

Original Research

Correlation Between Low THBS3 Expression in Peripheral Blood and Acute Myocardial Infarction

Yanqiu Chen^{1,2,3,4,5}, Heyu Meng^{1,2,3,4,5}, Xin Meng^{1,2,3,4,5}, Zhaohan Yan^{1,2,3,4,5},
Jingru Wang^{1,2,3,4,5}, Fanbo Meng^{1,2,3,4,5,*}

¹Department of Cardiology, China-Japan Union Hospital of Jilin University, 130033 Changchun, Jilin, China

²Jilin Provincial Precision Medicine Key Laboratory for Cardiovascular Genetic Diagnosis, 130033 Changchun, Jilin, China

³Jilin Provincial Engineering Laboratory for Endothelial Function and Genetic Diagnosis of Cardiovascular Disease, 130033 Changchun, Jilin, China

⁴Jilin Provincial Molecular Biology Research Center for Precision Medicine of Major Cardiovascular Disease, 130033 Changchun, Jilin, China

⁵Jilin Provincial Cardiovascular Research Institute, 130033 Changchun, Jilin, China

*Correspondence: mengfb@jlu.edu.cn (Fanbo Meng)

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Abstract

Background: Thrombospondin (THBS) 3 is an adhesive glycoprotein involved in cell-cell and cell-matrix interactions. The purpose of this study is to determine whether THBS3 expression in peripheral blood can be used as a biomarker to predict the risk of acute myocardial infarction (AMI). **Methods:** The peripheral blood of 111 patients with stable coronary artery disease (SCAD) and 112 patients with AMI was obtained. The experimental and the control cohorts were the AMI and SCAD groups, respectively. The expression of THBS3 mRNA and protein in both groups was determined using reverse-transcription polymerase chain reaction and enzyme-linked immunosorbent assay. **Results:** THBS3 expression (range) in the peripheral plasma of patients in the AMI group was lower than that of patients in the SCAD group (4.526 (3.748–5.521), 5.511 (4.726–6.334), respectively), which was 0.82 times lower than the control ($p < 0.001$). Furthermore, THBS3 mRNA level in the peripheral blood mononuclear cells of patients with AMI was 0.47 times lower than that in patients with SCAD ($p < 0.05$). AMI was associated with fasting blood glucose levels, platelet counts and low THBS3 expression. Logistic regression analysis revealed that decreased expression of THBS3 protein increased the probability of AMI by 4.076 times ($p < 0.01$). Additionally, high fasting blood glucose and high platelet counts increased the risk of AMI by 2.819 and 6.515 times, respectively ($p < 0.05$). **Conclusions:** THBS3 mRNA and protein levels in the peripheral blood of patients in the AMI group were much lower compared with those of patients in the SCAD group. Low THBS3 expression in peripheral blood was related to AMI and was an independent risk factor for AMI. Thus, low THBS3 expression in peripheral blood may be a novel, suitable molecular marker for the early detection of AMI.

Keywords: low thrombospondin 3 expression; risk factor; acute myocardial infarction; stable coronary artery disease; peripheral blood

1. Introduction

Coronary artery disease (CAD) is a widely and globally prevalent cardiovascular disease that can result in severe adverse cardiovascular events. If untreated, total occlusion of the coronary arteries can result in myocardial infarction and permanent damage to the underlying myocardium [1]. Atherosclerosis, the main cause of stable CAD (SCAD) and acute myocardial infarction (AMI), is distinguished by arterial wall inflammation and plaque buildup [2]. Acute coronary thrombosis is thought to be caused by the rupture of vulnerable atherosclerotic plaques and is the primary pathological source of AMI. Myocardial ischemia and hypoxia are caused by the rupture of atherosclerotic plaques and intramural thrombosis, which results in myocardial cell necrosis [3–5]. Thus, early detection and prompt treatment are critical in improving the prognosis of AMI.

Although cardiovascular troponin I, troponin T, myoglobin, and creatine kinase MB are currently used clinically

to diagnose AMI [6,7], these markers are not ideal for identifying the early stages of AMI in patients [8]. Therefore, new, sensitive, and specific biomarkers are required for the early detection of AMI. Protein detection has become indispensable in the diagnosis of CAD in recent years, and high-throughput proteomic studies have been used to identify the markers of early cardiovascular disease [9,10]. As the pathological basis of SCAD and AMI are similar, and SCAD is an early stage of AMI, it can be difficult to distinguish between the two [11,12]. Most studies have reported that the abnormal expression of molecular markers in peripheral blood can aid in the diagnosis of cardiovascular disease. For instance, high ADAMTS4 expression in peripheral plasma and mononuclear cells is a key factor aggravating the instability of atherosclerotic plaques [13], and an increase in interleukin-32 expression in peripheral plasma could play a role in the development of AMI from SCAD [14]. Elevated RORA and ABCB1 in leukocytes may be other suitable molecular indicators for the early diagnosis of AMI [15,16].



