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Original Article

Bioinformatics analysis of gene expression profile data to screen key genes involved in cardiac ischemia-reperfusion injury

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Abstract: In the present study, whole genome mRNA expression profiles in cardiac ischemia regions of rats with cardiac ischemia/reperfusion injury were analyzed to identify unique target genes. A total of 544 differentially expressed genes were identified between normal and cardiac ischemia samples. Our results identified ten key genes CXCR1, GLP1R, CCL21, GRK1, oxidative stress induced growth inhibitor 1 (OSGIN1), iroquois homeobox 1 (IRX1), opioid binding protein/cell adhesion molecule-like (OPCML), gi|672075288|ref|XR_595614.1|, NONRATT008949, XR_601753.1, NONRATT008080 were related to cardiac ischemia/reperfusion injury. These candidate genes were further examined in a cardiac ischemia/reperfusion model so as to provide important therapeutic targets for the treatment of local cardiac injury.

Keywords: Cardiac ischemia/reperfusion injury, differentially expressed genes, functional enrichment analysis, pathway analysis

Introduction

There is a complex community of specific molecules in cardiac ischemia regions, and these important genes may profoundly influence many aspects of development for ischemia/reperfusion (I/R) injury. Cardiac I/R injury is an alarming global public health problem [1-3]. Accumulating evidence suggests that I/R injury can increase the risk of many other life-threatening diseases, including certain types of heart disease. Further, I/R-induced autophagy increases the severity of cardiomyocyte injury [4].

Advances in high-throughput RNA sequencing techniques have rapidly expanded our knowledge about many biological processes [5-10]. The aim of this study was to identify gene expression profile data for screening key genes involved in cardiac I/R injury. This work helped us to understand the potential biological functions of mRNAs and lncRNAs and the pathophysiological mechanism involved in cardiac I/R injury.

Materials and methods

Animals and ethic statement

Male adult SD rats (250-300 g) were obtained from the Tongji Laboratory Animal Center. All rats were housed under a 12 h light/dark cycle with food and water provided *ad libitum*. The current study was performed in accordance with the guidelines of the National Institutes of Health. Animal care and experimental protocols were approved by the local Committee on Animal Care.

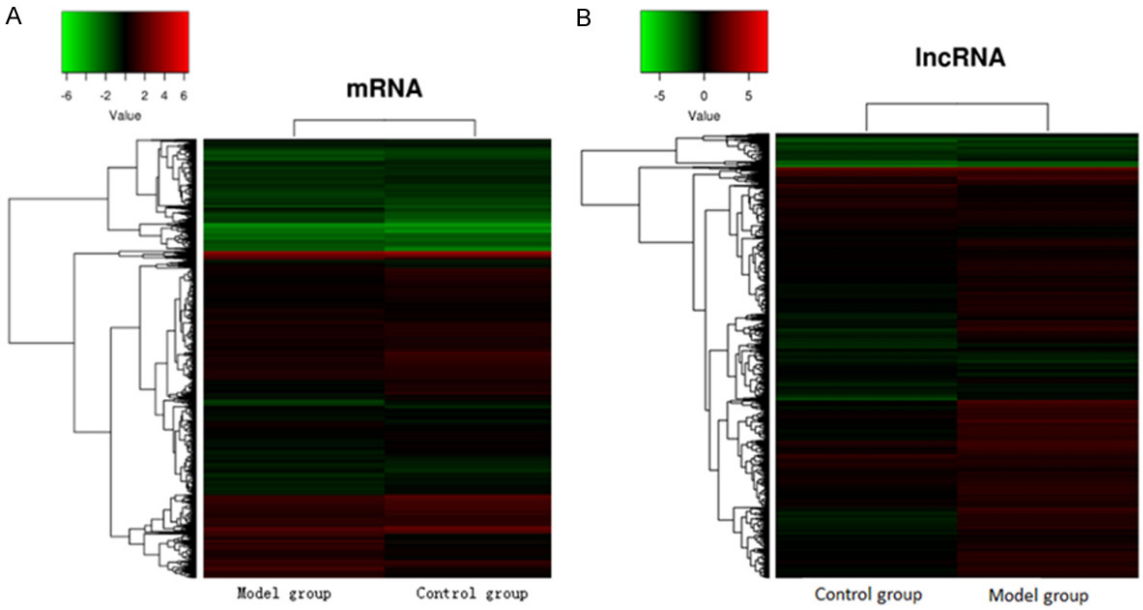

Figure 1. Heat maps and hierarchical clustering of expression ratios (log_2 scale) of mRNAs (A) and lncRNAs (B) in rat hearts 2 h after reperfusion. "Red" denotes high relative expression and "blue" denotes low relative expression. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article).

