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Proteomic evaluation of the thrombosis-inflammation interplay in STEMI with MVO

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Abstract

Background Coronary microvascular obstruction (MVO) occurs in up to half of acute myocardial infarction patients receiving successful primary percutaneous coronary intervention (pPCI) and is associated with a much worse outcome. Whereas the fluid phase cross-talk between thrombosis and inflammation is well appreciated, the pathophysiological implication is still scant.

Objectives This study sought to investigate the differentially expressed proteins and possible biological processes involved in MVO after pPCI in ST-segment elevation myocardial infarction (STEMI) patients based on thrombus proteomics.

Methods Aspirated thrombi and pPCI from 16 STEMI patients within 12 h of symptom onset were collected, including 8 MI with MVO (MVO+) and 8 MI without MVO (MVO-). 4D label-free proteomics was used to explore the differentially expressed proteins. Gene ontology enrichment analysis was performed using Metascape software and protein–protein interaction analysis was performed using Cystoscope software. Afterward, the Connectivity Map database was used to select drug candidates for MVO treatment.

Results We identified a total of 471 proteins with expression changes greater than 1.5-fold at $P < 0.05$, of which 50 were significantly upregulated and 421 were downregulated in the MVO + group compared with the MVO- group. Gene ontology enrichment analysis of significant differentially expressed proteins revealed the central role of platelet activation and neutrophil degranulation processes in patients with MVO. The protein-protein interaction network also confirmed the significant interaction of inflammation and platelet activation, which may mediate the role of thrombus-inflammation in the pathogenesis of MVO. Drug screening revealed 4 drug candidates for MVO treatment: D-64,131, TC-1, SB-431,542 and alvespimycin.

Conclusions Using the thrombus proteomic approach, we revealed the central role of the thrombus-inflammation interaction and potential drug candidates in STEMI with MVO. The findings from our study will contribute to the treatment of MVO in the future.

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Keywords STEMI, CMR, Microvascular obstruction, Thrombus–inflammation, Proteomics

Introduction

Acute ST-segment elevated myocardial infarction (STEMI) accounts for the leading cause and mortality of cardiovascular disease (CVD) worldwide, making it a major health-care challenge [1]. Acute luminal thrombosis in the coronary artery is one of the main causes of STEMI [2]. Emergency primary percutaneous coronary intervention (pPCI) is the preferred reperfusion strategy to restore the patency of the infarct-related artery, thereby limiting the irreversible damage of ischemia, reducing the reperfusion injury, and rescuing the survived myocardium [3–5]. Revascularization of epicardial vessels does not necessarily equate to myocardial reperfusion at the microvascular level. Up to half of patients may still experience coronary microcirculation obstruction (MVO), despite successful recanalization of the obstructed epicardial vessels [6, 7]. MVO, which can manifest as coronary no-reflow in the infarct-related artery, is a critical factor influencing the final infarct size and contributes significantly to poor prognosis in patients with STEMI [8]. Therefore, it is very important to explore the early pathogenesis mechanism of MVO and find new effective treatment approaches.

Studies have shown that the inflammatory storm caused by ischemic/reperfusion (I/R) injury is one of the important mechanisms leading to MVO formation [9]. The characteristics of the thrombus-inflammation paradigm deem STEMI as a result of a systemic proinflammatory state, which leads to endothelial dysfunction, coronary microvascular dysfunction, abnormal cardiac structure and function, and death [10]. However, the evidence supporting the link between thrombus characteristics and inflammation is primarily derived from animal studies and focuses on a limited number of proteins [11]. Because proteins are the functional executors of the cell, an in-depth characterization of the proteome and signal transduction of thrombi will lay a foundation for comprehensively understanding the molecular mechanisms of STEMI with MVO. High-throughput proteomic technologies enable the investigation of a large set of proteins in a single sample [10]. The resultant biomarker profiles are potentially useful for characterizing underlying pathophysiological pathways and lead to earlier and more accurate detection of cardiovascular syndromes.

In this study, we performed 4D label-free proteomic profiling of thrombi from 16 STEMI patients, both with and without MVO, to gain deeper insights into the molecular mechanisms underlying microvascular injury. Our research aimed to (1) provide a comprehensive profile of differentially expressed proteins among STEMI patients with and without MVO and (2) explore potential drugs to treat MVO.

Materials and methods

Ethics statement

This study was approved by the Nanjing Drum Ethics Committee (Ethics Number: 2021-531-01), and complied with the Declaration of Helsinki. All participants gave written informed consent.

Study design

A schematic flowchart the study design was illustrated in Fig. 1. We collected coronary thrombus tissue samples from STEMI patients receiving thrombus aspiration from January 2020 to October 2021 at the Department of Cardiology of Nanjing Drum Tower Hospital. The STEMI patients met the diagnostic criteria as outlined by the 2017 European Society of Cardiology (ESC) guidelines [12]. The inclusion criteria were as follows: (1) age ranging from 18 to 80 years; (2) admission within 12 h of chest pain onset; (3) diagnosis of STEMI with Thrombolysis in Myocardial Infarction (TIMI) 0–1 grade flow; (4) presence of a high thrombus burden (Grade 4 or 5) [13] on emergency angiography; (5) undergoing thrombus aspiration as part of primary PCI. Exclusion criteria were as follows: (1) current infections; (2) previous history of myocardial infarction or stroke, malignant tumors, severe liver and kidney dysfunction and rheumatic immune-related conditions; (3) non-completion of the cardiac magnetic resonance imaging (CMR) within 48 h post-pPCI; and (4) presence of secondary thrombus formation originating from sources such as the left ventricle.

MVO was diagnosed using CMR based on the following criteria: (1) absence of gadolinium uptake during first-pass perfusion; (2) absence of early gadolinium enhancement; and (3) absence of late gadolinium enhancement observed 10–15 min after contrast administration [14]. The groups were defined as STEMI with or without microvascular obstruction (MVO + and MVO-).