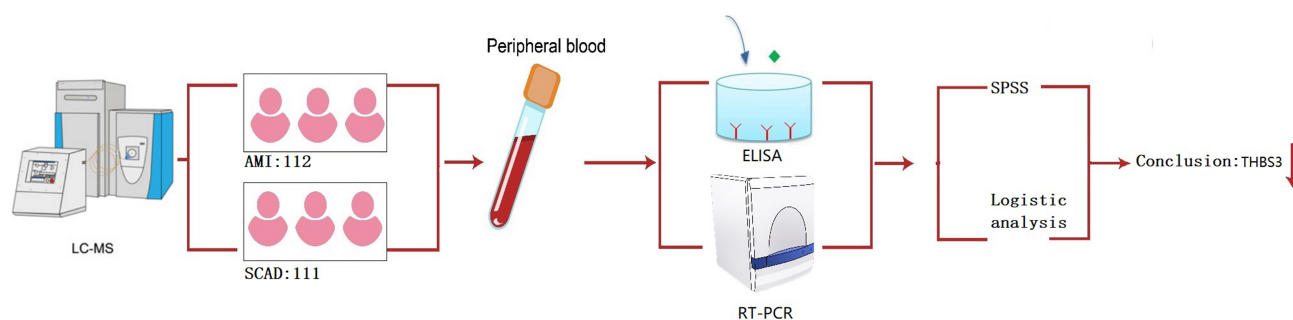


Fig. 1. Diagrammatic representation of the experimental scheme.

Thrombospondin 3 (THBS3) was originally discovered during DNA sequencing upstream of the transcription start site of the mouse *Muc1* (episialin) gene [17], a member of the THBS family that mediates cell-cell and cell-matrix interaction. Recent research shows that deleting THBS3 in mice improves the production and stability of integrin membranes, shielding the heart from disease-causing stimuli [18]. Similar to this, fatal cardiac atrophy was caused due to THBS1 (not THBS3) overexpression, which is another member of the THBS family [19]. Although THBS3 is abnormally expressed in cardiomyopathy, there are few studies on its role in CAD. Therefore, this study focuses on patients with AMI and SCAD, which may offer some unique perspectives on the diagnosis and treatment of this condition.

We screened differentially expressed proteins using proteomic studies in the early stage, and the results showed that the expression of THBS3 protein was lower in patients with AMI than in those with SCAD [20]. So, it was reasonable to speculate that AMI and low THBS3 expression in peripheral blood were related. Therefore, the aim of this study was to ascertain whether THBS3 expression was downregulated at the mRNA and protein levels in a larger cohort of patients with AMI, and to further evaluate if the relatively low THBS3 expression was related to the occurrence of AMI. Correlation analysis and logistic regression analysis further demonstrated that THBS3 might be used as a biomarker for identifying AMI.

2. Materials and Methods

2.1 Patients

This is a retrospective study. All patients in this study were enrolled from September 2017 to April 2019 and signed informed consent. The AMI team comprised 112 male patients who were acknowledged to the Department of Cardiology at China-Japan Union Hospital of Jilin University in China. These patients completed a 12-lead ECG as soon as possible at the first medical contact. The diagnosis of AMI was made in accordance with the European Society of Cardiology's globally accepted definition of myocardial infarction established in 2017 [21], with harsh stenosis and/or occlusion of the foremost coronary arteries (left and

right mains) and/or main branches (anterior descending and circumflex arteries).

In addition, 111 males with SCAD were included in the control group. An article posted in the *New England Journal of Medicine* in 2019 was used to determine the inclusion criteria [22]. The exclusion criteria were as follows: (1) patients with myocardial infarction associated with stent thrombosis and percutaneous coronary intervention/coronary artery bypass grafting infarction; (2) patients with myocardial infarction following cardiac or noncardiac surgery; (3) patients with secondary myocardial infarction; (4) patients with myocardial damage caused by severe stress cardiomyopathy; (5) patients with severe pulmonary embolism, heart failure, and other diseases. Age, history of hypertension, smoking history, diabetes history, blood lipid levels, and other relevant biochemical blood tests were meticulously recorded.

2.2 Research Measures

2.2.1 Experimental Scheme

Patients were divided into two groups: AMI and SCAD. Peripheral blood was collected from patients for ELISA and RT-qPCR to evaluate THBS3 expression at the protein and mRNA levels. Lastly, logistic regression and SPSS 25.0 software (IBM Corp, Armonk, NY, USA) were used for statistical analysis (Fig. 1).