Experimental protocol and myocardial ischemia/reperfusion injury model

SD rats were randomly divided into two groups: control group (Sham, n = 3) and I/R group (I/R, n = 3). Myocardial ischemia/reperfusion injury model was performed as previously described [11-14]. Briefly, rats were anesthetized and completely sedated, then tracheal intubation was set up. The left anterior descending coronary artery was then exposed and ligatured to form a reversible trap until myocardial ischemia occurred. After 30 min of myocardial ischemia, the trap was opened and myocardial reperfusion appeared. 2 h after reperfusion, the myocardial ischemic tissues were collected. For control group, animals, sutures were not tied undergoing a sham operation.

Tissue collection and RNA extraction

Following decapitation, the myocardial ischemic tissues were immediately dissected and washed thoroughly with sterile normal saline, and finally stored in liquid nitrogen for total RNA extraction. Total RNA was isolated using TRIzol (Invitrogen) according to manufacturer's instructions [6, 15-17]. RNA qualities were measured and RNA integrity was determined. The OD A260/A230 ratio was identified.

Microarray studies

Three myocardial tissues from each group were analyzed by lncRNAs + mRNAs Rat Gene Expression microarray (Agilent 8 × 60 K chips). After RNA quantities were measured, the samples were labeled and hybridized according to the manufacturer's guideline [18-20]. Significantly differentially expressed lncRNAs + mRNAs were then identified. Finally, hierarchical clustering was performed among the samples.

Bioinformatics analysis

For investigating the target genes of lncRNAs + mRNAs, some data were used. The lncRNAs and mRNAs expression profiles from the myocardial ischemic regions were screened by volcano plot filtering. The target genes were analyzed by GO (http://www.geneontology.org) and KEGG (http://www.genome.jp/dbget-bin).

Statistical analyses

Results are expressed as the mean ± SEM. The statistical analyses and graphs were performed using GraphPad Prism (GraphPad Software, Inc., La Jolla, CA). $P < 0.05$ was considered statistically significant.

Table 1. Detailed information of the top 50 up-regulated mRNAs in the ischemic region 2 h after reperfusion

