Identification of SLED1 as a Potential Predictive Biomarker and Therapeutic Target of Post-Infarct Heart Failure by Bioinformatics Analyses

Jiajia Zhang,¹ MB, Jun Wang,¹ PhD, Yong Wu,¹ MD, Wei Li,¹ MS,
Kaizheng Gong,¹ PhD and Pei Zhao,¹ MD

Summary

The aim of this study was to explore potential predictive biomarkers and therapeutic targets of post-infarct heart failure (HF) using bioinformatics analyses.

CEL raw data of GSE59867 and GSE62646 were downloaded from the GEO database. Differentially expressed genes (DEGs) between patients with ST-segment elevation myocardial infarction (STEMI) and those with stable coronary artery disease (CAD) at admission and DEGs between admission and 6 months after myocardial infarction (MI) in patients with STEMI were analyzed. A gene ontology (GO) analysis and a gene set enrichment analysis (GSEA) were performed, and a protein-protein interaction network was constructed. Critical genes were further analyzed.

In total, 147 DEGs were screened between STEMI and CAD at admission, and 62 DEGs were identified in patients with STEMI between admission and 6 months after MI. The results of GO and GSEA indicate that neutrophils, neutrophil-related immunity responses, and monocytes/macrophages play important roles in MI pathogenesis. SLED1 expression was higher in patients with HF than in those without HF at admission and 1 month after MI. GSEA indicates that mTORC1 activation, E2F targets, G2M checkpoint, and MYC targets v1 inhibition may play key roles in the development of post-infarct HF. Furthermore, SLED1 may be involved in the development of post-infarct HF by activating mTORC1 and inhibiting E2F targets, G2M checkpoint, and MYC targets v1.

SLED1 may be a novel biomarker of post-infarct HF and may serve as a potential therapeutic target in this disease.

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Key words: Acute myocardial infarction, Gene set enrichment analysis

Cardiovascular diseases are the leading causes of death worldwide, including in low-income and middle-income countries.¹⁾ In 2013, the total number of deaths resulting from cardiovascular diseases reached almost 33% of all deaths globally.²⁾ Among these, ischemic heart disease accounts for 47%.²⁾

Owing to the implementation of primary percutaneous coronary intervention and the prescription of evidence-based drug treatments, the prognosis of ST-segment elevation myocardial infarction (STEMI) has improved markedly over the past 20 years.³⁾ Despite these advances, STEMI leads to irreversible loss of myocardial cells and adverse left ventricular remodeling. Approximately 50% of the cases demonstrate a lower ejection fraction after myocardial infarction (MI).³⁾ Moreover, 20% of patients with anterior STEMI may suffer from heart failure (HF) within 1 year.⁴⁾ The long-term prognosis of patients with STEMI remains poor. Therefore, an in-depth understanding of the pathophysiology of STEMI and post-

infarct HF is still required to identify new prevention and treatment targets. In addition, effective and predictive biomarkers for post-infarct HF are urgently required for risk stratification, prognosis assessment, and individualized medicine.

The present study aimed to search for differentially expressed genes (DEGs) in patients with STEMI at admission that may be involved in the pathogenesis of STEMI and then evaluate these genes to identify reliable predictive biomarkers and therapeutic targets for post-infarct HF.

Methods

Microarray data information: CEL raw data files of GSE59867⁵⁾ and GSE62646⁶⁾ were downloaded from the GEO database,^{7,8)} and they were all based on the GPL6244 platform of [HuGene-1_0-st] Affymetrix Human Gene 1.0 ST Array [transcript (gene) version]. The GSE59867 dataset contained 436 peripheral blood mononuclear cell

From the ¹Department of Cardiology, The Affiliated Hospital of Yangzhou University, Yangzhou University, Yangzhou, China.

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Address for correspondence: Pei Zhao, MD, Department of Cardiology, The Affiliated Hospital of Yangzhou University, Yangzhou University, No. 368 Hanyang Middle Road, Yangzhou 225001, China. E-mail: zhaopei@yzu.edu.cn

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Figure 1. Flow chart of the selection process.