2.2.2 Separation of Plasma from Peripheral Blood

2.2.2.1 Acquisition of Peripheral Plasma. In the morning, 6 mL of peripheral blood was drawn from subjects who were fasting, and stored in anticoagulant tubes containing EDTA at 4 °C. Plasma was extracted within 4 h of specimen collection and centrifuged at 1000×g for 20 min. The uppermost layer of plasma was stored at -80 °C until it was analyzed using enzyme-linked immunosorbent assay (ELISA).

2.2.2.2 ELISA. The instructions listed in the THBS3 ELISA kit (Shanghai Enzyme Link Biotechnology Co., Ltd., Shanghai, China) were followed. Briefly, samples and horseradish peroxidase-labeled detection antibodies (3 replicates in each group) were added to the blank, sample,

and standard wells in the microwell plate and incubated for 60 min before being discarded and washed. Next, 50 μ L of substrates A and B were added to each well and incubated at 37 °C in the dark for 15 min, followed by the addition of the stop solution. The absorbance at 450 nm was determined using a microplate reader (Epoch; BioTek Instruments, Inc.). The linear equation was established using the standard curve. THBS3 content in plasma samples was determined. The internal difference coefficient of the kit was less than 10% and the inter-plate modification coefficient was less than 15%.

2.2.3 Acquisition and Processing of Peripheral Blood Mononuclear Cells (PBMCs)

2.2.3.1 Acquisition of PBMCs. Peripheral blood was obtained as described in section 2.2.2.1, and mononuclear cells were extracted using a peripheral blood lymphocyte isolation medium (Lymphoprep™, STEMCELL Technologies Inc., Vancouver, Washington, Canada). Fresh anticoagulant was mixed with an equal volume of 0.9% salt solution infusion, and the mixture was added to an equal volume of lymphocyte separation solution. The red blood cell layer, clear fluid layer (separated), opalescent mononuclear cell layer, and plasma layer were separated from the sample after centrifuging at 1000 \times g for 20 min. The mononuclear cell layer was collected and used for subsequent studies.

2.2.3.2 cDNA Synthesis from PBMCs. A total RNA extraction kit (RNAsimple Total RNA Kit, Tiangen Biochemical Technology Co., Ltd., Beijing, China) was used to extract total RNA. The manufacturer's instructions in the kit were meticulously followed to avoid RNA destruction or pollution during the process. The concentration and absorbance of items that fit the criteria were determined using a microplate reader. The A260/A230 value should be >2, and the 260/280 value should be 1.7–2.1. Instructions on the reverse-transcription (RT) kit were followed for reverse transcribing RNA (FastKing One Step genomic cDNA first-strand synthesis premix, Tiangen Biochemical Technology Co., Ltd., Beijing, China). Each sample had the same RNA concentration. Next, the cDNA samples were frozen at –80 °C and subjected to a quantitative polymerase chain reaction (PCR). Perform reverse transcription after meeting the requirements of the reverse transcription kit.

2.2.3.3 Reverse-Transcription Polymerase Chain Reaction (RT-PCR). RT-PCR was used for the amplification of the 5-fold diluted cDNA samples with the SYBR fluorescence quantitation kit (Sangon Fluorescence Quantitation Kit, Taq qPCR Synthesis Premix, Shanghai, China). The dissolution and amplification curves from 60–95 °C were recorded through multiplication, which comprised pre-denaturation, denaturation, and annealing steps. The melting curve obtained using ABI-FAST7500 software (Applied biosystems, Thermo Fisher Scientific, MA, USA) dictated the

Table 1. RT-PCR primer sequences.

Genes		Genes Primer sequence (5'-3')
<i>THBS3</i>	F ^a	CTCGGCAGATGGTAGCTGTG
	R ^b	TGGATAAGAGGTAGATGTCCCA
<i>GAPDH</i>	F ^a	GGAGCGAGATCCCTCCAAAAT
	R ^b	GGCTGTTGTCATACTTCTCATGG

F^a: Forward primers.

R^b: Reverse primers.

RT-PCR, Reverse-Transcription Polymerase Chain Reaction.

specificity of the amplification conditions. THBS3 and GAPDH were the target gene and internal reference gene, respectively, and the readings for each group were performed in triplicate. Each sample had a 2^{– Δ ct} RNA expression level (Δ ct = ct number of target gene–ct number of internal reference gene). The primer sequences for RT-PCR are listed in Table 1.

2.3 Statistical Analysis

Data were analyzed using SPSS 25.0 software. The retrieved data were checked for normality and, if they passed, were expressed as mean standard deviation ($p > 0.1$). Independent samples *t*-tests were used to assess differences between groups. Interquartile ranges and medians were used to depict non-normally distributed data ($p \leq 0.1$). The non-parametric rank-sum test of two independent samples was used to differentiate between groups. Count data were characterized by between-group differences and frequencies using the χ^2 test. Binary logistic regression was used to explore the risk variables for AMI. Statistical significance was defined as a two-sided $p < 0.05$.

