Heart Failure

LINC-PINT Activates the Mitogen-Activated Protein Kinase Pathway to Promote Acute Myocardial Infarction by Regulating miR-208a-3p

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Background: This study is performed to explore the differential expression of long intergenic non-coding-p53 induced non-coding transcript, miR-208a-3p and JUN in acute myocardial infarction (AMI) and their potential mechanisms.

Methods and Results: Gene Expression Omnibus, R software, Kyoto Encyclopedia of Genes and Genomes (KEGG) and gene ontology (GO) analysis were used for analyzing the differentially expressed genes (DEGs) and pathways. The differential expressions of LINC-PINT and miR-208a-3p were examined by qRT-PCR. The expressions of JUN and the mitogen-activated protein kinase (MAPK) pathway-related proteins were analyzed by Western blot. The triphenyltetrazolium chloride (TTC) staining and terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling assay (TUNEL) staining methods were used to measure the myocardial infarction size and tissue apoptosis respectively. The targeted relationships between miR-208a-3p and LINC-PINT or JUN were confirmed using a dual luciferase reporter assay. DEGs were significantly enriched in the MAPK signaling pathway. LINC-PINT could sponge miR-208a-3p, which targeted and regulated JUN. LINC-PINT and JUN were confirmed to be overexpressed in AMI tissues. Silencing LINC-PINT and JUN could exert a protective influence against AMI. The expression of miR-208a-3p was significantly decreased in AMI tissues, and miR-208a-3p reduced myocardial ischemia-reperfusion injury and apoptosis. Downregulation of LINC-PINT facilitated miR-208a-3p expression and suppressed the protein level of JUN, contributing to the inactivation of the MAPK pathway in the AMI tissues and thus generating protective effects.

Conclusions: Knockdown of LINC-PINT inactivated the MAPK pathway by releasing miR-208a-3p and suppressing the JUN, protecting the injury during the process of AMI.

Key Words: Acute myocardial infarction; JUN; LINC-PINT; MAPK pathway; miR-208a-3p

Acute myocardial infarction (AMI) is a subgroup of acute coronary syndromes, which is the major cause of mortality and morbidity in heart diseases, especially in industrialized countries. Cardiomyocyte death and myocardial remodeling are induced by the loss of blood flowing to the myocardium. Overall, 1.5 million people suffer from myocardial infarction in the USA every year. What’s worse, around the world, there are ~20 million people dying of the disease each year. Current treatments for AMI include the administration of thrombolytic drugs or percutaneous coronary intervention. The most viable option is heart transplantation, but this therapy lacks organ donors. Additionally, among the aging population, heart failure is highly generated. Massive efforts are needed to improve the therapy of AMI.

MicroRNAs (miRNAs) are a class of endogenic, non-coding single-stranded RNAs, approximately 21–25 nucleotides long. MiRNAs participate in many biological processes such as cell growth, proliferation, apoptosis and differentiation, and many miRNAs have been reported to be indicated in various oncological and cardiovascular diseases. Recent studies have shown that miR-1 and miR-208, which are the heart-specific or heart-enriched miRNAs, can be released into circulating blood after AMI. The miR-208 family includes miR-208a, miR-208b and miR-499. MiR-208a is encoded by the intron of the...
a-myosin heavy chain (Myh6), which is important in the regulation of myosin content, muscle fiber composition and muscle performance. In addition, there are some studies indicating that it seems to be intrinsic regulation between three miRNAs. MiR-208a could regulate miR-208b and miR-499 expression in rats. Long non-coding RNAs (lncRNAs), a family of RNAs longer than 200 nucleotides, cannot encode proteins. LncRNAs play important roles in various cellular processes, such as RNA alternative splicing, chromatin modification, apoptosis, invasion and so on. The two most important lncRNAs have been found to participate in cardiomyocyte differentiation. PINT is a human lncRNA, introduced by p53 and it has been confirmed that its overexpression inhibits the proliferation of tumor cells, including breast cancer, uterine corpus endometrial cancer, and lung squamous cell carcinoma. The study of PINT may expand our insights and help develop a better treatment.