Gene ID	Fold change (R/N)	GENE_SYMBOL	Gene name
NM_001134481	25.97118	Plcxd2	Phosphatidylinositol-specific phospholipase C, X domain containing 2
NM_001106165	25.46518	Car7	Carbonic anhydrase 7
XM_002727399	21.23528	Pilra	Paired immunoglobin-like type 2 receptor alpha
NM_001008339	21.07919	Nelfa	Negative elongation factor complex member A
XM_001081804	15.23514	Pde6g	Phosphodiesterase 6G, cGMP-specific, rod, gamma
XM_006249194	14.28584	RGD1561143	Similar to cell surface receptor FDFACT
NM_138504	13.01391	Osgin1	Oxidative stress induced growth inhibitor 1
NM_001107331	10.5432	Irx1	iroquois homeobox 1
NM_053848	10.10015	Opcml	Opioid binding protein/cell adhesion molecule-like
XM_006228512	8.814655	RGD1563034	Similar to ETS domain transcription factor ERF (Ets2 repressor factor)
NM_001109372	8.515041	Fam150b	Family with sequence similarity 150, member B
NM_001012224	8.093776	Nfe2	Nuclear factor, erythroid derived 2
NM_021688	8.069224	Kcnk1	Potassium channel, subfamily K, member 1
NM_020104	7.85615	Myl1	Myosin, light chain 1
XM_001074323	7.737585	Tnfsf18	Tumor necrosis factor (ligand) superfamily, member 18
NM_182952	7.403613	Cxcl11	Chemokine (C-X-C motif) ligand 11
NM_017019	7.245778	Il1a	Interleukin 1 alpha
NM_134372	7.220482	Acmsd	Aminocarboxymuconate semialdehyde decarboxylase
XM_006226563	7.156018	Cdhr4	Cadherin-related family member 4
NM_001109578	6.972716	LOC690326	Hypothetical protein LOC690326
NM_031327	6.843192	Cyr61	Cysteine-rich, angiogenic inducer, 61
NM_001108195	6.718838	Klhl40	Kelch-like family member 40
NM_181479	6.621698	Kir3dl1	Killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1
NM_001047866	6.594324	Cntnap5c	Contactin associated protein-like 5C
NM_001134481	6.215656	Plcxd2	Phosphatidylinositol-specific phospholipase C, X domain containing 2
NM_001106165	6.109064	Car7	Carbonic anhydrase 7
XM_002727399	5.92121	Pilra	Paired immunoglobin-like type 2 receptor alpha
NM_001008339	5.667461	Nelfa	Negative elongation factor complex member A
XM_001081804	5.640686	Pde6g	Phosphodiesterase 6G, cGMP-specific, rod, gamma
XM_006249194	5.442043	RGD1561143	Similar to cell surface receptor FDFACT
NM_138504	5.379033	Osgin1	Oxidative stress induced growth inhibitor 1
NM_001107331	5.352544	Irx1	iroquois homeobox 1
NM_053848	5.347504	Opcml	Opioid binding protein/cell adhesion molecule-like
XM_006228512	5.144021	RGD1563034	Similar to ETS domain transcription factor ERF (Ets2 repressor factor)
NM_001109372	5.078389	Fam150b	Family with sequence similarity 150, member B
NM_001012224	5.060686	Nfe2	Nuclear factor, erythroid derived 2
NM_021688	5.022726	Kcnk1	Potassium channel, subfamily K, member 1
NM_020104	4.914793	Myl1	Myosin, light chain 1
XM_001074323	4.900176	Tnfsf18	Tumor necrosis factor (ligand) superfamily, member 18
NM_182952	4.871627	Cxcl11	Chemokine (C-X-C motif) ligand 11
NM_017019	4.866426	Il1a	Interleukin 1 alpha
NM_134372	4.727727	Acmsd	Aminocarboxymuconate semialdehyde decarboxylase
XM_006226563	4.721964	Cdhr4	Cadherin-related family member 4
NM_001109578	4.706878	LOC690326	Hypothetical protein LOC690326
NM_031327	4.699767	Cyr61	Cysteine-rich, angiogenic inducer, 61
NM_001108195	4.689268	Klhl40	Kelch-like family member 40
NM_181479	4.649013	Kir3dl1	Killer cell immunoglobulin-like receptor, three domains, long cytoplasmic tail, 1
NM_001047866	4.638201	Cntnap5c	Contactin associated protein-like 5C
NM_001029927	4.525999	Cpvl	Carboxypeptidase, vitellogenic-like
NM_017358	4.451633	Cdon	Cell adhesion associated, oncogene regulated

Values are fold changes (FC) in the reperfusion groups (reperfusion 2 h) over the control group (N > F2.0-fold; P < 0.05 by analysis of variance).

Table 2. Detailed information of the top 50 down-regulated mRNAs in the ischemic region 2 h after reperfusion