3. Results

3.1 Acquisition of Baseline Data

Table 2 shows the results of the clinical data analysis. There were significant changes in age, smoking history, fasting blood glucose, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and platelet (PLT) levels ($p < 0.05$) between patients in the AMI and SCAD groups.

3.2 Analysis of THBS3 Protein in Plasma

It can be seen in Fig. 2 that THBS3 protein expression in the AMI group was 4.526 (3.748–5.521), whereas that in the SCAD group was 5.511 (4.726–6.334). The expression of THBS3 protein in the AMI group was lower than that in the control group, with a relative expression that was 0.82 times that of the control group ($p < 0.001$) and statistically significant.

3.3 mRNA level of THBS3 in PBMCs

Analysis of peripheral blood RNA and the amplification curve of the *THBS3* gene showed a smooth “S-shape”. The single peak of the dissociation curve (Fig. 3) is thought

Table 2. Baseline data of patients in the AMI and SCAD groups.

Clinical indicators	AMI group	SCAD group	$t/z/x^2$	p
	N = 112	N = 111		
Hypertension (%)	52 (46.43)	37 (33.33)	1.297	0.255
Type 2 diabetes (%)	31 (27.68)	21 (18.92)	1.365	0.243
TG (mmol/L)	1.610 (1.110–2.235)	1.510 (1.078–1.998)	−1.307	0.191
HDL-C (mmol/L)	1.120 (0.890–1.225)	1.040 (0.918–1.185)	−0.132	0.895
Fasting blood glucose (mmol/L)	7.360 (6.095–9.055)	5.950 (5.408–6.848)	−4.824	<0.001
TC (mmol/L)	4.730 (4.005–5.440)	3.650 (3.098–4.055)	−7.063	<0.001
LDL-C (mmol/L)	3.212 ± 0.848	2.000 (1.728–2.350)	−8.221	<0.001
PLT ($10^9/L$)	237.000 (202.500–284.500)	223.530 ± 51.598	−2.288	0.022
Age	59.376 ± 10.498	50.000 (48.000–53.000)	−6.945	<0.001
Smoking history (%)	65 (58.04)	52 (46.85)	7.096	0.008

The normal distribution is expressed as the mean ± standard deviation. Non-normally distributed data are presented as interquartile ranges and medians. $p < 0.05$ was considered statistically significant. TG, triglycerides; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PLT, platelet counts.

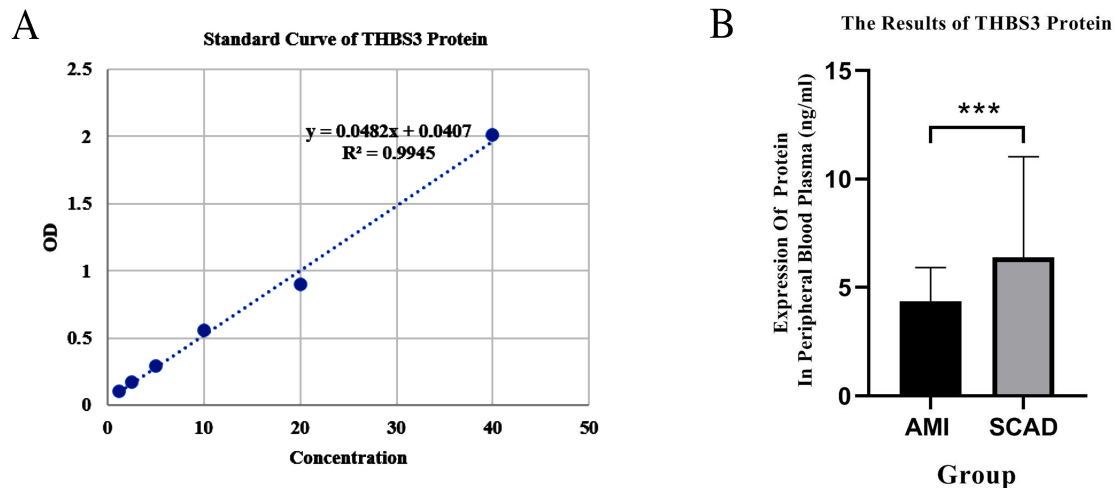


Fig. 2. ELISA standard curve and THBS3 protein levels. (A) Standard curve. (B) THBS3 protein expression. ELISA, Enzyme-linked immunosorbent assay; *** $p < 0.001$.

to be specific for the amplified product. The Δct values are the average of three RT-PCR tests per sample. The $2^{-\Delta\text{ct}}$ value of the AMI group was 0.014 (0.009–0.039) and that of the SCAD group was 0.030 (0.019–0.098) and the difference was significantly different between the two ($p < 0.05$). The mRNA expression of *THBS3* in PBMCs in the AMI group was significantly lower and the relative expression was 0.47 times that of the control group (Fig. 4).