The mitogen-activated protein kinase (MAPK) is a critical signaling pathway. It plays an important role in controlling embryogenesis, cell differentiation, proliferation and cell death. This family includes three main modules: extracellular regulated kinases (ERK), stress-activated protein kinases (SAPK, also known as c-Jun N-terminal kinase, JNK) and p38 signaling families. The ERK MAPK plays a role in cell protection, while the JNK and p38 MAPK cascades promote apoptosis or differentiation. Nevertheless, two serine/threonine kinases and one double specificity threonine/tyrosine kinase are the basic compositions in these families. JNK plays a major role in cell transformation and metastasis in different types of cancers. It is activated by the phosphorylation on Thr183 and Tyr185 residues, and it is reported to phosphorylate many substrates, including c-Jun, p53 and so on. C-Jun is a transcription factor that has been reported to be highly expressed and activated in various cancers. Activation of c-Jun will influence the MAPK pathway, thereby mediating many cellular events. However, the roles of c-Jun in the regulation of MAPK in AMI remain unknown.

Our study focused on the investigation of lncRNAs, miRNAs and MAPK in myocardial infarction tissues and explored the potential mechanisms. We explore the effect of LINC-PINT, miR-208a-3p and JUN in AMI tissues, and analyzed expressions of LINC-PINT, miR-208a-3p and JUN to explain the mechanism of AMI. We hypothesized that the knockdown of LINC-PINT could facilitate miR-208a-3p expression and suppress JUN, leading to the inactivation of the MAPK pathway. Therefore, the knockdown of LINC-PINT or JUN might make contribute to AMI therapy.

Methods

Gene Expression Profiles

The data of gene expression related to AMI were downloaded from the Gene Expression Omnibus (GEO) platform (ftp://ftp.ncbi.nlm.nih.gov/geo/series/GSE66360). Gene expressions in circulating endothelial cells from patients with AMI (n=28) and healthy cohorts (n=28) were measured using the HG-133U_PLUS_2 microarray on a GPL570 platform.

Filtration of Differentially Expressed mRNAs and IncRNAs

R software was used to screen the differentially expressed genes (DEGs) between AMI samples and normal samples. First, we used a box plot to conduct data quality detection and quantile normalization to parallel experimental deviation elimination. Hierarchical clustering analysis diagram was based on “affy”, “affyPLM”, “RColorBrewer”, and “heatmap” packages. Second, the data were processed by “limFit”. The Empirical Bayes method was used to filter DEGs.

mRNAs Processing

Total normalized mRNAs expression data were uploaded by GSEA v3.0 software. The gene ontology (GO) term gene set and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway gene set were used to explore enriched pathways and functions. Statistical results for default weighted enrichment were used to manipulate data 1,000 times using P<0.05; this was considered as significantly enriched. Next, the 7 top and bottom results from the gene set enrichment analysis (GSEA) reports were chosen for curve determination using the “ggplot2” package in R software.

We also used the R software “GSEABase” package to conduct data processing. The gene sets containing 10–500 genes were obtained. The Benjamini-Hochberg procedure was used to adjust P values. A permutation test conducted 1,000 times was performed to differentiate the different pathways and GO terms with threshold P<0.05. We then used Cytoscape software to construct pathways and the GO terms network. We also adopted “joyplot” and “dotplot” to visualize their distribution.

Correlation Network Analysis of DEGs

The correlation of gene expression pattern between DEGs in MARK pathway and differentially expressed lncRNAs. The Pearson method in the “psych” package was used to probe into the correlations among DEGs in a chosen pathway, and Benjamini-Hochberg (BH) was used to adjust “P.Value” to “adjust.P.Value”. Then Cytoscape software was used to establish networks in which nodes represented DEGs, and the edges represented co-expression existence.