Gene ID	Fold change (R/N)	GENE_SYMBOL	Gene name
XM_001065496	-38.1959	Mmrn1	Multimerin 1
NM_031737	-33.0016	Nkx6-1	NK6 homeobox 1
XM_006223594	-29.4102	LOC688778	Similar to fatty aldehyde dehydrogenase-like
NM_001000011	-23.3124	Olr1428	Olfactory receptor 1428
NM_001008513	-22.6481	Ccl21	Chemokine (C-C motif) ligand 21
NM_001000856	-21.9947	Olr386	Olfactory receptor 386
NM_022544	-19.1504	Defb4	Defensin beta 4
NM_019310	-15.8884	Cxcr1	Chemokine (C-X-C motif) receptor 1
NM_012728	-14.7924	Glp1r	Glucagon-like peptide 1 receptor
NM_012535	-14.7229	Prl3b1	"Prolactin family 3, subfamily b, member 1"
NM_019329	-14.5408	Cntn3	Contactin 3 (plasmacytoma associated)
NM_001000693	-13.3859	Olr24	Olfactory receptor 24
NM_001106017	-13.1395	Myl7	Myosin, light chain 7, regulatory
NM_001134799	-13.0844	Tmem95	Transmembrane protein 95
NM_001109370	-11.8416	Dgat2l6	Diacylglycerol O-acyltransferase 2-like 6
XM_001065496	-11.6631	Mmrn1	Multimerin 1
NM_031737	-11.5115	Nkx6-1	NK6 homeobox 1
XM_006223594	-11.3629	LOC688778	Similar to fatty aldehyde dehydrogenase-like
NM_001000011	-11.1664	Olr1428	Olfactory receptor 1428
NM_001008513	-11.023	Ccl21	Chemokine (C-C motif) ligand 21
NM_001000856	-10.8804	Olr386	Olfactory receptor 386
NM_022544	-10.5916	Defb4	Defensin beta 4
NM_019310	-10.3024	Cxcr1	Chemokine (C-X-C motif) receptor 1
NM_012728	-9.81794	Glp1r	Glucagon-like peptide 1 receptor
NM_012535	-9.79548	Prl3b1	Prolactin family 3, subfamily b, member 1
NM_019329	-9.69249	Cntn3	Contactin 3 (plasmacytoma associated)
NM_001109370	-9.24467	Dgat2l6	Diacylglycerol O-acyltransferase 2-like 6
XM_001065496	-9.23634	Mmrn1	Multimerin 1
NM_031737	-9.13645	Nkx6-1	NK6 homeobox 1
XM_006223594	-9.11597	LOC688778	Similar to fatty aldehyde dehydrogenase-like
NM_001134799	-9.39616	Tmem95	Transmembrane protein 95
NM_001017501	-9.09525	Hormad2	HORMA domain containing 2
NM_001109520	-8.92488	Kcnk16	Potassium channel, subfamily K, member 16
NM_173333	-8.92021	Olr1361	Olfactory receptor 1361
NM_001105884	-8.89532	Hoxd1	Homeo box D1
XM_002726648	-8.74563	Pramef17	PRAME family member 17
NM_001000019	-8.73589	Olr1448	Olfactory receptor 1448
XM_002726602	-8.37797	Catsper4	Cation channel, sperm associated 4
NM_001134845	-8.26427	LOC688613	Hypothetical protein LOC688613
XM_003751638	-8.03944	LOC100912252	Uncharacterized LOC100912252
NM_001161846	-7.99213	Nipsnap3a	Nipsnap homolog 3A (C. elegans)
NM_022274	-7.92157	Birc5	Baculoviral IAP repeat-containing 5
NM_001271453	-7.89264	Gbx1	Gastrulation brain homeobox 1
XM_001081217	-7.84544	RGD1564031	Similar to transcription elongation factor B (SIII), polypeptide 2
NM_013098	-7.82849	G6pc	Glucose-6-phosphatase, catalytic subunit
NM_176078	-7.78195	Clic6	Chloride intracellular channel 6
NM_001004079	-7.77497	Bpi	Bactericidal/permeability-increasing protein
NM_001000112	-7.76414	Olr5	Olfactory receptor 5
NM_198790	-7.76106	Rgsl2h	Regulator of G-protein signaling like 2 homolog (mouse)
NM_001105776	-7.71202	Foxi1	Forkhead box I1
NM_001109089	-7.69987	Msantd1	Myb/SANT-like DNA-binding domain containing 1
NM_001130581	-7.61227	Fam227a	Family with sequence similarity 227, member A

Values are fold changes (FC) in the reperfusion groups (reperfusion 2 h) over the control group (N > 2.0-fold; P < 0.05 by analysis of variance).

Table 3. Detailed information of the top 50 up-regulated lncRNAs in the ischemic regions 2 h after reperfusion