Thrombus aspiration procedure

All patients received a loading dose of aspirin (300 mg) and clopidogrel (300 mg) orally before the procedure, as per standard STEMI management guidelines. Unfractionated heparin was administered intravenously before PCI to achieve adequate anticoagulation. A guiding catheter was used to engage the infarct-related artery (IRA). A 6 F Export AP aspiration catheter (Medtronic, USA) was introduced over a guidewire and advanced to the site of the thrombus. Negative pressure was applied using a 20 mL syringe, allowing for thrombus aspiration.

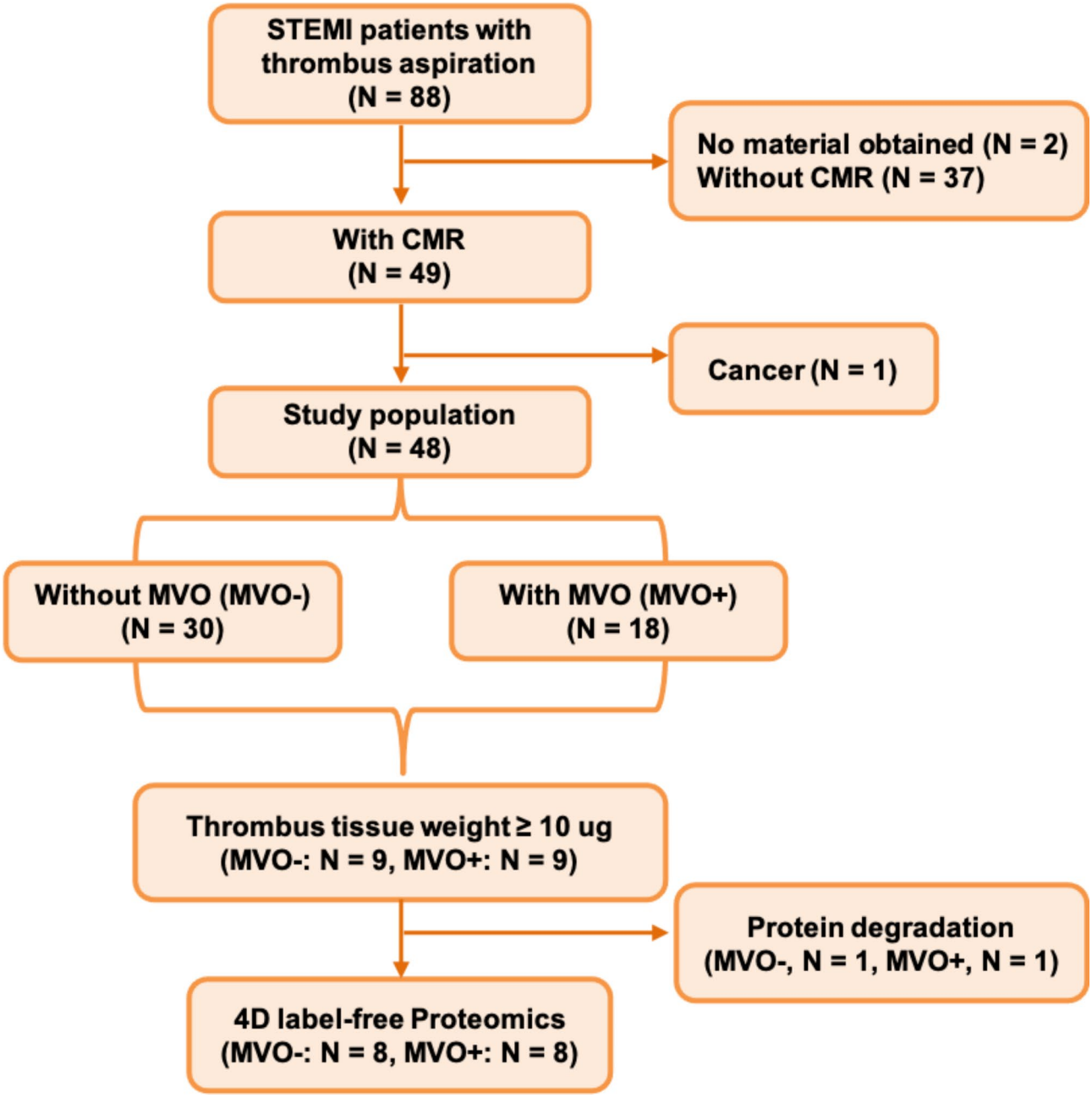

Fig. 1 A schematic flowchart of the experimental design

The aspiration process was repeated if residual thrombus was observed. The catheter was then withdrawn while maintaining continuous aspiration to prevent distal embolization. The coronary flow was reassessed based on TIMI flow grade and Thrombus Grade Reduction criteria. Drug-eluting stents (DES) were implanted when indicated.

Sample collection

Aspirated blood and intracoronary thrombus material were collected in a collection bottle with a filter. The aspirated thrombus material was divided into three portions: one was embedded in (Optimal Cutting Temperature compound) OCT (Sakura, USA) for pathology, one was stored in glutaraldehyde for electron microscopy imaging, and the other was frozen at -80 °C for proteomic analysis. Approximately 3 ml of coronary blood was collected in a tube containing Ethylenediaminetetraacetic Acid (EDTA) and centrifuged at 3000 rpm for 20 min at 4℃. The supernatant containing plasma was collected and stored at -80℃ until analysis.