Animal Samples

Male Sprague-Dawley (SD) rats (10 weeks old, 300–400 g body weight) were provided by Suzhou Kowloon Hospital Affiliated to Shanghai Jiao Tong University School of Medicine. All experiments in this study were approved by the Ethics Committee for Use of Animal Samples of Suzhou Kowloon Hospital Affiliated to Shanghai Jiao Tong University School of Medicine, and the methods were carried out in line with approved guidelines.

Adenovirus Construction and Infection

Three first-generation type 5 recombinant adenoviruses (Ad) were used in these studies: Ad.βgal, Ad.PL, and Ad.EGFP. The adenovirus expression vectors of miR-208a-3p and silence adenovirus expression vectors of LINC-PINT (shLINC-PINT) and JUN (shJUN) were constructed according to following the instructions for the Adeno-XTM Expression Systems 2 kit (Clontech, Mountain View, CA, USA). The adenoviruses were purified using cesium chloride gradient ultracentrifugation and titrated via a standard plaque assay. Rats were anesthetized with xylazine (5 mg/kg i.p. [intraperitoneally]) and ketamine (80 mg/kg, intraperitoneally), and their pericardia were opened through the third intercostal space. Then, the pulmonary and aorta artery were identified. A 23-gauge...
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Male SD rats were randomly divided into sham, AMI, ischemic post conditioning (IPC) and AMI+shLINC-PINT, AMI+shJUN, AMI+miR-208a-3p groups; there were 8 rats in each treatment group. Rat AMI and IPC models were established via left anterior descending coronary artery (LAD) ligation. Rats were anaesthetized with xylazine (5 mg/kg of body weight, i.p.) and ketamine (80 mg/kg of body weight, i.p.). An antecedent transmural AMI was created by occlusion of the LAD with a silk suture in a sterile environment. Rats undergoing sham-operations were used as control groups. IPC was achieved through 4 cycles of 5 min of LAD occlusion then 5 min of reperfusion cycles. All surgical processes were conducted in a sterile environment. Twelve hours after occlusion, the heart was removed for Langendorff perfusion and the tissues within the ischemic zone, border zone and non-ischemic zone distal to the ischemic zone were dissected to measure the levels of LINC-PINT, miR-208a-3p and MAPK pathway protein.

Reverse Transcription-Quantitative Polymerase Chain Reaction (PCR)

In total, 20 μg of total RNA was extracted from myocardial tissues and reversely transcribed to cDNA using Superscript II reverse transcriptase (Life Technologies). Controls that did not undergo reverse transcription were performed to ensure the absence of genomic DNA amplification during PCR. Real-time PCR was performed with IQ SYBR Green supermix in a CFX96 apparatus (Bio-rad, Nazareth, Belgium). PCR primers were designed using Beacon

| Gene name       | Primer sequence (5′–3′)                  |
|-----------------|-----------------------------------------|
| LINC-PINT forward | 5'-CGTGGGAGCCCCCTTTAATGT-3'            |
| LINC-PINT reverse  | 5'-GGGAGCTGGCGATGTTTCTC-3'              |
| miR-208a-3p forward  | 5'-TGCGGATAGAGCGACAAAAAG-3'            |
| miR-208a-3p reverse  | 5'-ACAAGCTTTTTGCTCGTCTAT-3'            |
| U6 forward       | 5'-CTGCTCTCGCCAGACATA-3'               |
| U6 reverse       | 5'-AAGATTACGAATTGCTG-3'                |
| JUN forward      | 5'-CGGATCAAGGGGAGAGAAGTTTTTT-3'        |
| JUN reverse      | 5'-CCTGAGCATTATGGCCAGTCGAC-3'          |
| β-actin forward  | 5'-GGCTCTGAGCAAGAGATGG-3'              |
| β-actin reverse  | 5'-AGCACTGTGTTGCGATACAG-3'             |