(PBMC) samples from 111 patients with STEMI at four time points (admission, discharge, 1 month after MI, and 6 months after MI) and from 46 patients with stable coronary artery disease (CAD) at admission as the control. Among the patients with STEMI, nine were diagnosed with HF, and eight were excluded from HF. The GSE 62646 dataset included 98 PBMC samples from 28 patients with STEMI at three time points (admission, discharge, and 6 months after MI) and from 14 patients with CAD at admission as the control. From the two datasets, all patients with STEMI underwent coronary angiography and angioplasty of the infarct-related artery, and all patients with CAD were confirmed using coronary angiography (at least one stenosis exceeding 50%) or by previous coronary angioplasty, coronary artery bypass graft, or non-invasive tests (positive exercise test).

In this study, PBMC gene expression data from 139 patients with STEMI (111 from GSE59867 and 28 from GSE62646) at admission were compared with those from 60 patients with CAD (46 from GSE59867 and 14 from GSE62646) to identify DEGs in patients with STEMI at admission for subsequent analysis. Among the 139 patients with STEMI, 111 patients (83 from GSE59867 and 28 from GSE62646) had gene expression data at both admission and 6 months after STEMI. The paired data of the 111 patients were analyzed to screen for DEGs in patients with STEMI at admission for subsequent analysis. In total, complete gene expression data at four time points (admission, discharge, 1 month after MI, and 6 months after MI) were available from seven patients with HF and six patients without HF. These data were used to identify

biomarkers for post-infarct HF for further analysis. The clinical characteristics and medicines of patients for post-infarct HF analysis were analyzed in the original author's paper.⁵⁾ There was no significant difference between patients with and without HF, except for left ventricular ejection fraction, NT proBNP, and diuretics. (Figure 1)

Microarray data preprocessing: CEL raw data of GSE 59867 and GSE62646 were merged and preprocessed using the affy package⁹⁾ in R Bioconductor for background correction, normalization, and calculation expression. The sva¹⁰⁾ and limma^{11,12)} packages in R Bioconductor were used for removal of batch effects and for normalization between arrays, respectively. The probes were converted into gene symbols according to the probe annotation information. Probes without the corresponding gene symbols were removed. If multiple probes corresponded to the same gene symbol, the probe with the highest expression value was retained.

DEGs analysis: DEGs between patients with STEMI and patients with CAD at admission were identified using the limma package. The paired *t*-test of the limma package was used to identify DEGs between admission and 6 months after MI in patients with STEMI. A fold change of a gene expression ratio > 1.0 and adjusted *P*-value (adj. *P*) < 0.05 were used as cut-off criteria. Genes with a fold change of gene expression ratio > 2.0 were chosen as top DEGs. DEGs between HF and non-HF at the four time points were also identified using the limma package. A fold change of a gene expression ratio > 1.0 and *P* < 0.05 were used as the threshold value. Subsequently, DEGs of patients with STEMI at admission were visualized as vol-

Figure 2. Identification of DEGs. **A:** Volcano plot of mRNA expression profiles discriminating STEMI from CAD. **B:** Heatmap from cluster analysis of 147 DEGs between STEMI and CAD at admission. **C:** Volcano plot of mRNA expression profiles between admission and 6 months after MI in patients with STEMI. **D:** Heatmap from cluster analysis of 62 DEGs between admission and 6 months after MI in patients with STEMI. Red and blue dots in volcano plots represent upregulated and downregulated mRNAs, respectively. Green dots are the top DEGs. Each column in heatmaps represents a sample, and each row represents a gene. The gradual color change from blue to red indicates the changing process from downregulation to upregulation in heatmaps. A fold change of gene expression ratio > 1.0 and adj. $P < 0.05$ were considered statistically significant.

cano plots and heatmaps using the ggplot2¹³⁾ and pheatmap (<https://cran.r-project.org/web/packages/pheatmap/>) packages, respectively, in R.

Gene ontology (GO) functional enrichment analysis of DEGs: To explore the potential function of DEGs in patients with STEMI, DEGs were subjected to GO enrichment analysis to determine the associated biological process (BP), molecular function (MF) and cellular component (CC) terms using the clusterProfiler package¹⁴⁾ in R Bioconductor. Adj. $P < 0.05$ and enrichment count ≥ 2 were used as the threshold values.