ProbeName	FC (abs)	Log_2 FC	Length (bp)
gi|672030539|ref|XR_598272.1|	11.25695	3.492744	823
gi|672022169|ref|XR_601936.1|	11.02217	3.462337	9453
gi|672075288|ref|XR_595614.1|	10.97383	3.455996	1023
NONRATT008949	10.93034	3.450266	593
gi|672021685|ref|XR_601753.1|	10.49752	3.391977	3534
NONRATT008080	10.44272	3.384425	639
NONRATT016029	10.30865	3.365783	856
NONRATT016422	10.15852	3.344618	876
NONRATT028588	10.14481	3.34267	345
gi|672075969|ref|XR_359418.2|	9.935386	3.312576	2073
NONRATT010658	9.540987	3.254139	737
NONRATT013997	9.101868	3.186163	584
NONRATT010660	9.099189	3.185738	739
gi|672029961|ref|XR_341569.2|	8.762763	3.131386	1557
NONRATT023075	8.715472	3.123579	746
gi|672084663|ref|XR_597212.1|	8.47595	3.083375	344
NONRATT009756	8.330487	3.058401	500
NONRATT017646	8.206457	3.036759	1112
NONRATT002658	7.978861	2.996183	720
NONRATT006492	7.693932	2.943721	345
gi|672017126|ref|XR_600286.1|	7.499788	2.90685	931
gi|672056839|ref|XR_593095.1|	7.364812	2.880649	4960
gi|672040804|ref|XR_590723.1|	7.343456	2.876459	1718
NONRATT027869	7.312231	2.870312	617
NONRATT010300	7.309803	2.869833	504
NONRATT019811	7.211921	2.850384	344
NONRATT027180	7.191667	2.846326	352
gi|672083774|ref|XR_361470.2|	7.186728	2.845335	473
gi|672029852|ref|XR_598075.1|	7.15444	2.838839	323
gi|672024868|ref|XR_338678.2|	7.136673	2.835252	2991
gi|672016311|ref|XR_599992.1|	6.644287	2.732114	374
NONRATT025404	6.484052	2.696896	420
NONRATT025357	6.268275	2.648069	266
gi|564335313|ref|XR_351412.1|	6.241109	2.641802	589
NONRATT012880	6.239899	2.641523	322
NONRATT024390	6.190577	2.630074	385
NONRATT013400	6.115241	2.612409	373
NONRATT006836	6.071511	2.602056	772
gi|672028592|ref|XR_340666.2|	5.80575	2.537482	1887
NONRATT014969	5.718843	2.515723	315
NONRATT001619	5.707868	2.512952	1341
gi|672029992|ref|XR_598146.1|	5.476558	2.453269	1793
gi|672032741|ref|XR_589772.1|	5.468528	2.451153	517
NONRATT013679	5.439823	2.44356	1082
NONRATT030203	5.389799	2.430231	1073
uc.284	5.379776	2.427546	209
NONRATT001867	5.283186	2.401408	323

Results

Hierarchical clustering analysis of mRNAs and lncRNAs

After extracting the expression values of the differentially expressed genes, hierarchical clustering analysis was conducted for the mRNAs and lncRNAs 2 h after I/R-induced cardiac injury. As shown in the heat maps and hierarchical clustering (**Figure 1**), the differentially expressed genes could clearly distinguish the myocardial ischemic samples from the normal myocardial samples. “Red” denotes high relative expression and “blue” denotes low relative expression. In myocardial ischemic samples, there were more down-regulated genes than up-regulated genes (**Figure 1**).

The up-regulated mRNAs in the myocardial ischemic regions 2 h after I/R-induced cardiac injury

mRNA expression was quantified using RPKM value to identify the gene expression changes in T1-T4 spinal cord upon I/R-induced cardiac injury. The top 50 up-regulated mRNAs are listed in **Table 1**. Our results show that the up-regulated mRNAs in the myocardial ischemic regions include phosphatidylinositol-specific phospholipase C, X domain containing 2 (PLCXD2), carbonic anhydrase 7 (CAR7), paired immunoglobin-like type 2 receptor alpha (PILRA), negative elongation factor complex member A (Nelfa), oxidative stress induced growth inhibitor 1 (OSGIN1), iroquois homeobox 1 (IRX1), and opioid binding protein/cell adhesion molecule-like (OPCML).

gi|672014436|ref|XR_343345.2|	5.281367	2.400912	1039
NONRATT012717	5.280564	2.400692	426
NONRATT017465	5.255965	2.393956	292
gi|672030539|ref|XR_598272.1|	11.25695	3.492744	823
gi|672022169|ref|XR_601936.1|	11.02217	3.462337	9453

Values are fold changes (FC) in the reperfusion groups (reperfusion 2 h) over the control group (N > 2.0-fold; P < 0.05 by analysis of variance).