**Figure 1.** Dysregulated long non-coding RNAs (lncRNAs) and mRNAs during acute myocardial infarction (AMI). (A) Volcano plot indicated differentially expressed lncRNAs in AMI. (B) Heat map displayed the top 10 under-expressed lncRNAs in AMI, including LINC-PINT. (C) Volcano plot showed differentially expressed mRNAs in AMI. (D) Heat map manifested the top 10 upregulated and downregulated lncRNAs in AMI.
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Western Blotting
Collected tissues were lysed with RIPA Lysis Buffer (Beyotime, Beijing). Total proteins in cells were centrifuged to be purified and then an equal amount of them was separated via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins from the gel were transferred to a polyvinylidene fluoride (PVDF) membrane. After being blocked with 5% non-fat milk (diluted in Tris-buffered saline, TBS), proteins in membrane were incubated with rabbit anti-p38 (Abcam, Cambridge, UK; diluted 1:1,000 in TBS), rabbit anti-PERK (Abcam, Cambridge, UK; diluted 1:100 in TBS), rabbit anti-JUN (Abcam, Cambridge, UK; diluted 1:1,000 in TBS) and anti-β-Actin antibodies overnight at 4°C, washed with TBS containing 0.1% Tween-20 and incubation with anti-rabbit IgG (immunoglobulin G)-horseradish peroxidase conjugate. Ultimately, proteins were visualized using enhanced chemiluminescence (Beyotime) in a E-Gel Imager (Thermo Fisher). The gene expression of β-Actin is the internal reference for JUN and MAPK pathway-related proteins.

Luciferase Constructs and Transfection
The LINC-PINT and JUN 3′-UTR were amplified from genomic DNA (gDNA) by PCR and inserted into a pmir-RB-REPORT vector (Ribobio, Guangzhou, China) at the Designer software (Premier Biosoft, USA). PCR conditions were as follows: 3 min at 95°C, 30 s at 95°C, and 1 min annealing extension (40-fold). The specificity of the PCR reaction was confirmed by melting curve analysis. Both U6 and β-actin were chosen as the internal controls for normalization. Expression levels were calculated by using the relative quantification method (2−ΔΔCt) via CFX Manager 2.1 software (Bio-Rad). All primers used are shown in Table.

Figure 2. Function annotations for deregulated genes in acute myocardial infarction (AMI). (A) Gene ontology (GO) term enrichment analysis indicated 7 most significantly enriched biological pathways in AMI. (B) A dotplot suggested the distributions of biological pathway gene sets. (C) A joyplot suggested the distributions of some biological pathway gene sets.
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Measurement of Myocardial Infarct Size
Pathological staining was used to test the size of myocardial infarction size. At 6 and 24h after coronary occlusion, rats were anaesthetized and then 6mL of 10% Evans Blue dye was injected into the rat vena cava in order to define the region that was not supplied by the LAD. Areas that had a lack of blue staining were identified as a myocardial ischaemic area at risk (IAR). Heart ventricles were transversely sliced into 2-mm-thick slices. The slices were cultured in 1% triphenyltetrazolium chloride (TTC) at 37°C for 10min to locate the infarcted and non-infarcted areas. The TTC

![Image](image_url)

Figure 3. Sequence of dysregulated pathways in acute myocardial infarction (AMI). (A) The gene set enrichment analysis (GSEA) report displayed the top 7 upregulated and downregulated pathways in AMI. The percentage beside the bar represents the proportion of differential genes in the pathway gene set. (B) A dotplot suggested the distributions of some Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways gene sets in all differential genes. (C) A joyplot suggested the distributions of some KEGG pathways gene sets in all differential genes. (D) GSEA results plot showed most genes of the KEGG mitogen-activated protein kinase (MAPK) signaling pathway were discovered in the region where genes were overexpressed in AMI.
ischemia and reperfusion at 24 h and centrifuged for 10 min under 1,200 g/min in 4°C to separate the serum. Serum levels of creatine phosphokinase (CK), creatine kinase MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH) were detected by using an AU480 automatic biochemistry analyser (Beckman Coulter, Inc., USA) following the instructions.