Protein extraction

Thrombus samples ($n = 8$ each group) were prepared for 4D label-free proteomics analysis. A total of 10–50 mg of each sample was ground in liquid nitrogen into a cell powder and then disrupted in 4-fold volumes of lysis buffer (8 M urea, 1% protease inhibitor cocktail), sonicated three times on ice (high intensity ultrasonic processor, Scientz), and centrifuged at 12,000 × g for 10 min at 4 °C. The supernatant was collected and measured with the BCA method. A total of 20 μg of protein extract from the thrombus was loaded in 10% SDS−PAG.

4D label-free proteomics

Tandem mass spectrometry (MS/MS) analysis was performed using an EASY (Enhanced Automated Sample Preparation System)-nLC 1000 ultraperformance liquid chromatography (UPLC) system with buffer A (0.1% formic acid and 2% acetonitrile) and B (0.1% formic acid and 100% acetonitrile) coupled to a Q Executive Plus Orbitrap mass spectrometer at a resolution of 17,500 via an EASY-Spray nanoelectrospray ionization source (Thermo Fisher, USA). Peptide (0.5 μg) was injected into the column, and the gradient was used to perform separation at a flow rate of 450 nL/min: 6–24% B (0–70 min), 24–35% B (70–84 min), 35–80% B (84–87 min), and 80% B (87–90 min). MS/MS data analysis was conducted via the MaxQuant search engine (1.6.15.0). Tandem mass spectra were searched against the human UniProt database concatenated with a reverse decoy database. The peptide false-discovery rate (FDR) was ≤ 1%.

Bioinformatics analysis of quantifiable proteins

Data generated were compared by using Student's test for two group comparisons. Proteins with a fold change ≥ 1.5 and a P value < 0.05 were considered significantly differentially expressed proteins. For functional enrichment of the identified proteins, Metascape was used to map proteins into the Gene Ontology (GO terms) and Kyoto Encyclopedia of Genes and Genomes (KEGG) databases. Protein-protein interaction (PPI) network analysis was performed using the STRING database and visualized using Cytoscape.

Screening of potential drug molecules

Connectivity Map is a database that explores the relationships between bioactive small molecules and gene expression profiles. This study utilized the Connectivity Map website (https://broadinstitute.org/CMap) to predi ct small molecule compounds that target MVO. Subsequently, 50 upregulated proteins were entered into the website for drug screening. The prediction score represented the correlation between the drug and DEGs (Differentially Expressed Genes), and a negative correlation indicated that the drug was suitable for the treatment of MVO. In this study, − 60 was selected as the threshold of the prediction score to screen drug candidates for MVO treatment.

Scanning electron microscopy (SEM)

Thrombi were fixed in 2.5% glutaraldehyde for 1 h, rinsed in saline and fixed in 1% osmium tetroxide for 1 h. Then samples were dehydrated in a graded series of ethyl alcohol solutions. Dehydrated specimens were critical-point dried, mounted on studs and sputter coated before SEM imaging.

Histology

Thrombi were embedded in OCT and serially cut at a thickness of 5 μm. Samples were stained with hematoxylin and eosin (H&E) and imaged (10X and 40X magnification). Immunohistochemical analysis was performed with antibodies against CD11b (granulocytes) and CD61 (platelets). After blocking nonspecific reactions with 10% donkey serum and 2% Triton X-100, the sections were incubated with primary antibodies at 4 °C overnight. The next day, secondary antibody conjugated with HRP/DAB (Horseradish Peroxidase / 3,3'-Diaminobenzidine) was used for 1 h.

Statistical analysis

For the statistical analysis of the clinical characteristics of STEMI patients with versus without MVO, a Mann-Whitney U test was used for continuous variables, and a chi-squared test was used for categorical variables. Data were statistically analyzed using GraphPad Prism 8.4.0 software, and a p value less than 0.05 was indicative of statistical significance.

Results

STEMI patients with MVO demonstrated worse cardiac function

A schematic of the experimental design is shown in Fig. 1. The present study collected a total of 16 thrombi samples from STEMI patients with thrombosis aspiration. Patient characteristics in this study are described in Table 1. There were no statistically significant differences between the STEMI groups (with and without MVO) in terms of age, sex, and BMI (all $P > 0.05$). Compared with the MVO - group, the MVO + group showed poorer cardiac function (LVEF, $P = 0.0179$) and higher inflammatory markers (CRP, $P = 0.0437$). This suggested that the poor cardiac function in MVO patients was partially related to the inflammatory response.

Thrombotic tissue from MVO patients shows increased neutrophil infiltration and decreased fibrin content

We observed thrombi aspirated from STEMI patients without or with MVO under the macroscopic view

Table 1 Baseline clinical characteristics between STEMI patients with or without MVO