Table 4. Detailed information of the top 50 down-regulated lncRNAs in the ischemic regions 2 h after reperfusion

ProbeName	FC (abs)	Log_2 FC	Length (bp)
NONRATT004082	-23.196	-4.53581	470
NONRATT000894	-23.0546	-4.52698	540
NONRATT028709	-22.9795	-4.52228	995
NONRATT015658	-21.8799	-4.45153	755
gi|672070370|ref|XR_358071.2|	-21.5694	-4.43092	498
NONRATT001791	-21.1837	-4.40488	735
NONRATT023521	-21.1414	-4.4020	833
gi|672082871|ref|XR_596918.1|	-20.6647	-4.3691	1655
NONRATT014802	-20.3956	-4.35019	1014
NONRATT008376	-20.178	-4.33471	858
NONRATT028780	-20.0827	-4.32788	390
uc.367	-19.8568	-4.31156	298
NONRATT030839	-19.6054	-4.29318	305
NONRATT018033	-19.5257	-4.2873	840
NONRATT014624	-19.5141	-4.28644	744
NONRATT013112	-19.0972	-4.25529	438
NONRATT014824	-19.0196	-4.24942	579
NONRATT014480	-18.956	-4.24459	300
NONRATT010526	-18.6505	-4.22114	744
NONRATT009061	-18.6214	-4.21889	545
NONRATT013001	-18.4618	-4.20647	528
NONRATT008744	-18.3621	-4.19866	477
uc.437	-18.3618	-4.19864	215
NONRATT005800	-18.2419	-4.18918	720
NONRATT010464	-18.2309	-4.18831	288
gi|672033376|ref|XR_589489.1|	-18.1298	-4.1803	778
NONRATT010825	-17.9918	-4.16927	891
gi|672089372|ref|XR_597918.1|	-17.8159	-4.15509	506
gi|672031982|ref|XR_589619.1|	-17.6334	-4.14024	4919
NONRATT022292	-17.5927	-4.13691	387
NONRATT023015	-17.5174	-4.13072	418
NONRATT007424	-17.4914	-4.12857	251
NONRATT027491	-17.3819	-4.11952	1137
NONRATT024410	-17.2341	-4.1072	371
NONRATT005822	-17.2261	-4.10652	923
gi|672023024|ref|XR_348601.2|	-17.0555	-4.09216	1125

Expression profiling of down-regulated mRNAs in the myocardial ischemic regions 2 h after I/R-induced cardiac injury

Expression profiling of down-regulated mRNAs was identified in myocardial ischemic samples relative to control samples. The top 50 down-regulated mRNAs are listed in **Table 2**. Our results showed that the down-regulated mRNAs in the myocardial ischemic regions included multimerin 1 (MMRN1), NK6 homeobox 1 (NKX6-1), olfactory receptor 1428 (OLR1428), chemokine (C-C motif) ligand 21 (CCL21), olfactory receptor 386 (OLR386), defensin beta 4 (DEFB4), chemokine (C-X-C motif) receptor 1 (CXCR1), and glucagon-like peptide 1 receptor (GLP1R).

Expression profiling of up-regulated lncRNAs in the myocardial ischemic regions 2 h after I/R-induced cardiac injury

High-throughput RNA sequencing technique for the expression profiling of up-regulated lncRNAs from rats with I/R-induced cardiac injury was analyzed to acquire key lncRNAs associated with I/R. The top 50 up-regulated lncRNAs are listed in **Table 3**. Our results showed that the up-regulated lncRNAs in the myocardial ischemic regions mainly included XR_598272.1, XR_601936.1, NONRATT016029, gi|672075288|ref|XR_595614.1|, NONRATT008949, XR_601753.1, NONRATT008080, NONRATT016422, NONRATT028588.

uc.391	-16.9984	-4.08733	311
NONRATT003963	-16.9707	-4.08498	352
NONRATT010147	-16.9354	-4.08197	565
NONRATT009885	-16.8779	-4.07706	345
NONRATT013237	-16.668	-4.05901	693
NONRATT030311	-16.6182	-4.0547	948
NONRATT013031	-16.3546	-4.03162	416
gi|672034555|ref|XR_350029.2|	-16.3519	-4.03138	2403
NONRATT007621	-16.3285	-4.02932	725
NONRATT007868	-16.2052	-4.01839	896
NONRATT001032	-16.1868	-4.01675	721
NONRATT022178	-16.154	-4.01382	368
NONRATT004973	-16.1106	-4.00994	630
NONRATT006935	-16.1068	-4.00959	334
NONRATT004082	-23.196	-4.53581	470
NONRATT000894	-23.0546	-4.52698	540

Values are fold changes (FC) in the reperfusion groups (reperfusion 2 h) over the control group (N > 2.0-fold; P < 0.05 by analysis of variance).