Statistical Analysis
The data are presented as mean±standard deviation (x±s) by Graphpad 6.0 (Version 6; CA, USA). The data of two sets were compared by using a t-test. The differences among 3 or more groups were analyzed using a one-way analysis of variance (ANOVA). All experiments were operated thrice. P<0.05 signified statistical significance.

Results
Differentially Expressed Genes, IncRNAs in AMI
Differential gene expression analysis showed that 719 unstained area (white color) was defined as an infarcted area. The percentage of the IAR represented infarct size.

TUNEL Assay
We determined the myocardial apoptosis cells by using the terminal deoxynucleotidyl transferase mediated dUTP nick-end (TUNEL) method. Briefly, after routine deparaffinization and treatment using 3% H2O2 for 20 min, the sections were digested with pepsin at 37°C for 30 min and treated with a TUNEL reaction mixture at an indoor temperature for 60 min. Hematoxylin dye was used for counterstaining. Apoptotic cells were quantified by counting TUNEL-positive nuclei. For each section, the number of TUNEL-positive cells was analyzed by using a light microscope system at 100× magnification in a blinded manner. Six representative fields were randomly selected for counting at least 100 cells from at least 3 separate experiments per condition.

Determination of Myocardial Enzyme
Femoral artery blood (3 mL) was taken after myocardial ischemia and reperfusion at 24 h and centrifuged for 10 min under 1,200 g/min in 4°C to separate the serum. Serum levels of creatine phosphokinase (CK), creatine kinase MB isoenzyme (CK-MB) and lactate dehydrogenase (LDH) were detected by using an AU480 automatic biochemistry analyser (Beckman Coulter, Inc., USA) following the instructions.

Statistical Analysis
The data are presented as mean±standard deviation (x±s) by Graphpad 6.0 (Version 6; CA, USA). The data of two sets were compared by using a t-test. The differences among 3 or more groups were analyzed using a one-way analysis of variance (ANOVA). All experiments were operated thrice. P<0.05 signified statistical significance.
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KEGG Pathway Enrichment Analysis of DEGs

The GSEA report displayed the top 7 upregulated and downregulated pathways in AMI (Figure 3A). The activated and inactivated pathways are shown in Figure 3B and 3C, in which the MAPK pathway was activated in AMI. The specific gene expression related to the MAPK pathway was confirmed in Figure 3D. Furthermore, JUN was identified to be highly expressed in MAPK pathway (Figure 4A).

Targeted Relationship Between LINC-PINT and JUN

The correlation network in Figure 4B indicated the relationship between LINC-PINT and multiple genes including JUN. TargetScan and difference analysis showed that miR-208a-3p could be one of the binding sites of LINC-PINT, and LINC-PINT was overexpressed, whereas miR-208a-3p was downexpressed in AMI. Hence, we think that LINC-PINT might competitively inhibit miR-208a-3p expression. A dual luciferase reporter assay indicated that miR-208a-3p could hinder the luciferase of the wild-type LINC-PINT plasmid, while no significant influence was detected after co-transfection with LINC-PINT mutant-reporting plasmids, which also validated the targeted relationship between LINC-PINT and miR-208a-3p (Figure 4C,D, P<0.01). Similarly, after transfection with miR-208a-3p mimics, the wild-type fluorescence activity was reduced by a large degree compared with control/NC.
Expression of LINC-PINT, miR-208a-3p and JUN in AMI