Characteristic	MVO– (*n* = 30)	MVO + (*n* = 18)	*P* value
Demographics			
Age, yrs	56.5 (49.0, 67.5)	61.0 (53.0, 70.5)	0.4582
Men, %	28 (93.33)	16 (88.89)	0.6244
BMI, kg/m^2	24.73 (22.17, 26.20)	24.18 (22.85, 26.96)	0.8146
SBP, mmHg	116 (102, 134)	115 (103,130)	0.8063
DBP, mmHg	77 (62, 85)	73 (65, 80)	0.8123
Prior conditions			
Angina pectoris, %	11 (36.67)	5 (27.78)	0.5271
Hypertension, %	16 (53.33)	12(66.67)	0.3643
Diabetes mellitus, %	4 (13.33)	4 (22.22)	0.6892
Stroke, %	3 (10.00)	3 (16.67)	0.8217
Smoking history, %	14 (46.67)	7 (38.89)	0.5990
Medications			
Aspirin, %	30	18	-
Clopidogrel, %	30	18	-
Unfractionated heparin, %	30	18	-
Laboratory values			
Fasting glucose, mmol/L	7.18 (4.54, 9.33)	6.76 (5.43, 9.56)	0.5182
Triglycerides, mmol/L	2.32 (1.13, 4.03)	1.87 (1.02, 3.97)	0.5730
Total cholesterol, mmol/L	4.16 (3.06, 5.83)	3.95 (3.14, 6.03)	0.5834
HDL-C, mmol/L	0.90 (0.75, 1.12)	0.98 (0.79, 1.27)	0.2871
LDL-C, mmol/L	2.31 (1.59, 3.12)	2.12 (1.58, 2.91)	0.6823
TnT, μg/L	4.98 (2.81, 6.84)	5.47 (3.60, 7.47)	0.7269
Leukocyte count, 10*9/L	10.12 (6.71, 12.09)	11.22 (8.59, 14.67)	0.3192
Neutrophil count, 10*9/L	7.72 (5.69, 9.79)	9.26 (7.05, 12.5)	0.7743
Monocyte count, 10*9/L	0.66 (0.43, 0.82)	0.68 (0.49, 0.93)	0.8143
Erythrocyte count, 10*12/L	4.57 (4.14, 5.21)	4.25 (3.65, 4.78)	0.1312
Neutrophile/RBC	1.93 (1.39, 2.16)	2.37 (1.72, 3.05)	**0.0341**
Platelet count, 10*9/L	201.8 (143.8, 259.3)	198.9 (145.2, 272.7)	0.4718
Hemoglobin, g/L	148.5 (124.0, 155.5)	138.0 (111.5, 146.5)	0.0845
CRP, mg/L	14.52 (2.27, 23.93)	31.06 (8.58, 56.49)	**0.0437**
Infarct-related arteries			0.1793
LAD, %	12 (40.00)	12 (66.67)	
LCX, %	5 (16.67)	1 (5.56)	
RCA, %	13 (43.33)	5 (27.78)	
LVEF, %	49.26 (42.71, 57.39)	42.41 (30.18, 51.75)	**0.0179**

BMI: Body Mass Index; SBP: systolic blood pressure; DBP: diastolic blood pressure; HDL-C: High-Density Lipoprotein Cholesterol; LDL-C: Low-Density Lipoprotein Cholesterol; TNT: Cardiac Troponin T; RBC: Red Blood Cell; CRP: C-Reactive Protein; LAD: Left Anterior Descending Artery; LCX: Left Circumflex Artery; RCA: Right Coronary Artery; LVEF: Left Ventricular Ejection Fraction

(Fig. 2A-B). SEM revealed that patients with MVO exhibited a weaker fibrin network structure and increased infiltration of granulocytes in thrombus tissue compared to patients without MVO (Fig. 2C). H&E staining results showed that thrombus tissues in MVO + patients had increased granulocyte infiltration and less fibrin compared to those in MVO- patients (Fig. 2D). MOVAT staining showed that compared to patients without MVO, patients with MVO had fewer fibrinogen components in thrombus tissue. Immunohistochemistry showed that compared to patients without MVO, patients with MVO had more severe granulocyte infiltration and increased platelet recruitment in the thrombus tissue (Fig. 2F-G). Hence, these findings indicated that the instability of thrombosis and the inflammatory response are associated with poor microvascular perfusion, leading to adverse cardiac remodeling and worse clinical outcomes.

Differentially expressed proteins in thrombotic tissues from STEMI patients with or without MVO

To further investigate the mechanism of thrombosis formation in STEMI patients with or without MVO, after appropriate sample quality control and normalization procedures, a total of 2538 quantified proteins were identified in both groups using 4D liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based