Expression profiling of down-regulated lncRNAs in the myocardial ischemic regions 2 h after I/R-induced cardiac injury

Expression profiling of down-regulated lncRNAs from rats with I/R-induced cardiac injury was analyzed by high-throughput RNA sequencing techniques to acquire key lncRNAs associated with I/R. The top 50 down-regulated lncRNAs are listed in **Table 4**. Our results showed that the down-regulated lncRNAs in the myocardial ischemic regions mainly included NONRATT004082, NONRATT000894, NONRATT028709, NONRATT015658, gi|672070370|ref|XR_358071.2|, NONRATT001791, NONRATT023521, gi|672082871|ref|XR_596918.1|, NONRATT014802, NONRATT008376.

Gene ontology and KEGG Pathway enrichment analysis

The top six enriched GO biological processes (**Table 5**) in the I/R group mainly included neurological system process (*P*-Value = 3.94E-13), sensory perception (*P*-Value = 5.80E-13), system process (*P*-Value = 5.20E-12), G-protein coupled receptor signaling pathway (*P*-Value = 5.62E-12), cell surface receptor signaling pathway (*P*-Value = 2.79E-11), and detection of stimulus (*P*-Value = 1.25E-10). The top six enriched GO molecular function (**Table 6**) in the I/R group included G-protein coupled receptor activity (*P*-Value = 4.45e-12), signaling receptor activity (*P*-Value = 5.17e-12), transmembrane signaling receptor activity (*P*-Value = 1.91e-11), receptor activity (*P*-Value = 1.04e-10), signal transducer activity (*P*-Value = 1.50e-10), and molecular transducer activity (*P*-Value = 1.93e-09). The top six enriched GO cellular components (**Table 7**) in the I/R group included integral component of membrane (*P*-Value = 2.02e-08), cell periphery (*P*-Value = 1.23e-07), plasma membrane (*P*-Value = 1.74e-07), extracellular space (*P*-Value = 2.51e-07), membrane part (*P*-Value = 1.80e-05), and intrinsic component of plasma membrane (*P*-Value = 2.27e-05). The top five enriched KEGG pathways (**Table 8**) of STRING database in the I/R group mainly included olfactory transduction (*P*-Value = 7.03e-11), neuroactive ligand-receptor interaction (*P*-Value = 0.0002), cytokine-cytokine receptor interaction (*P*-Value = 0.0009), and steroid hormone biosynthesis (*P*-Value = 0.0015), retinol metabolism (*P*-Value = 0.0015). The top five enriched KEGG pathways (**Table 9**) of PANTHER database in the I/R group included Nicotine pharmacodynamics pathway (*P*-Value = 0.042), and heterotrimeric G-protein signaling pathway-rod outer segment phototransduction (*P*-Value = 0.045).

Discussion

In this study, chip data for the myocardial tissue samples from rats with I/R-induced cardiac injury was analysis. We found that the top 50 up-regulated mRNAs/lncRNAs and top 50 down-regulated mRNAs/lncRNAs were identified in I/R cardiac samples relative to normal cardiac samples. Among which, ten key genes CXCR1, GLP1R, CCL21, GRK1, oxidative stress induced growth inhibitor 1 (OSGIN1), iroquoishomeobox 1 (IRX1), opioid binding protein/cell adhesion molecule-like (OPCML), gi|672075288|ref|XR_595614.1|, NONRATT008949, XR_601753.1, and NONRATT008080 were related to cardiac ischemia/reperfusion injury.

Table 5. Biological process analyses of the Gene Ontology (GO) terms enriched for the differentially expressed genes (DEGs) involved in the protein-protein interaction (PPI) network constructed by STRING database

Term		Gene number	*P*-value
GO:0050877	Neurological system process	272	3.94e-13
GO:0007600	Sensory perception	235	5.80e-13
GO:0003008	System process	321	5.20e-12
GO:0007186	G-protein coupled receptor signaling pathway	277	5.62e-12
GO:0007166	Cell surface receptor signaling pathway	431	2.79e-11
GO:0051606	Detection of stimulus	194	1.25e-10
GO:0050906	Detection of stimulus involved in sensory perception	182	2.59e-10
GO:0007606	Sensory perception of chemical stimulus	181	2.19e-09
GO:0050907	Detection of chemical stimulus involved in sensory perception	171	3.75e-09
GO:0007608	Sensory perception of smell	169	7.47e-09
GO:0050911	Detection of chemical stimulus involved in sensory perception of smell	165	8.26e-09
GO:0009593	Detection of chemical stimulus	173	8.93e-09
GO:0032501	Multicellular organismal process	691	2.88e-08
GO:0044707	Single-multicellular organism process	669	4.86e-08
GO:0042221	Response to chemical	458	4.97e-07
GO:0044700	Single organism signaling	576	2.66e-06
GO:0023052	Signaling	576	2.71e-06
GO:0009605	Response to external stimulus	219	2.91e-06
GO:0007165	Signal transduction	540	3.66e-06
GO:0007154	Cell communication	584	4.62e-06
GO:0050877	Neurological system process	272	3.94e-13