Gene set enrichment analysis (GSEA): GSEA¹⁵⁾ is used to determine whether a predefined gene set is significantly different between two groups. In this study, GSEA was performed using hallmark gene sets (<http://software.broadinstitute.org/gsea/msigdb/collections.jsp#H>). To explore the mechanism of MI and post-infarct HF, GSEA was con-

ducted using the clusterProfiler package in R Bioconductor. The entire gene lists were ranked according to the log 2 fold change of gene expression ratios between STEMI and CAD at admission and between admission and 6 months after MI in patients with STEMI, respectively. Adj. $P < 0.05$ was considered statistically significant.

Protein-protein interaction (PPI) networks of DEGs: To screen for crucial genes associated with STEMI, DEGs were mapped into the Search Tool for the Retrieval of Interacting Genes database (STRING; Version 11.0)¹⁶⁾ to predict PPI pairs with a combined score > 0.4. The PPI networks were visualized using Cytoscape software (Version 3.6.1).¹⁷⁾ Nodes with a degree of interaction ≥ 10 were considered as hub genes. Additionally, the Molecular Complex Detection plugin (MCODE, Version 1.5.1, <http://apps.cytoscape.org/apps/mcode>) in Cytoscape was applied to identify a function module in the PPI network with the

Table I. The Overlapping GO Terms Enriched by DEGs in Patients with STEMI

GO	ID	Description	DEGs (MI-CAD)		DEGs (MI-Follow)	
			Count	adj. <i>P</i>	Count	adj. <i>P</i>
BP	GO: 0042119	neutrophil activation	17	0.00028598	12	0.0000245
BP	GO: 0002283	neutrophil activation involved in immune response	16	0.0002963	11	0.0000796
BP	GO: 0032103	positive regulation of response to external stimulus	13	0.0002963	10	0.0000245
BP	GO: 0043312	neutrophil degranulation	16	0.0002963	11	0.0000796
BP	GO: 0002446	neutrophil mediated immunity	16	0.00031838	11	0.0000828
BP	GO: 0002683	negative regulation of the immune system process	14	0.00139748	6	0.02523409
BP	GO: 1902106	negative regulation of leukocyte differentiation	7	0.0024105	3	0.03087566
BP	GO: 0060326	cell chemotaxis	11	0.00300653	8	0.00046973
BP	GO: 0050727	regulation of the inflammatory response	13	0.00316498	11	0.0000329
BP	GO: 0097529	myeloid leukocyte migration	9	0.00316498	7	0.00045122
BP	GO: 0071677	positive regulation of mononuclear cell migration	4	0.00341911	2	0.02523409
BP	GO: 0030595	leukocyte chemotaxis	9	0.00531431	7	0.00053106
BP	GO: 0071674	mononuclear cell migration	6	0.00595344	4	0.00436075
BP	GO: 1903706	regulation of hemopoiesis	12	0.00785638	6	0.02227269
BP	GO: 0045088	regulation of the innate immune response	11	0.00986956	6	0.01497313
BP	GO: 0060670	branching involved in the labyrinthine layer morphogenesis	3	0.00986956	3	0.000604
BP	GO: 0002548	monocyte chemotaxis	5	0.01180963	3	0.01633613
BP	GO: 0002688	regulation of leukocyte chemotaxis	6	0.01480557	4	0.00943495
BP	GO: 0014912	negative regulation of smooth muscle cell migration	4	0.01480557	2	0.04260046
BP	GO: 1902105	regulation of leukocyte differentiation	9	0.01480557	6	0.00491112
BP	GO: 0071675	regulation of mononuclear cell migration	4	0.02338211	3	0.00825707
BP	GO: 0002687	positive regulation of leukocyte migration	6	0.02368844	4	0.01199788
BP	GO: 0031349	positive regulation of the defense response	11	0.02462593	7	0.00840837
BP	GO: 0090026	positive regulation of monocyte chemotaxis	3	0.02462593	2	0.01985968
BP	GO: 0002685	regulation of leukocyte migration	7	0.02723273	4	0.02617203
BP	GO: 0002690	positive regulation of leukocyte chemotaxis	5	0.02723273	4	0.00431101
BP	GO: 0050921	positive regulation of chemotaxis	6	0.02723273	4	0.01361885
BP	GO: 0060713	labyrinthine layer morphogenesis	3	0.03156755	3	0.00165716
BP	GO: 0002573	myeloid leukocyte differentiation	7	0.03740316	4	0.02971064
BP	GO: 0032102	negative regulation of response to external stimulus	9	0.03965365	5	0.03087566
BP	GO: 0090025	regulation of monocyte chemotaxis	3	0.03965365	2	0.02763815
BP	GO: 0001960	negative regulation of the cytokine-mediated signaling pathway	4	0.0397247	3	0.01361885
BP	GO: 0042063	gliogenesis	8	0.0397247	5	0.01907026
BP	GO: 0050900	leukocyte migration	11	0.0397247	8	0.00342591
BP	GO: 0006700	C21-steroid hormone biosynthetic process	3	0.03984278	2	0.02824021
BP	GO: 0030593	neutrophil chemotaxis	5	0.04116033	5	0.00100868
BP	GO: 0050920	regulation of chemotaxis	7	0.04116033	4	0.03377804
BP	GO: 0060669	embryonic placenta morphogenesis	3	0.04116033	3	0.00268583
BP	GO: 0001959	regulation of the cytokine-mediated signaling pathway	6	0.04251596	4	0.01907026
BP	GO: 0060761	negative regulation of response to cytokine stimulus	4	0.04251596	3	0.01497313
BP	GO: 0001819	positive regulation of cytokine production	10	0.04430991	7	0.00817951
BP	GO: 1901342	regulation of vasculature development	10	0.04430991	9	0.00053106
BP	GO: 0045444	fat cell differentiation	7	0.04439439	6	0.00222828
CC	GO: 0070820	tertiary granule	8	0.00306951	6	0.0009739
CC	GO: 0042581	specific granule	7	0.01032844	5	0.0056212
CC	GO: 0030667	secretory granule membrane	9	0.01174884	5	0.01970976
CC	GO: 0035579	specific granule membrane	5	0.01766586	4	0.0056212
CC	GO: 0101003	ficolin-1-rich granule membrane	4	0.02434274	3	0.01242826
MF	GO: 0038024	cargo receptor activity	5	0.029224	4	0.02420311
MF	GO: 0004896	cytokine receptor activity	5	0.02984443	3	0.03924022

