulation of Sesn2 decreased oxidized low density lipoprotein-induced reactive oxygen species (ROS) secretion and alleviated macrophage apoptosis in vitro [4]. In addition, Yi L et al. found that Ang II-induced ROS secretion and endothelial toxicity could be reversed by Sesn2 up-regulation [6]. In HL-1 cells, Sesn2 up-regulation by siRNA significantly decreased ROS expression and alleviated fibrosis [16]. Silencing of Sesn2 was also reported to increase circulating ROS and elevate blood pressure [12]. To explore the mechanisms by which Sesn2 participated in the occurrence of AD, we detected the circulating SOD and MDA levels, and the results showed that increased oxidative stress was observed in AD patients, and circulating Sesn2 levels were positively correlated with the severity of oxidative stress. These results were consistent with previous conclusions and suggested that Sesn2 may be involved in AD by regulating oxidative stress.

SMCs constantly synthesize and degrade the extracellular matrix (ECM) and can also sense hemodynamic pressure and maintain cytoskeletal and ECM remodeling, which are important for maintaining the dynamic balance of ECM and the normal structure and function of the aorta [24,25]. Excessive loss of SMC which is mediated by sensors,

Table 4
Plasma Sesn2, SOD and MDA levels in each group.

Characteristic	NAD	AD		
		Total	Stanford A	Stanford B
Sesn2 (ng/ml)	0.83 (0.61, 1.20)	0.97 (0.70, 1.54)*	0.92 (0.69, 1.69)*	1.00 (0.74, 1.32)*
SOD (μg/dl)	0.98 (0.79, 1.26)	0.73 (0.57, 0.94)*	0.72 (0.52, 0.93)*	0.77 (0.63, 1.03)*
MDA (nmol/ml)	0.33 (0.27, 0.42)	0.76 (0.62, 0.93)*	0.74 (0.59, 0.88)*	0.77 (0.63, 0.94)*

* $p < 0.05$ vs. the NAD group.

Table 5
Association between Sesn2, SOD, MDA clinical characteristics and the presence of acute AD were analyzed by univariate analysis and multivariate linear regression analysis.

Variables	Univariate			Multivariate		
	β	95% CI	P value	β	95% CI	P value
Sesn2	0.274	0.123 to 0.425	< 0.001	−0.162	−0.260 to −0.055	0.003
SOD	−0.529	−0.662 to −0.395	< 0.001	−0.192	−0.299 to −0.086	< 0.001
MDA	0.743	0.638 to 0.849	< 0.001	0.578	0.454 to 0.703	< 0.001
PBPC	0.218	0.065 to 0.372	0.006	0.070	−0.023 to 0.162	0.138
Glu	0.321	0.173 to 0.470	< 0.001	0.103	0.007 to 0.199	0.035
WBC	0.543	0.411 to 0.675	< 0.001	0.161	0.055 to 0.267	0.003
CRP	0.225	0.072 to 0.378	0.004	0.293	0.081 to 0.109	0.770
D-dimer	0.418	0.276 to 0.561	< 0.001	0.156	0.059 to 0.253	0.002
Smoking	0.073	−0.084 to 0.229	0.917			
TC	0.022	−0.135 to 0.179	0.780			
LDL-C	−0.017	−0.174 to 0.140	0.829			

including ischemic, denatured, and necrotic signals, could reduce the amount of ECM in the aorta and result in dysfunction of the aortic structure and function, which is the leading cause of AD [26,27].In addition, SMCs were found to be significantly decreased in the aortas collected from AD patients, and the middle layer showed the most obvious change [26,27]. The myosin heavy chains of SMCs were also observed to increase in the blood after the occurrence of AD [28,29]. This evidence demonstrated that excessive loss of SMCs plays a leading role in the occurrence and development of AD.A previous study reported that oxidative stress was closely related to SMC apoptosis [30]. Therefore, to further determine the mechanisms by which Sesn2 involved in AD, the effect of Ang II on Sesn2 secretion and Sesn2 on Ang II-induced SMC apoptosis was detected. The results showed that Ang II dose dependently increased Sesn2 levels in macrophages, and these effects could be prevented by SOD treatment. These results are consistent with previous reports in which Sesn2 levels were determined by oxidative stress levels. In addition, we found that co-culture with macrophages increased SMC apoptosis, although Ang II increased Sesn2 expression in macrophages. The possible reason for this phenomenon is that the elevated Sesn2 expression was not sufficient to combat Ang II-induced oxidative stress levels. Further up regulation of Sesn2 by cDNA-Sesn2 in macrophages could reduce SMC apoptosis, and these results supported our speculation above. Sens2 was also play shown to have an antioxidant role by promoting Nrf2 protein secretion [8,31]. Therefore, Nrf2 expression in SMCs was down-regulated by SiNrf2, and the protective role of Sesn2 in SMC apoptosis was reversed. These data demonstrated that Sesn2 up-regulated Nrf2 secretion, paly an antioxidant role, protected against SMC apoptosis and reduced the occurrence of AD.