The LINC-PINT was obviously highly expressed in AMI compared with that in the sham group, but was expressed at low levels in both IPC and AMI+shLINC-PINT groups, showing that knockdown of LINC-PINT and ischemic preconditioning could reduce the expression of LINC-PINT compared to the levels in the AMI group (Figure 5A, P<0.01). MiR-208a-3p was expressed at a low level in the AMI group compared with the sham group, while its expression increased significantly in the IPC and AMI+miR-208a-3p groups (Figure 5B, P<0.01). JUN was significantly upregulated in AMI group versus the sham group, and it was significantly downregulated in both the IPC and AMI+shJUN groups versus the AMI group (Figure 5C, P<0.01). Expression of myocardial enzymes CK, CK-MB, LDH in serum was significantly increased in the AMI group compared with the sham-operated group, while considerably decreased in the IPC, AMI+shLINC-PINT, AMI+miR-208a-3p and AMI+shJUN groups in contrast to the AMI group (Figure 5D–F, P<0.01). The myocardial infarction size in the IPC, AMI+shLINC-PINT, AMI+shJUN and AMI+miR-208a-3p groups was relatively smaller than that in the AMI group (Figure 6A, B, P<0.01). A TUNEL assay showed that the low expression of LINC-PINT and JUN could suppress myocardial infarction apoptotic cells. Overexpression of miR-208a-3p can also decrease the level of cardiomyocyte apoptosis. **P<0.01 compared with the sham group, ##P<0.01 compared with the AMI group.

Figure 6. Function of LINC-PINT, miR-208a-3p and JUN in acute myocardial infarction (AMI). (A,B) In each group, 3 rats were executed and the cardiac tissues were extracted for Evans blue and triphenyl tetrazolium chloride (TTC) staining. The myocardial infarction size (white area) in the ischemic post conditioning (IPC), AMI+shLINC-PINT, AMI+shJUN and AMI+miR-208a-3p groups was relatively smaller than that in the AMI group. **P<0.01 compared with the sham group; ##P<0.01 compared with the AMI group. (C,D) Terminal deoxynucleotidyl transferase-mediated dUTP nick-end (TUNEL) assay was performed on 5 slices from 5 rats in each group, showing that the low expression of LINC-PINT and JUN could suppress myocardial infarction apoptotic cells. Overexpression of miR-208a-3p can also decrease the level of cardiomyocyte apoptosis. **P<0.01 compared with the sham group, ##P<0.01 compared with the AMI group.
has a similar function on cardiomyocytes compared with the AMI group, which included the the most number of cardiomyocytes (Figure 6C, 6D, P<0.01).

MAPK Pathway Was Activated in AMI

From Western blot experiments, MAPK pathway-related proteins, PERK, JNK and p38 were overexpressed in the AMI group vs. the sham group, whereas they were remarkably restrained after knockdown of LINC-PINT and JUN in comparison with the AMI group. Furthermore, overexpression of miR-208a-3p could also suppress MAPK pathway-related protein expression. Taken together, silencing LINC-PINT could inactivate the MAPK pathway through indirectly releasing miR-208-3p and suppressing the expression of JUN, which also suggested that the knockdown of LINC-PINT exerted a protective function during AMI progression (Figure 7A, B, P<0.01).

Discussion

In this study, we identified that LINC-PINT and JUN were overexpressed in AMI through analyzing differentially expressed genes. KEGG pathway analysis revealed that the MAPK signaling pathway was significantly enriched. The correlation network displayed the association between LINC-PINT and JUN. Our experiments verified that LINC-PINT was overexpressed, while miR-208a-3p was under expressed in AMI. A decreased miR-208a-3p level could increase the expression level of JUN, contributing to the activation of the MAPK pathway in AMI tissues. Our research demonstrated that LINC-PINT could exert a certain influence on the MAPK pathway and AMI progression via regulation of miR-208a-3p and JUN.