DEGs (MI-CAD) indicates DEGs between STEMI and coronary artery disease at admission; DEGs (MI-Follow), DEGs between admission and 6 months after myocardial infarction in patients with STEMI; BP, biological process; CC, cellular component; DEGs, differentially expressed genes; GO, gene ontology; MF, molecular function; and STEMI, ST-segment elevation myocardial infarction.

following parameters: degree cut-off: 2, node score cut-off: 0.2, k-core: 2, maximum depth: 100. Modules with MCODE scores ≥ 4.5 and nodes ≥ 5 were used for further analysis.

Biomarker screening for post-infarct HF and function analysis: To seek biomarkers of post-infarct HF, top

DEGs, hub genes, and genes in the selected function modules were further analyzed to elucidate their roles in post-infarct HF during follow-up. Biomarkers were screened by crossing these genes with DEGs between patients with HF and those without HF at four time points. The biomarkers were further subjected to GSEA to ex-

Figure 3. Mechanism analysis of STEMI. GO function enrichment analysis of DEGs between STEMI and CAD at admission (A) and between admission and 6 months after MI in patients with STEMI (B). The change of color from red to blue indicates the adj. *P* from low to high. The horizontal axes represent gene counts of enriched genes in corresponding GO terms. Adj. *P* < 0.05 was considered statistically significant. GSEA of entire genes according to the log₂ fold change of the gene expression ratio between STEMI and CAD at admission (C) and between admission and 6 months after MI in patients with STEMI (D). The size and the color intensity of a circle represent the numbers of enriched genes and adj. *P*, respectively. Adj. *P* < 0.05 was considered statistically significant.

plore their function in HF. The entire gene lists were ranked according to the correlation coefficient of a biomarker and other genes of gene expression ratio between patients with and without HF at admission. Adj. *P* < 0.05 was considered statistically significant.

Results

Identification of DEGs: Raw data comprised 32,321 probes. A total of 18822 genes remained following data preprocessing. Subsequently, 147 DEGs were screened between STEMI and CAD at admission, including 79 upregulated and 68 downregulated genes (Figure 2A and B). As shown in Figure 2A, SOCS3, ERCP, and HP were the top DEGs. There were 62 DEGs in patients with STEMI between admission and 6 months after MI. Interestingly, 95% (59 out of 62) of the DEGs were upregulated. SOCS3, HP, and SLED1 were identified as the top DEGs. The results are presented in volcano plots and heatmaps in Figure 2C and D.