3.4 Correlation Analysis of *THBS3* Protein Expression and Patient Characteristics

THBS3 expression, age, smoking history, fasting blood glucose, TC, LDL-C, and PLT levels were significantly different between the AMI and SCAD groups (Table 3). Thus, we investigated whether *THBS3* protein expression was related to the aforementioned factors. Patients were divided into two groups: older (≥ 65 years) and younger (< 65 years); smoking and non-smoking groups;

high fasting blood glucose (≥ 7 mmol/L) and normal fasting blood glucose groups (< 7 mmol/L); high TC (≥ 6.2 mmol/L) and normal TC groups (< 6.2 mmol/L), high LDL-C (≥ 4.1 mmol/L) and normal LDL-C groups (< 4.1 mmol/L); and high PLT group ($\geq 300 \times 10^9/L$) and normal PLT group ($< 300 \times 10^9/L$) [23–25]. *THBS3* protein expression was significantly different ($p < 0.05$) with respect to age and fasting blood glucose, but not with other factors.

3.5 Logistic Regression Analysis to Determine the Relationship between High Fasting Blood Glucose, High LDL-C, High TC, and High PLT Levels; Smoking History, and AMI

Based on the correlation between *THBS3* expression and AMI, the maximum value of sensitivity and specificity was taken as the cut-off value, which was 4.612. We divided all subjects into two groups: high expression (concentration > 4.612) and low expression (concentration

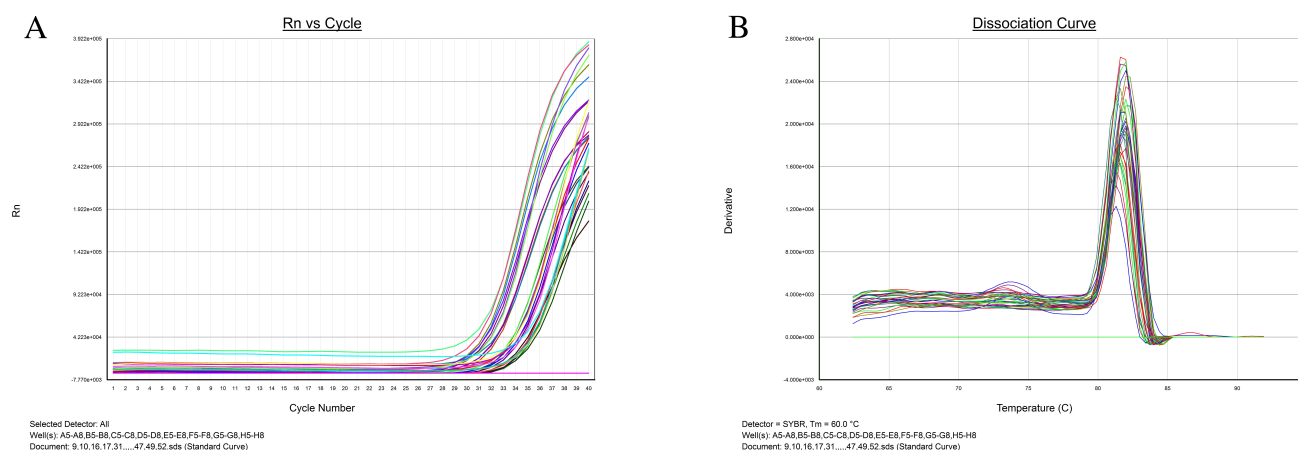


Fig. 3. Amplification using RT-PCR and dissociation curve of the *THBS3* gene. (A) Amplification curve. (B) Lysis curve.

Table 3. Correlation analysis.

Groups	THBS3 expression	Z	p value
High TC group	5.316 ± 2.998	-0.414	0.679
Normal TC group	4.954 (4.259–5.819)		
High LDL-C group	4.736 ± 2.735	-1.047	0.295
Normal LDL-C group	4.979 (4.342–5.898)		
High PLT group	5.006 (4.207–5.802)	-0.274	0.784
Normal PLT group	4.840 (4.093–5.779)		
Smoking group	4.851 (4.311–5.879)	-1.090	0.276
Non-smoking group	4.703 ± 1.522		
High age	4.481 (3.896–5.354)	-2.600	0.009
Young age	4.954 (4.259–6.012)		
High fasting blood glucose group	4.662 (4.039–5.343)	-2.938	0.003
Normal fasting blood glucose group	4.982 (4.200–6.173)		

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; PLT, platelet.