Differential gene expression analysis revealed that 719 mRNAs showed significant different expressions, with 405 upregulated and 314 downregulated, and JUN was expressed highly remarkably in AMI. In the study of Cheng et al, there were approximately 913 genes upregulated and 1060 genes downregulated in the AMI group found through bioinformatics analysis. What’s more, hsa-miR-30c-5p and hsa-miR-30c-5p were decreased in AMI, as determined by RT-PCR. In addition, there were 107 lncRNAs differently expressed, and obviously LINC-PINT was over-expressed in our study. Furthermore, these results suggested that lncRNAs were positively connected with miRNA. Wu et al had demonstrated that differentially expressed IncRNAs had high connectivity with differentially expressed mRNAs in MI. Cheng al detected 26 pathways, some of which were important signaling pathways in myocardial infarction, including the Calcium signaling pathway and the MAPK signaling pathway. KEGG pathway-enriched analysis of DEGs also indicated that the MAPK signaling pathway was remarkably enriched. The GO enrichments showed that the overexpressed genes were enriched with granulocyte migration, phagocytosis, and myeloid leukocyte migration, and the downregulated genes were prominently enriched in ciliary tip, isotype switching, snRNA processing, etc.

In previous studies, thousands of lncRNAs have been identified in AMI, but their functions in diseases are still poorly understood. lncRNAs not only play a critical role in physiological processes, pathological conditions, and as preoperative predictors, but also could serve as novel biomarkers in cancer. In 2013, Wang et al found a cardiac apoptosis-associated lncRNA, CARL, inhibited mitochondrial fission and apoptosis induced by anoxia in cardiomyocytes by upregulating miR-539-dependent PHB2 expression. In their later study, they found another 2 lncRNAs, CHRF (cardiac hypertrophy-related factor) and APF (autophagy promoting factor), and claimed that CHRF could induce cardiac hypertrophy by targeting miR-489, and that APF could promote myocardial infarction and autophagy by regulating miR-188-3p. Correlation network analysis of miRNA-lncRNA-mRNA indicated that lncRNAs were positively associated with miRNAs that had been known to be regulated in infarcted myocardium, but negatively associated with mRNA levels. However, our study displayed a different relationship between lncRNAs and mRNAs. LINC-PINT was over-expressed and miR-208a-3p was under-expressed in AMI. There was a negative correlation between IncRNA level and the mRNA level. As for these results, we verified that LINC-PINT inhibited miR-208a-3p expression and miR-208a-3p reduced...
myocardial ischemia-reperfusion injury and apoptosis. Similarly, Liu et al also confirmed that the expression of miR-208a-3p was decreased at the late stage after myocardial infarction.22

The MAPK signal pathway has been extensively studied as an essential signaling mechanism in plants and mammals.38 MAPKs-JNKs were deleterious for post-ischemic myocardial apoptosis and the recovery of cardiac function.24 Active MAPK alters myocardial gene expression, resulting in myocyte hypertrophy.26 With reference to a recent report, the expression levels of miR200c in tumors were significantly lower, whereas the JNK2 expression was higher, which was shown to activate c-Jun, ATF2 and Elk-1.26 In addition, overexpression of c-Jun or c-fos could activate the MAPK pathway.27 Similar to our findings, the overexpression of JUN could activate the MAPK pathway.

In conclusion, our findings indicate that LINC-PINT and JUN were overexpressed in AMI. Further analysis showed that overexpression of LINC-PINT inhibited miR-208a-3p and mediated the expression of JUN. There were several limitations existed in this study, the uncertain role of miR-208a-3p in the regulation of JUN. And the interaction between miR-208a and miR-208b need to be investigated or verified in clinical studies. But our study has revealed the relationships among LINC-PINT, miR-208a-3p and JUN, and provided novel therapeutic targets for AMI. Meanwhile, the specific connection among LINC-PINT, miR-208a-3p and JUN also offered a deeper insight into AMI progression.

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Supplementary Files

Supplementary File 1

Table S1. Differentially expressed miRNAs in endothelial cells between AMI patients and healthy cohorts

Table S2. Differentially expressed IncRNAs in endothelial cells between AMI patients and healthy cohorts

Please find supplementary file(s) at: http://dx.doi.org/10.1253/circj.CJ-18-0396