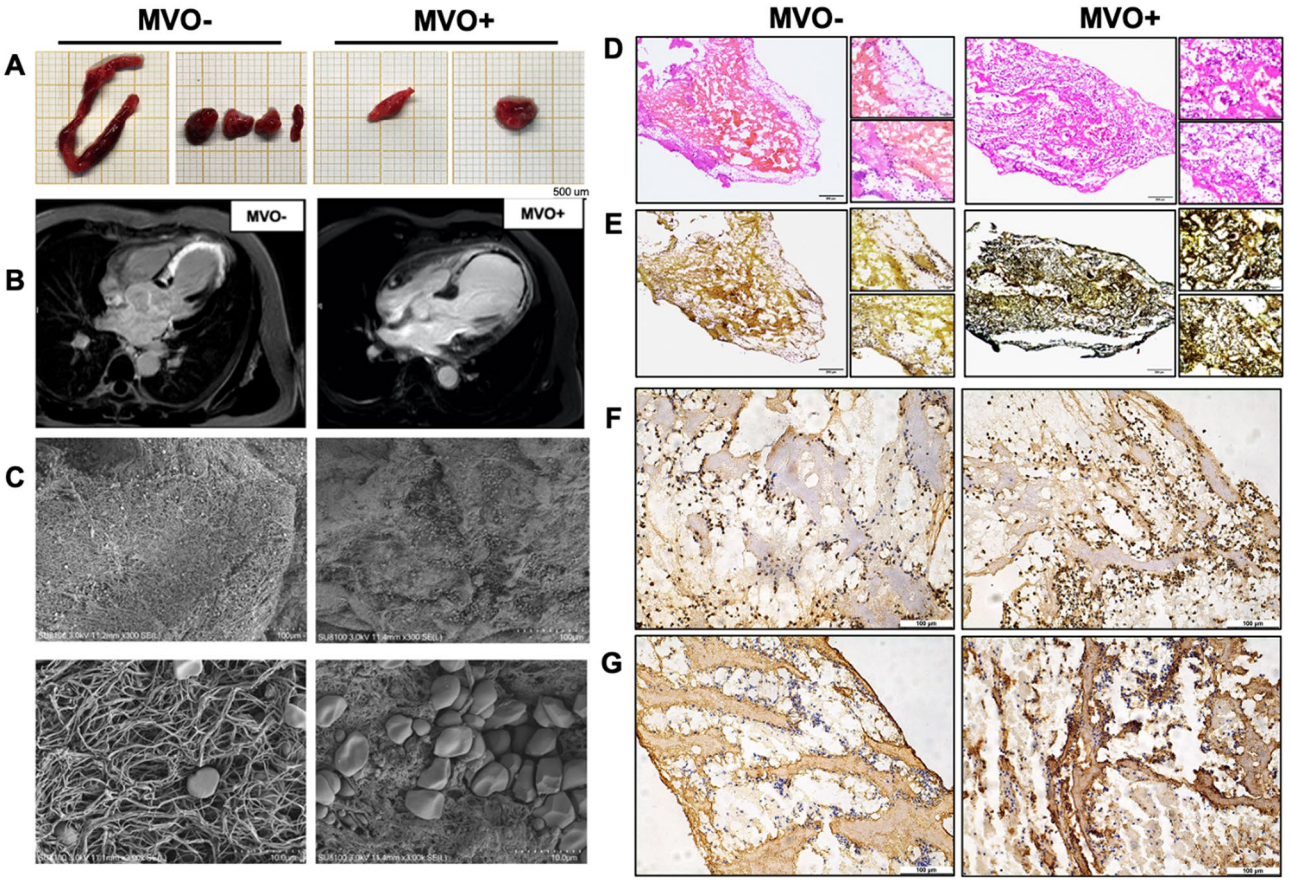

Fig. 2 Histology of the thrombus. **A**-**B**, Macroscopic view and representative cardiac magnetic resonance imaging of STEMI patients without or with MVO who underwent thrombus aspiration (scale bar: 500 μm). **C**, Transmission electron microscopy (TEM) image of a blood thrombus aspirated in STEMI patients without or with MVO (scale bar: 100 μm). **D**, Hematoxylin and eosin (H&E) staining of thrombotic tissue (scale: 100 μm). **E**, MOVAT staining of thrombotic tissue (scale: 100 μm). **F**, Immunohistochemical images of granulocytes markers (CD11b) in patients with or without MVO in thrombotic tissue. **G**, Immunohistochemical images of platelets markers (CD61) in patients with or without MVO in thrombotic tissue (scale bar: 100 μm)

label-free quantification proteomics. There were 471 proteins with expression changes greater than 1.5-fold at $P < 0.05$ between the MI with MVO and MI without MVO groups, including 50 upregulated proteins and 421 downregulated proteins in the MVO group (Fig. 3). The top 5 upregulated proteins included CYP20A1, IL6ST, DSG1, COL4A1 and TNS1, while the top 5 downregulated proteins included CA13, COX6C, EFR3A, RNF11 and TEX2. The upregulation and downregulation of specific proteins suggest a complex interaction between various biological pathways that contribute to the development and progression of MVO.

Inflammatory response participates in the pathogenesis of thrombosis in MVO patients

We further used Metascape (https://Metascape.org) to explore the functional enrichment of upregulated proteins. As shown in Fig. 4, the enriched terms included O2/CO2 exchange in erythrocytes, neutrophil degranulation, platelet activation, complement and coagulation cascades and acute inflammatory response. The functional enrichment results suggested that coagulation and inflammation processes were involved in the pathogenesis of MVO.

SLC4A1 and SLC2A1 were involved in thrombosis formation in STEMI patients with MVO

To explore the interaction of upregulated proteins, we constructed a PPI network using Cytoscape. As shown in Fig. 5, significant interactions were observed. We had identified the core roles of SLC4A1 and SLC2A1 in thrombosis formation in MVO patients, both of which are involved in coagulation and inflammatory responses.

Screening potential therapeutic drugs for MVO

50 upregulated genes in the MVO group were uploaded to Connectivity MAP, and then the scores of drug candidates that could be used for MVO treatment were obtained. A total of 4 drug candidates were screened out with scores < −60 (Table 2), including the tubulin inhibitor D-64,131, beta secretase inhibitor TC-1, TGF-beta receptor inhibitor SB-431,542, and HSP inhibitor