≤ 4.612). Logistic regression analysis revealed that low expression of *THBS3*, fasting blood glucose and PLT levels were related to the occurrence of AMI (Table 4). Reduced *THBS3* protein expression was found to be an independent risk factor for AMI, which increased the risk of AMI by 4.076 times ($p < 0.01$). Furthermore, high fasting blood glucose and high platelet counts increased the risk of AMI by 2.819 and 6.515 times, respectively ($p < 0.05$). TC and LDL-C levels and smoking history, on the other hand, were not independent predictors for AMI ($p > 0.05$) (Table 4).

4. Discussion

THBS3 protein expression in peripheral blood was examined between the AMI and SCAD groups. *THBS3* protein expression in AMI was significantly lower than that in SCAD, and the relative expression level was 0.82 times that in the SCAD group.

In our previous proteomic research [20], we found that patients with AMI had lower *THBS3* expression in their peripheral plasma than patients with SCAD ($p < 0.05$). Based on this finding, we further verified that *THBS3* expression in the peripheral plasma of patients with AMI was lower

than that in patients in the SCAD group ($p < 0.001$). Recent studies show that the *THBS3* gene in PBMCs is abnormally expressed in patients with poor coronary flow [26]. However, *THBS3* gene expression in the AMI and SCAD groups has not been studied. Thus, we were eager to discover if *THBS3* gene was aberrantly expressed in these groups. We obtained PBMCs from the study participants and using RT-PCR, we found that *THBS3* gene expression in patients in the AMI group was lower than that in the controls ($p < 0.05$). In conclusion, *THBS3* expression in the peripheral blood of patients with AMI was lower than that in patients with SCAD at the mRNA and protein levels.

Five members make up the glycoprotein family *THBS*, which codes for the extracellular matrix (ECM). *THBS1* and *THBS2* form trimers, whereas *THBS3*, *THBS4*, and *THBS5* form pentamers. Their shared domain is intricate [27,28]. As the five members are the byproducts of distinct genes on distinct chromosomes, their regulated DNA and mRNA regions are also distinct. Thus, they can perform several functional roles in cell adhesion, proliferation, migration, PLT aggregation, angiogenesis, and wound healing [29–32]. Single nucleotide polymorphisms

Table 4. Logistic regression analysis.

	B	Standard variation	Wald	Degree of freedom	<i>p</i> value	OR	95% CI	
Low expression of THBS3	1.405	0.456	9.502	1	0.002	4.076	1.668	9.958
High fasting blood glucose	1.036	0.468	4.902	1	0.027	2.819	1.126	7.054
High PLT	1.874	0.722	6.730	1	0.009	6.515	1.581	26.842
High TC	1.316	1.730	0.579	1	0.447	3.728	0.126	110.662
High LDL-C	−1.955	1.357	2.074	1	0.150	0.142	0.010	2.025
Smoking History	−0.791	0.486	2.653	1	0.103	0.453	0.175	1.174

TC, total cholesterol; PLT, platelet counts; OR, odds ratio; CI, confidence interval.