In summary, we found that aortic macrophages were the main source of Sesn2 in aortas of AD patients. Sesn2 promoted Nrf2 secretion, had an antioxidant role and regulated the occurrence of AD. However, there were some limitations to our study. First, the inflammatory response was closely related to the presence and

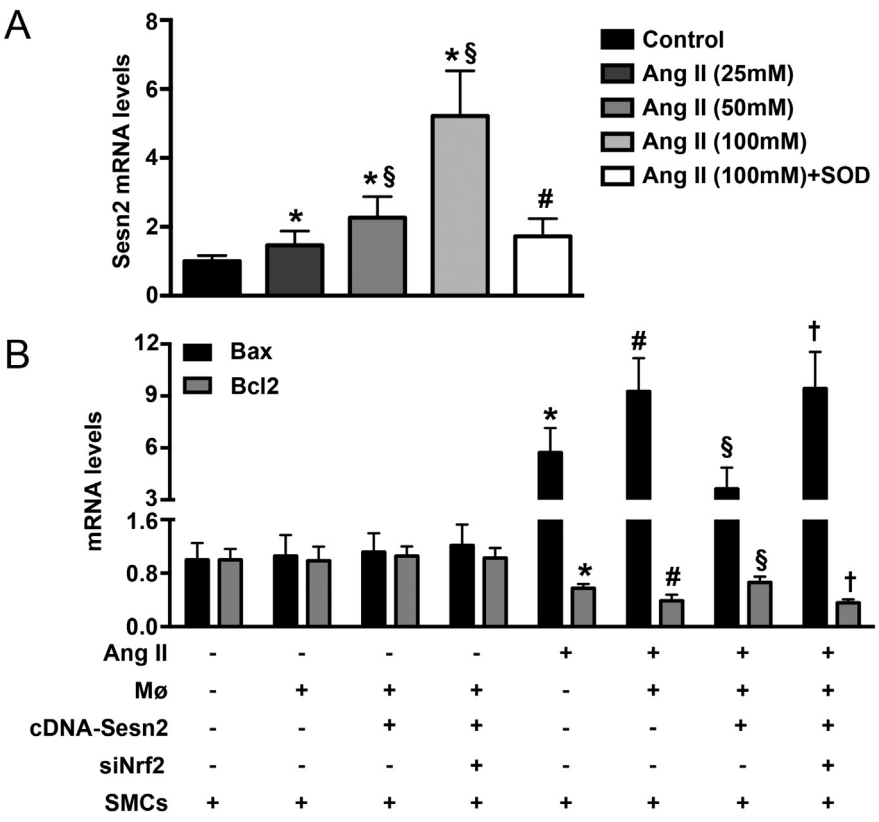

Fig. 3. Effect of Sesn2 on Ang II-induced SMC apoptosis. (A). Different doses of Ang II and SOD on Sesn2 expression in macrophages; N = 5 of each group;* $p < 0.05$ vs. the control group, § $p < 0.05$ vs. the previous group, # $p < 0.05$ vs. the Ang II (100 mM) group. (B). Effect of macrophages, Sesn2, and Nrf2 on the Bax, and Bcl2 mRNA expression in SMCs; N = 5 for each group; N = 5 for each group; * $p < 0.05$ vs. the SMCs, SMCs + Mø, SMCs + Mø + cDNA-Sesn2, and SMCs + Mø + cDNA-Sesn2 + SiNrf2 group, # $p < 0.05$ vs. the Ang II + SMCs groups, § $p < 0.05$ vs. the Ang II + Mø + SMCs group, † $p < 0.05$ vs. the Ang II + Mø + cDNA-Sens2 group.

progression of AD, and Sesn2 could also regulate the inflammatory response. Therefore, we did not investigate the effect of Sesn2 on the inflammatory response. Second, the sample size in the histologic analysis was quite small and needed to be increased. More studies are needed to further investigate what the role of Sesn2 in AD.

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