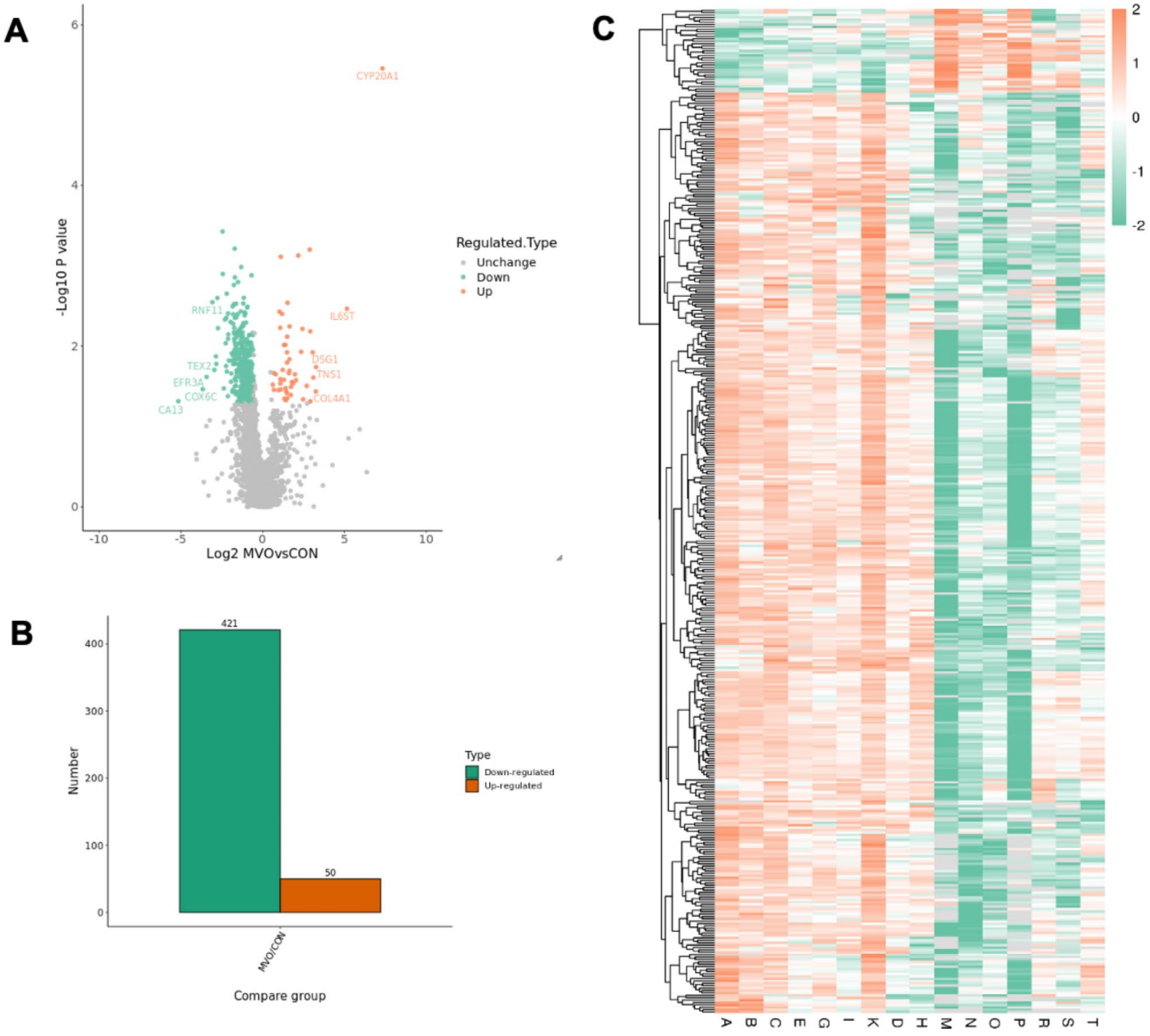

Fig. 3 Identification of differentially expressed proteins. Left is the volcano plot. The heatmap plot is shown on the right

alvespimycin. These drug candidates highlight potential therapeutic avenues for mitigating the adverse effects associated with MVO, targeting key pathways involved in thrombus formation and inflammation.

Discussion

In our proteomic analysis of the relationship between thrombus and inflammation, we identified 50 upregulated proteins and 421 downregulated proteins linked to MVO. The upregulated proteins were significantly enriched in O2/CO2 exchange in erythrocytes, neutrophil degranulation, platelet activation, complement and coagulation cascades and acute inflammatory response. Protein–protein interaction analysis revealed the central role of SLC4A1 and SLC2A1, which are both involved in the coagulation and inflammation response. Moreover, drug screening discovered 4 drug candidates for MVO treatment: D-64,131, TC-1, SB-431,542 and alvespimycin. Our study provides evidence supporting the hypothesis of inflammation's role in STEMI patients with MVO, utilizing high-throughput proteomics, and confirms inflammation as a key mediator between thrombosis and MVO.

Thrombosis-inflammation paradigm in STEMI

In STEMI patients with high thrombus burden, thrombectomy during primary PCI did not further reduce CV death, MI (Myocardial Infarction), cardiogenic shock, or Class IV heart failure compared with PCI alone, but did significantly increase stroke [15, 16]. Additionally,