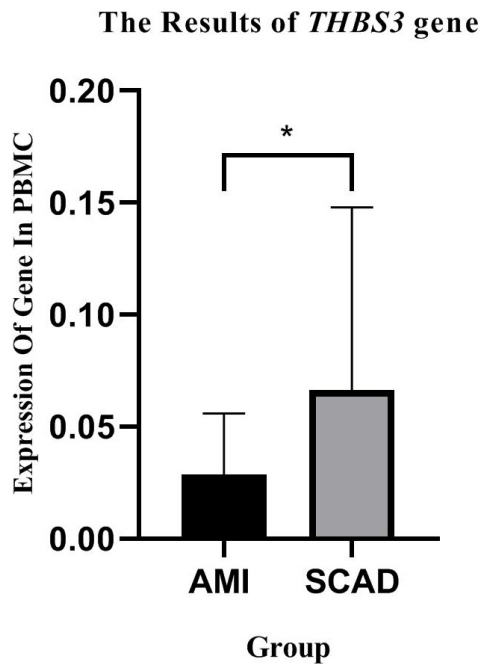


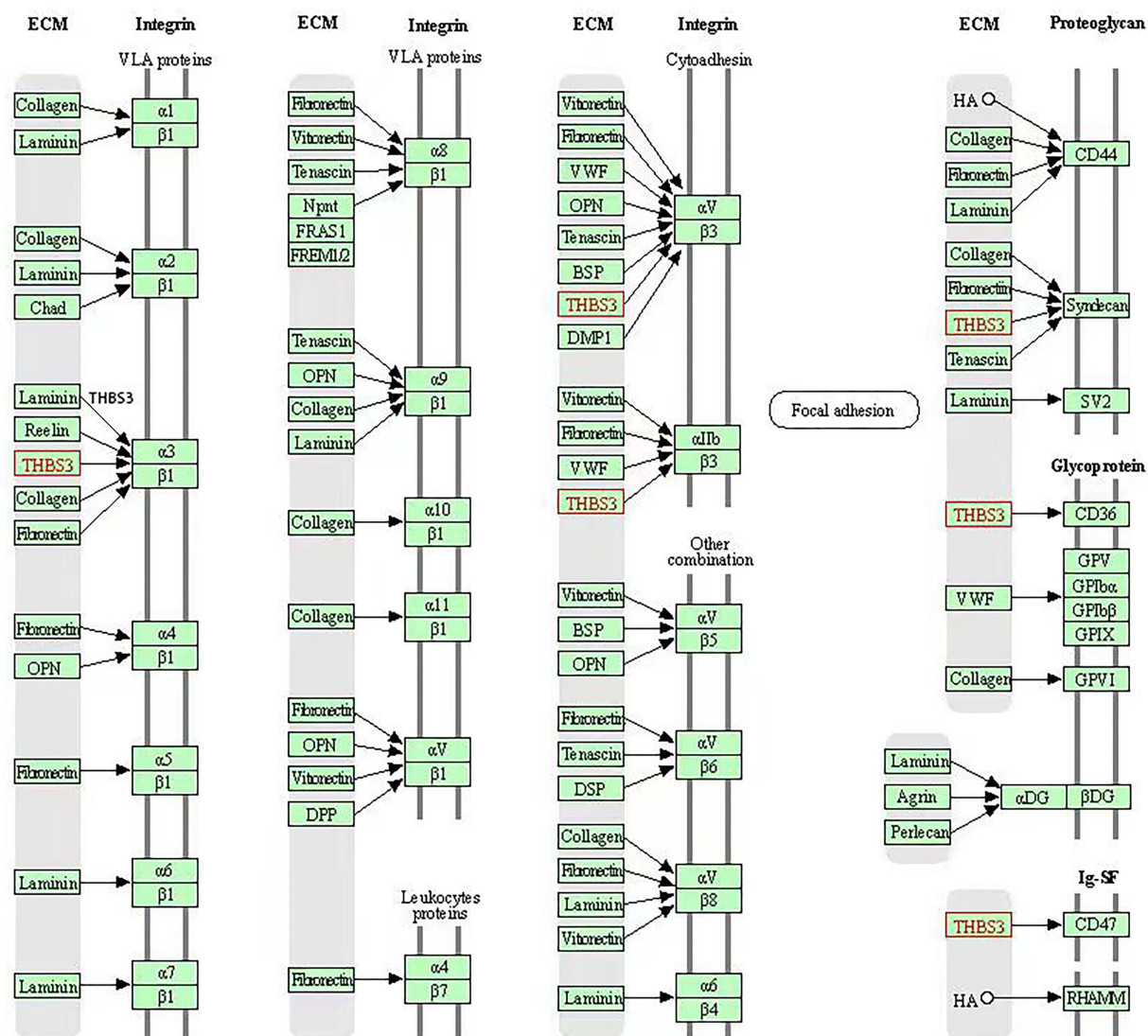
Fig. 4. Relative expression of the *THBS3* gene at the mRNA level. AMI, Acute myocardial infarction; SCAD, Stable coronary artery disease; PBMC, Peripheral blood mononuclear cell. * $p < 0.05$.

in THBS1, THBS 2, and THBS 4 have been reported to be associated with an increased risk of early myocardial infarction [33]. As a member of the same family, Vanhoutte [19] proposed that THBS1 overexpression can atrophy the heart, ultimately leading to fatal cardiomyopathy. This appears to be a little different from what we concluded. First off, while both cardiomyopathy and coronary heart disease were conditions affecting the circulatory system, their pathogenic mechanisms were different. The former can be caused by genetic variation of genes and has been the focus of clinical research so far. The latter's pathophysiology was based on atherosclerosis, which caused blood vessel stenosis or obstruction as a result of thrombus formation in blood vessels. Second, the relationship between myocardial atrophy and myocardial infarction was still unclear. The control group utilized by Vanhoutte was not used in this investigation. Instead of a healthy control group, our study included the SCAD group as the control group. Finally, basic studies

of Vanhoutte have confirmed *in vivo* and *in vitro* that overexpression of THBS1 led to myocardial atrophy and that THBS1^{−/−} led to cardiac hypertrophy. The purpose of our study was to show the correlation between low THBS3 expression and AMI. The working basis of the two studies was slightly different, and the same or different molecules from the same family may be differentially expressed in different diseases. Likewise, the mechanism leading to the lower expression of THBS3 in AMI than in SCAD deserved further investigation. Nonetheless, the present study on THBS3 is insufficient.