Mechanism analysis of STEMI: 66 GO terms were enriched in DEGs between STEMI and CAD, whereas 319 GO terms were enriched in DEGs between admission and 6 months after MI in patients with STEMI. There were 50 overlapping GO terms between the two sets of DEGs (Ta-

ble I), which more likely indicate the functions of DEGs in STEMI. The top 8 enrichment terms are shown in graphs for each part of the GO analyses (if any) for the two sets of DEGs (Figure 3A and B). As illustrated in Figure 3A and B, BP enrichment analyses show that DEGs chiefly participated in neutrophil-related biological processes, such as neutrophil activation, neutrophil degranulation, and neutrophil-mediated immunity. In CC enrichment analysis, DEGs were predominantly involved in the construction of tertiary granule, specific granule, and secretory granule membrane. In MF terms, DEGs were mainly related to cargo receptor activity.

Twenty-seven hallmark gene sets were chosen for STEMI when compared with CAD, and 30 hallmark gene sets were screened for STEMI when compared with 6 months after MI. There were 21 overlapping hallmark gene sets enriched in STEMI (Table II), which may be related to the development of MI. As illustrated in Figure 3 C and D, the overlapping activated hallmark gene sets mainly included TNF- α signaling via NF- κ B, the inflammatory response, the P53 pathway, complement, and glycolysis. By contrast, E2F targets, the interferon alpha response, and allograft rejection were the overlapping suppressed hallmark gene sets.

PPI network construction and analysis: A PPI network

Table II. The Overlapping Hallmark Gene Sets Enriched in Patients with STEMI

Description	MI-CAD		MI-Follow	
	enrichmentScore	adj. <i>P</i>	enrichmentScore	adj. <i>P</i>
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.522071286	0.00628457	0.698989769	0.00448029
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.49293494	0.00628457	0.593655818	0.00448029
HALLMARK_INFLAMMATORY_RESPONSE	0.482613528	0.00628457	0.595852774	0.00448029
HALLMARK_REACTIVE_OXYGEN_SPECIES_PATHWAY	0.477174119	0.01036055	0.685752564	0.00448029
HALLMARK_P53_PATHWAY	0.445908687	0.00628457	0.575456712	0.00448029
HALLMARK_XENOBIOTIC_METABOLISM	0.40940348	0.00628457	0.536417408	0.00448029
HALLMARK_MYOGENESIS	0.403026887	0.00628457	0.373873455	0.02232855
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.393257704	0.00628457	0.478674726	0.00448029
HALLMARK_HYPOXIA	0.388079457	0.00628457	0.5407072	0.00448029
HALLMARK_COAGULATION	0.387177088	0.00628457	0.461673775	0.00448029
HALLMARK_COMPLEMENT	0.381198929	0.00628457	0.49184231	0.00448029
HALLMARK_UV_RESPONSE_UP	0.37520927	0.00628457	0.510961287	0.00448029
HALLMARK_ADIPOGENESIS	0.369588076	0.00628457	0.578860962	0.00448029
HALLMARK_DNA_REPAIR	0.334003156	0.02342579	0.415918467	0.02076412
HALLMARK_GLYCOLYSIS	0.329119185	0.01043841	0.486867778	0.00448029
HALLMARK_ESTROGEN_RESPONSE_LATE	0.320294174	0.01506932	0.430635724	0.00448029
HALLMARK_APOPTOSIS	0.309286511	0.03063726	0.438760087	0.00448029
HALLMARK_KRAS_SIGNALING_UP	0.291704593	0.03238866	0.422024238	0.00448029
HALLMARK_ALLOGRAFT_REJECTION	-0.343867483	0.00628457	-0.365514253	0.01707262
HALLMARK_INTERFERON_ALPHA_RESPONSE	-0.378088157	0.01036055	-0.575753057	0.00562873
HALLMARK_E2F_TARGETS	-0.445985931	0.00628457	-0.360133485	0.01218621

MI-CAD indicates a gene set enrichment analysis between STEMI and coronary artery disease at admission; MI-Follow, a gene set enrichment analysis between admission and 6 months after myocardial infarction in patients with STEMI; and STEMI, ST-segment elevation myocardial infarction.

was constructed using STRING with DEGs between STEMI and CAD, including 72 (49 upregulated and 23 downregulated) nodes and 150 edges (Figure 4A). Furthermore, seven hub genes and two modules were screened from the PPI network (Figure 4B). Another PPI network was built with DEGs between admission and 6 months after MI in patients with STEMI, including 43 (41 upregulated and 2 downregulated) nodes and 105 edges (Figure 4C). In addition, five hub genes and one module were chosen from the PPI network (Figure 4D).