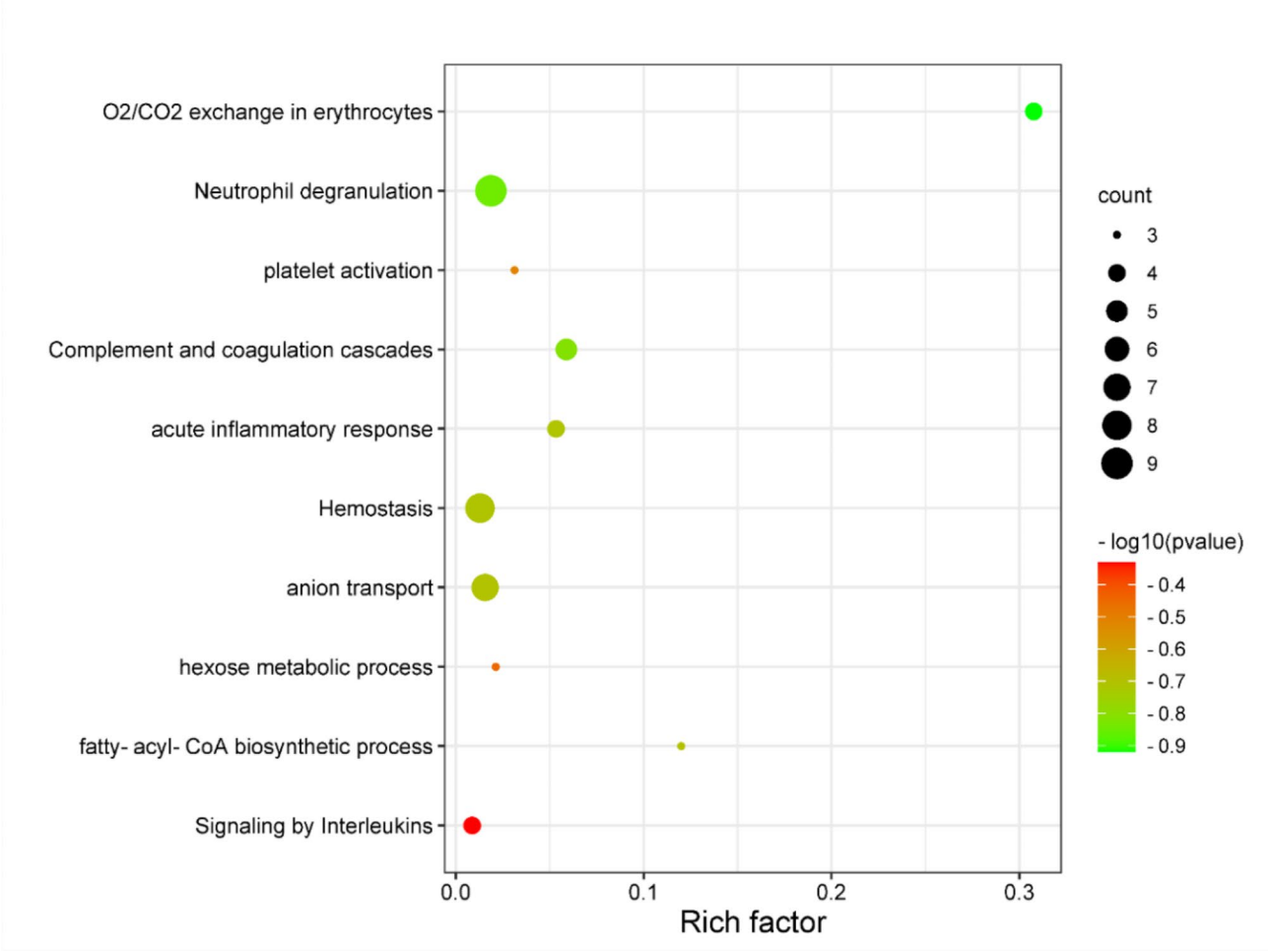

Fig. 4 The functional classification of upregulated proteins in the MVO group

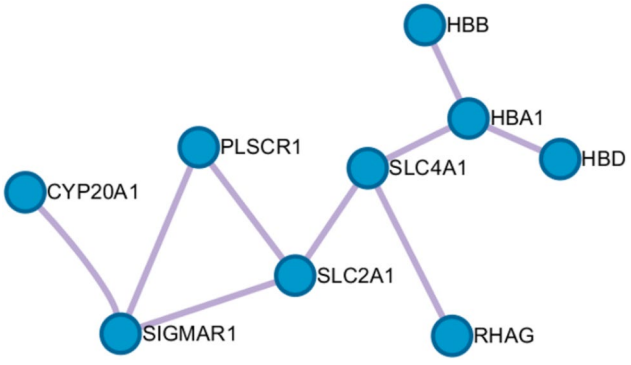

Fig. 5 Protein-protein interaction analysis of upregulated proteins

antithrombotic regimens cannot completely prevent thrombotic events [17, 18]. Conversely, the highly dynamic and coordinated inflammatory response is quickly triggered at the site of injury, potentially exacerbating myocardial damage [19, 20]. This implies that a therapeutic gap due to a third, not yet adequately addressed mechanism, inflammation, has received considerable attention owing to the coronavirus disease COVID-19 pandemic [21, 22]. Therefore, an emerging concept is of particular interest to target thromboinflammation—that is, the aberrant and excessive activation of immunothrombosis—contributes to preventing thrombotic complications in CVD [23]. From Table 1, the statistics of baseline clinical characteristics, we found that the number of neutrophil and the ratio of neutrophils/RBCs were higher in the MVO + group than in the MVO- group, which indicated that the degree of polarization of inflammatory cells and the pathophysiology mechanisms in the microenvironment of thrombosis may vary in the two groups.

SLC4A1 and SLC2A1 May be mediators between inflammation and thrombosis

Previous studies have suggested that MVO by CMR imaging is a major contributor to final infarct size and provides independent prognostic value [8]. Mechanistically, microvascular dysfunction is primarily caused by endothelial swelling, coronary microembolization of

Table 2 Predicted drug candidates based on connectivity MAP analysis

Rank	Compound	MOA	Score	SMILES
1	D-64,131	Tubulin inhibitor	-62.58	COC1 = CC2 = C(C = C1)NC(= C2)C(= O)C3 = CC = CC = C3
2	TC-1	Beta secretase inhibitor	-62.45	C1C2C3C4C1C5C2C6C3C4C5(N6CCC7 = CC(= CC = C7)F)O
3	SB-431,542	TGF-beta receptor inhibitor	-62.34	C1OC2 = C(O1)C = C(C = C2)C3 = C(NC(= N3)C4 = CC = C(C = C4)C(= O)N)C5 = CC = CC = N5
4	alvespimycin	HSP inhibitor	-61.47	C[C@H]1 C[C@@H]([C@@H]([C@H](/C = C(/[C@@H]([C@H](/C = C\C = C(\C(= O)NC2 = CC(= O)C(= C(C1)C2 = O)NCCN(C)C)/C)OC)OC(= O)N)\C)C)O)OC

MOA: mechanism of action; SMILES: Simplified Molecular Input Line Entry System; TGF: Transforming Growth Factor; HSP: Heat Shock Protein

atherosclerotic debris, cardiomyocyte swelling, vasomotion dysfunction, aggregation of leukocytes, platelets and erythrocytes, and capillary destruction and hemorrhage [9, 24]. Therefore, we hypothesize that some factors relating to immune thrombosis are directly or indirectly involved in MVO formation. From the 4D high-throughput proteomics data of coronary thrombi, we identified that the enriched terms included neutrophil degranulation, platelet activation, complement and coagulation cascades, acute inflammatory response, and O2/CO2 exchange in erythrocytes. The functional enrichment results strongly suggested that coagulation and inflammation processes were involved in the pathogenesis of MVO.