Atherosclerosis is the pathological basis of coronary heart disease. Its progression and the formation of coronary plaques contribute to the occurrence and development of various outcomes in CAD. Previous research has shown that the rupture of vulnerable plaques can result in the formation of an acute thrombus, leading to acute cardiovascular events [34,35]. Studies have also shown that the THBS family can mediate the function of the ECM [36,37], which is composed of glycoproteins such as collagen and laminin [38]. Numerous cell receptors, including integrin and cadherin, interact directly with the components of the ECM [39]. The common aspects of regulation include cell proliferation, adhesion, and migration [40–42]. For instance, THBS1 regulates adhesion and promotes movement in smooth muscles and endothelial cells by antagonizing the assembly of adhesion plaques in response to ECM components such as fibronectin [28]. The ability of laminin to stimulate embryonic retinal cell adhesion and neurite development is improved by THBS4 [43]. THBS3 also plays a role in cardiomyopathy by participating in the regulation of integrin expression and function and increasing disease-induced decompensation to promote myomembrane instability [18]. Ridley *et al.* [44] reported that mediating the ECM can regulate cell migration and advance the onset of atherosclerosis, in which metalloproteinases (MMPs) play a crucial role [45,46]. ECM metalloproteinase inducer (EMMPRIN) can promote plaque instability by inducing ECM degradation and MMP synthesis, leading to AMI [47]. Moreover, EMMPRIN can stimulate MMP-9 in monocytes and MMP-2 in smooth muscle cells, which are essential for regulating MMP activity in cardiovascular diseases [48]. Lindsey *et al.* [49] also found that THBS3 expression increased when the collagen Iα1 frag-

ECM-RECEPTOR INTERACTION



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Fig. 5. THBS3 KEGG pathway diagram.

ment formed by MMP-2 and MMP-9 cleavage was used to treat patients after an MI episode. By querying the KEGG (<https://www.kegg.jp/>) database, we found that THBS3 is closely related to the ECM receptor interaction pathway (Fig. 5). We may, therefore, safely deduce from our data inquiry that THBS3 may affect differential expression in the peripheral blood of patients with AMI and SCAD via the ECM receptor interaction pathway and participate in the regulation of AMI.

Baseline analysis revealed a significant difference in fasting blood glucose levels between patients in the AMI and SCAD groups, where patients in the AMI group had higher levels than those in the SCAD group. Subsequently, the correlation between low THBS3 protein expression and high fasting glucose levels was also found. Elevated fasting

blood glucose levels were also a risk factor for AMI (odds ratio [OR] = 2.819, $p < 0.05$). However, there were no significant differences with respect to the history of diabetes between patients in the two groups, which may have occurred due to the stress-induced increase in blood glucose after AMI.

THBS3 is a glycoprotein secreted by PLTs. Although baseline analysis showed that the PLT counts in the peripheral blood was significantly different between the two groups ($p < 0.05$), there was no correlation between PLT counts and the low expression of THBS3 protein. Moreover, PLT was an independent risk factor for AMI (OR = 6.515, $p < 0.01$). Although PLT aggregation occurs in AMI, the *in vivo* coagulation system is complicated and flexible; thus, a change in PLT levels in the peripheral blood

might not be the deciding factor leading to AMI. Logistic analysis demonstrated that low THBS3 protein expression was a distinct risk factor for AMI (OR = 4.076, $p < 0.01$). Additionally, patients with low THBS3 expression, high PLT counts and high fasting blood glucose levels were found to be more prone to developing AMI.

Our study has some limitations. As a retrospective study, if a healthy control group is added, it is more meaningful to compare the differential expression of THBS3 among the three groups. At the same time, if the correlation analysis of myocardial markers (troponin I, troponin T, myoglobin, etc.) and THBS3 expression can be carried out, this study will be more comprehensive and valuable. The ECM as well as processes such as cell aggregation, adhesion, and glucose metabolism can contribute to atherosclerosis. Currently, we can only hypothesize how THBS3 might cause AMI and, accordingly, provide a new and reliable biomarker for the diagnosis of AMI. In the future, we intend to further explore disease pathogenesis based on *in vitro* and *in vivo* studies and provide new directions and ideas that may offer novel perspectives and suggestions related to THBS3 for the treatment of cardiovascular diseases.

5. Conclusions

mRNA and protein levels of THBS3 in the peripheral blood of patients with AMI were significantly lower than those of patients in the SCAD group. Low THBS3 expression in peripheral blood was associated with AMI and was an independent risk factor for AMI. Thus, low THBS3 expression in peripheral blood may be a new molecular marker for the early diagnosis of AMI.

Author Contributions

YQC, HYM and FBM designed the research study; YQC and FBM performed the research; HYM, XM and ZHY conducted the work and involved in data collection; XM and JRW analyzed the data; YQC and FBM wrote the manuscript; All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Informed consent forms were signed by all patients in this investigation and the guidelines of the Declaration of Helsinki were followed. The protocol was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University (approval number: 2016WJW017).

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Not applicable.

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Conflict of Interest

The authors declare no conflict of interest.

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