Mechanism analysis of post-infarct HF: GSEA indicated that 29 hallmark gene sets were enriched in patients with HF. The top 15 gene sets are depicted in Figure 5D. Compared with GSEA between STEMI and CAD, mTORC1 signaling activation was specific for post-infarct HF. In addition, E2F targets, G2M checkpoint, and MYC targets v1 were the suppressed hallmark gene sets.

Biomarkers of post-infarct HF and function analysis: A total of 29 genes, including top DEGs, hub genes, and genes in the selected function modules, were selected for further analysis to elucidate their role in post-infarct HF. At admission, the expression levels of CD163, FPR2, IL1 R2, SLED1, and VSIG4 were higher in patients with HF than in those without it (Figure 5A). Patients with HF showed higher expression of FOS and lower expression of KLRF1 than those in patients without HF at discharge (Figure 5B). The expressions of PTGS2 and SLED1 were upregulated in patients with HF 1 month after MI compared to those in patients without HF (Figure 5C). There was no expression difference among the 29 genes between patients with and without HF 6 months after MI. SLED1 was upregulated in patients with HF at admission and 1 month after MI when compared to that in patients without

HF. Therefore, SLED1 may be a potential biomarker of post-infarct HF.

GSEA of SLED1 was carried out between patients with and without HF at admission to explore the function of SLED1 in post-infarct HF, which showed that 32 hallmark gene sets were related to SLED1. Interestingly, there were 28 overlapping hallmark gene sets (including mTOR 1 signaling) between the hallmark gene sets associated with SLED1 and those enriched in HF (Table III). Moreover, the overlapping hallmark gene sets activated and inhibited in HF were also positively and negatively correlated with SLED1 expression, respectively (Figures 5D, 5E). As illustrated in Figures 5D, 5E, the hallmark gene sets inhibited in HF and SLED1 were completely consistent, including E2F targets, the G2M checkpoint, and MYC targets v1.

Discussion

In the present study, 147 DEGs were identified between STEMI and CAD at admission, and 62 DEGs were screened for STEMI between admission and 6 months after MI. GO functional enrichment analyses were conducted to explore the potential functions of the two sets of DEGs. There were 50 overlapping GO terms between the two sets of DEGs, indicating that DEGs for STEMI were chiefly involved in neutrophil-related biological processes, such as neutrophil activation, neutrophil degranulation, and neutrophil-mediated immunity. GSEA revealed that most overlapping hallmark gene sets for STEMI were related to activation and/or infiltration of monocytes/macrophages. These results suggest that neutrophils and monocytes/macrophages may play vital roles in the pathogene-

Figure 4. PPI networks construction of DEGs. **A:** The PPI network of DEGs between STEMI and CAD at admission was constructed using Cytoscape. **B:** Top two significant modules were obtained from PPI of DEGs between STEMI and CAD with scores of 4.8 and 4.5, respectively. **C:** The PPI network of DEGs between admission and 6 months after MI in patients with STEMI was built. **D:** The top significant module (score = 5.0) was selected from PPI of DEGs between admission and 6 months after MI in patients with STEMI. The nodes represent the genes, whereas the lines represent interactions between genes. The gradual color change of nodes from blue to red indicates the changing process of genes from downregulation to upregulation. The width of the line denotes the combined score of the predicted interaction. The size of the nodes indicates the degree of interaction in the PPI network (**A**, **B**). Nodes with the black outline stand for the hub genes.