In addition, protein–protein interaction analysis revealed the central role of SLC4A1 and SLC2A1. The SLC4A1 gene located on chromosome 17q21-q22 encodes two proteins: the longer erythrocyte SLC4A1 protein and the shorter kidney SLC4A1 protein. It is a transmembrane glycoprotein involved in anion exchange as Na+-independent CI-/HCO3-exchanges [25]. It is widely expressed in erythrocytes, kidney, heart and colon [26]. SLC4A1 has been previously reported to be related to thromboembolism disease and diabetes mellitus [27, 28]. In addition, SLC4A1 was also associated with inflammation [5] and oxidative stress [26]. However, the effect of SLC4A1 in myocardial infarction has not been reported. SLC2A1, also known as GLUT1 is the major inducible glucose transporter for glucose uptake to fuel the glycolytic pathway and works in almost all mammalian cells, especially in activated leukocytes [29]. Neutrophils are the first leukocytes to massively invade the myocardium after AMI, and their inflammatory function relies greatly on glycolysis [30]. In response to immunological stimulation, neutrophil increase the surface expression of SLC2A1 for glucose uptake, as an energy source for neutrophil extracellular trap (NET) formation [31, 32]. Moreover, glucose metabolism transformation allows for Nicotinamide Adenine Dinucleotide Phosphate (NADPH) production, thereby fueling NADPH oxidase to produce superoxide and NET release [33]. Collectively, inhibition of SLC4A1, SLC2A1 or both may attenuate the formation and release of NETs, alleviating reperfusion injury and MVO formation.

Clinical impact and future research

Traditional risk assessment tools are only modestly prognostic in people with known cardiovascular disease, or elderly individuals without confirmed cardiovascular disease [34]. The insensitivity to improvements in traditional risk factors and imaging measurements is a problem for both clinical trials and medical practice [35]. Individualizing residual cardiovascular risk assessment enables precisive allocation and monitoring of the benefit of cardioprotective therapies [36]. Inflammation biomarkers may have potential in screening, diagnostic, and prognostic purposes for STEMI with MVO [37, 38]. Several inflammation-related proteins in our study deserve further investigation, either a biomarker for screening or as potential therapeutic targets in preclinical practice. Furthermore, we screened potential therapeutic drugs for treating MVO. Considering that increased systemic inflammation is a precursor of STEMI and the progression of recovery, which is supported by our results, it would be worthwhile to investigate whether early modulation of inflammatory blood cells prevents or delays the onset of cardiovascular frailty.

Limitations

Our study has several limitations. First, our study included a relatively small number of patients (only 48 STEMI patients, particularly for proteomic analysis (only 16 STEMI patients' thrombi were analyzed). This limited sample size may reduce the statistical power and generalizability of the findings. A larger cohort would help validate the identified proteomic changes in STEMI patients with and without MVO. The study primarily focused on acute-phase thrombus proteomics and MVO identification. Additionally, long-term clinical outcomes were not assessed. Further studies with extended follow-up would provide insights into the prognostic value of identified proteins. Finally, our study identified D-64131, TC-1, SB-431542, and alvespimycin as potential drug candidates for MVO treatment. However, their precise mechanisms in thrombus formation and inflammation remain unclear. There is no direct clinical evidence supporting their use in cardiovascular disease. Future preclinical and clinical studies are required to assess their therapeutic potential and safety profiles.

Conclusions

Overall, we identified 50 upregulated proteins and 421 downregulated proteins in the MVO group. These proteins were significantly enriched in neutrophil degranulation and platelet activation, suggesting an interaction of thrombosis-inflammation. Our study provides new proof-of-concept of the proteomics characteristics of STEMI patients with MVO.

Abbreviations

BMI	Body Mass Index
CMR	Cardiac magnetic resonance imaging
CRP	C-Reactive Protein
CVD	Cardiovascular disease
DAB	3,3′-Diaminobenzidine
DBP	Diastolic blood pressure
DEGs	Differentially Expressed Genes
DES	Drug-eluting stents
EASY	Enhanced Automated Sample Preparation System
EDTA	Ethylenediaminetetraacetic Acid
ESC	European Society of Cardiology
FDR	False-discovery rate
GO	Gene Ontology
HDL-C	High-Density Lipoprotein Cholesterol
HRP	Horseradish Peroxidase
HSP	Heat Shock Protein
H&E	Hematoxylin and eosin
IRA	Infarct-related artery
KEGG	Kyoto Encyclopedia of Genes and Genomes
LAD	Left Anterior Descending Artery
LCX	Left Circumflex Artery
LDL-C	Low-Density Lipoprotein Cholesterol
LVEF	Left Ventricular Ejection Fraction
MI	Myocardial Infarction
MOA	Mechanism of action
MS/MS	Tandem mass spectrometry
MVO	Microvascular obstruction
NAPDH	Nicotinamide Adenine Dinucleotide Phosphate
NET	Neutrophil extracellular trap
OCT	Optimal Cutting Temperature compound
pPCI	Primary percutaneous coronary intervention
PPI	Protein-protein interaction
RBC	Red Blood Cell
RCA	Right Coronary Artery
SBP	Systolic blood pressure
SEM	Scanning electron microscopy
SMILES	Simplified Molecular Input Line Entry System
STEMI	ST-segment elevation myocardial infarction
TGF	Transforming Growth Factor
TIMI	Thrombolysis in Myocardial Infarction
TNT	Cardiac Troponin T
UPLC	Ultraperformance liquid chromatography

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12872-025-04694-9.

Supplementary Material 1

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Author contributions

K.W., L.K. and J.C. designed the study and applicated the ethics, and all coauthors participated in the collection of the clinical samples. Y.Q. and Y.L. performed the statistical analysis and pathologic experiment and wrote the manuscript. All the authors have read and approved the final manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Consent for publication

Not applicable. This study does not include any individual person's data in any form (including any identifiable images, videos, or personal information requiring consent).

Competing interests

The authors declare no competing interests.

Generative AI and AI-assisted technologies in the writing process

AI-assisted technology was not used in the preparation of this work (except checking grammar and spelling).

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