sis of MI. After MI, neutrophils are the first cell line to massively invade the myocardium, followed by monocytes,¹⁸⁾ which further differentiate into M1 or M2 macrophages. Neutrophils and the M1 subtype dominate the early inflammatory phase and secrete pro-inflammatory cytokines, causing damage and removing dead cells and debris, whereas the M2 subtype accumulates during the resolution of inflammation, promoting wound healing.¹⁹⁻²¹⁾ However, excessive neutrophil activation may release toxic

products such as proteolytic enzymes and oxygen radicals, thus, injuring cardiomyocytes.²²⁾ A clinical study revealed that a high neutrophil-to-lymphocyte ratio predicts large infarct sizes and poor clinical outcomes in patients with AMI who undergo PCI.²³⁾ Animal experiments have indicated that neutrophil depletion reduces the myocardial infarct size, suggesting neutrophil-mediated injury.^{24,25)} Besides, previous studies indicated that excessive activation of M1 macrophages promotes ventricular remodeling and

Figure 5. Biomarkers and mechanism analysis of post-infarct HF. DEGs between patients with and without HF were identified from 29 crucial genes at admission (A), discharge (B), and 1 month after MI (C). $P < 0.05$ was considered statistically significant. D: GSEA of entire genes according to the log2 fold change of the gene expression ratio between patients with HF and those without HF at admission. E: GSEA of entire genes according to the correlation coefficient of SLED1 and other genes of the gene expression ratio between patients with and without HF at admission. The size and the color intensity of a circle represent the numbers of enriched genes and adj. P , respectively (D, E). Adj. $P < 0.05$ was considered statistically significant.

HF after MI, and strategies modulating macrophage polarization have been proven to improve ejection fraction after MI,^{26,27)} suggesting a pivotal role of M1 macrophages in post-infarct HF.

SLED1 is a pseudogene of the lncRNA class. This study demonstrated that SLED1 was upregulated in patients with post-infarct HF. Ishii and her colleagues found that SLED1 was significantly upregulated in peripheral blood cells of patients with systemic lupus erythematosus and speculated that it may be associated with systemic inflammation.²⁸⁾ Also, the inflammatory response plays an important role in adverse remodeling and heart failure following MI.^{29,30)} Therefore, we speculate that SLED1 may contribute in the progression of post-infarct HF through the inflammatory response. In the present study, we found that activation of mTORC1 is specific for post-infarct HF, and SLED1 might activate mTORC1. Interestingly, mTORC1 activity is increased in both SLE and HF, and

its blockade is therapeutic.³¹⁻³³⁾ Studies indicated that mTORC1 regulates macrophage polarization by promoting the M1 subtype and inhibiting the M2 subtype.^{34,35)} Disruption of mTORC1 activation in macrophages inhibits M1 macrophage-mediated inflammation.³⁶⁾ Considering a key role of M1 macrophages in post-infarct HF, we considered that SLED1 may promote M1 macrophage polarization and inflammation by activating mTORC1, leading to post-infarct HF. Autophagy is generally considered to be cardioprotective. mTORC1 has been shown to inhibit autophagy and promote heart failure, whereas inhibition of mTORC1 activation enhances autophagy and then mitigates HF.³⁷⁾ Obviously, SLED1 may also promote heart failure after MI by regulating autophagy.

In addition, GSEA showed that genes involved in E2F targets, the G2M checkpoint, and MYC targets v1 were inhibited in post-infarct HF. All of these are involved in cell cycle regulation. For example, the E2F family

Table III. The Overlapping Hallmark Gene Sets Involved in Heart Failure and SLED1

Description	HF		SLED1	
	enrichmentScore	adj. <i>P</i>	enrichmentScore	adj. <i>P</i>
HALLMARK_TNFA_SIGNALING_VIA_NFKB	0.624618258	0.00484778	0.571373887	0.004609994
HALLMARK_ANGIOGENESIS	0.591437343	0.00484778	0.538982289	0.008753501
HALLMARK_COAGULATION	0.59135656	0.00484778	0.44302767	0.004609994
HALLMARK_IL6_JAK_STAT3_SIGNALING	0.581241779	0.00484778	0.442838107	0.004609994
HALLMARK_INFLAMMATORY_RESPONSE	0.571731609	0.00484778	0.449214934	0.004609994
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION	0.532854975	0.00484778	0.356951265	0.004609994
HALLMARK_XENOBIOTIC_METABOLISM	0.530068998	0.00484778	0.32658307	0.004609994
HALLMARK_REACTIVE_OXIGEN_SPECIES_PATHWAY	0.500315914	0.01100352	0.380427613	0.043232458
HALLMARK_COMPLEMENT	0.500106618	0.00484778	0.458650994	0.004609994
HALLMARK_CHOLESTEROL_HOMEOSTASIS	0.450403792	0.01310444	0.343018847	0.034482759
HALLMARK_P53_PATHWAY	0.444597839	0.00484778	0.373765243	0.004609994
HALLMARK_FATTY_ACID_METABOLISM	0.444396361	0.00484778	0.296492338	0.020926339
HALLMARK_KRAS_SIGNALING_UP	0.441842638	0.00484778	0.346315356	0.004609994
HALLMARK_PROTEIN_SECRETION	0.43900199	0.00484778	0.498234018	0.004609994
HALLMARK_APOPTOSIS	0.423140738	0.00484778	0.403838539	0.004609994
HALLMARK_UV_RESPONSE_UP	0.418568493	0.00484778	0.400571245	0.004609994
HALLMARK_HYPOXIA	0.413140925	0.00484778	0.393659186	0.004609994
HALLMARK_INTERFERON_GAMMA_RESPONSE	0.413120536	0.00484778	0.353415511	0.004609994
HALLMARK_MTORC1_SIGNALING	0.398188677	0.00484778	0.308128459	0.004609994
HALLMARK_ADIPOGENESIS	0.386008633	0.00666845	0.318599108	0.004609994
HALLMARK_HEME_METABOLISM	0.38273054	0.00666845	0.301756507	0.004609994
HALLMARK_GLYCOLYSIS	0.365533466	0.01210287	0.305862863	0.004609994
HALLMARK_ESTROGEN_RESPONSE_LATE	0.348634823	0.0139237	0.284630006	0.014697237
HALLMARK_IL2_STAT5_SIGNALING	0.345822688	0.0188253	0.272466523	0.014697237
HALLMARK_APICAL_JUNCTION	0.321123528	0.03390892	0.271723022	0.014697237
HALLMARK_MYC_TARGETS_V1	-0.380791823	0.00666845	-0.370638076	0.004609994
HALLMARK_G2M_CHECKPOINT	-0.43698821	0.00666845	-0.260710001	0.039840637
HALLMARK_E2F_TARGETS	-0.503420367	0.00666845	-0.381706116	0.004609994

HF indicates a gene set enrichment analysis between heart failure and non-heart failure at admission, and SLED1, a gene set enrichment analysis of SLED1 in heart failure at admission.

regulates cell cycle progression through the G1/S transition,³⁸⁾ and inhibition of E2F targets may lead to cell cycle arrest. The G2M checkpoint prevents damaged cells and thus prevented them from entering the mitotic M phase.³⁹⁾ The G2M checkpoint arrest induces cells to directly enter the M phase without repair and poses a high risk of apoptosis. Abbate and colleagues have confirmed by autopsy that the apoptotic rate at infarct sites in patients with post-infarct HF was almost four times that in patients without HF,⁴⁰⁾ highlighting the importance of apoptosis in post-infarct HF. Collectively, these data suggest that cell cycle disorder and apoptosis may play a pathophysiological role in post-infarct HF. Surprisingly, the inhibited hallmark gene sets involved in HF and SLED1 were completely consistent, suggesting that upregulated SLED1 may also be involved in HF attack by interfering with cell cycle and promoting apoptosis through inhibiting E2F targets, the G2M checkpoint, and MYC targets v1.

These data indicate that SLED1 may be a novel biomarker for post-infarct HF. Owing to its roles in the development of post-infarct HF, SLED1 may also serve as a potential therapeutic target in this disease. However, we did not conduct experiments to validate our conclusions. Further extensive investigation and experimentation are thus warranted to confirm whether SLED1 could be a biomarker of post-infarct HF and to clarify its potential mechanisms and therapeutic value.

Conclusions

Patients with STEMI and high SLED1 expression at admission may be at a higher risk of HF and are more likely to suffer from HF during follow-up. Furthermore, upregulated SLED1 may be involved in the development of post-infarct HF by activating mTORC1 and inhibiting E2F targets, the G2M checkpoint, and MYC targets v1. In summary, SLED1 may be a novel biomarker of post-infarct HF and a potential therapeutic target for this disease.

Disclosure

Conflicts of interest: None.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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