Table 6. Molecular function analyses of the Gene Ontology (GO) terms enriched for the differentially expressed genes (DEGs) involved in the protein-protein interaction (PPI) network constructed by STRING database

Term		Gene number	*P*-value
GO:0004930	G-protein coupled receptor activity	245	4.45e-12
GO:0038023	Signaling receptor activity	283	5.17e-12
GO:0004888	Transmembrane signaling receptor activity	270	1.91e-11
GO:0004872	Receptor activity	291	1.04e-10
GO:0004871	Signal transducer activity	301	1.50e-10
GO:0060089	Molecular transducer activity	309	1.93e-09
GO:0004984	Olfactory receptor activity	165	8.26e-09
GO:0046873	Metal ion transmembrane transporter activity	59	2.86e-05
GO:0005125	Cytokine activity	33	5.10e-05
GO:0042379	Chemokine receptor binding	15	7.88e-05
GO:0005261	Cation channel activity	45	8.35e-05
GO:0008009	Chemokine activity	13	0.0001
GO:0005216	Ion channel activity	54	0.0001
GO:0022843	Voltage-gated cation channel activity	27	0.0001
GO:0022838	Substrate-specific channel activity	55	0.0001
GO:0022832	Voltage-gated channel activity	32	0.0002
GO:0005244	Voltage-gated ion channel activity	32	0.0002
GO:0022836	Gated channel activity	45	0.0003
GO:0022803	Passive transmembrane transporter activity	56	0.0003
GO:0015267	Channel activity	56	0.0003

Previous studies showed that the inflammatory response was likely to be the main factor for I/R-induced cardiac injury [21]. It is well-known that chemokine family was related to inflammatory response. A study of Zouggari et al. [22] identified a crucial interaction between mature B lymphocytes and monocytes after acute myocardial ischemia, and showed that high circulating concentrations of CCL7 and BAFF in patients with acute myocardial infarction predicted increased risk

Table 7. Cellular component analyses of the Gene Ontology (GO) terms enriched for the differentially expressed genes (DEGs) involved in the protein-protein interaction (PPI) network constructed by STRING database

Term		Gene number	*P*-value
GO:0031224	Intrinsic component of membrane	549	3.50e-09
GO:0016021	Integral component of membrane	533	2.02e-08
GO:0071944	Cell periphery	494	1.23e-07
GO:0005886	Plasma membrane	484	1.74e-07
GO:0005615	Extracellular space	147	2.51e-07
GO:0044425	Membrane part	607	1.80e-05
GO:0031226	Intrinsic component of plasma membrane	100	2.27e-05
GO:0005887	Integral component of plasma membrane	92	6.22e-05
GO:0034702	Ion channel complex	39	0.0006
GO:1902495	Transmembrane transporter complex	40	0.0018
GO:0034703	Cation channel complex	24	0.0019
GO:1990351	Transporter complex	40	0.002
GO:0032281	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid selective glutamate receptor complex	9	0.002
GO:0045095	Keratin filament	14	0.002
GO:0005882	Intermediate filament	20	0.003
GO:0009986	Cell surface	78	0.003
GO:0005891	Voltage-gated calcium channel complex	8	0.004
GO:0044459	Plasma membrane part	181	0.004
GO:0031225	Anchored component of membrane	15	0.011
GO:0043235	Receptor complex	35	0.015

Table 8. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched for the differentially expressed genes (DEGs) involved in the protein-protein interaction (PPI) network constructed by STRING database

Term		Gene number	*P*-value
rno04740	Olfactory transduction	194	7.03e-11
rno04080	Neuroactive ligand-receptor interaction	49	0.0002
rno04060	Cytokine-cytokine receptor interaction	37	0.0009
rno00140	Steroid hormone biosynthesis	18	0.0015
rno00830	Retinol metabolism	18	0.0015
rno05321	Inflammatory bowel disease (IBD)	15	0.0021
rno05323	Rheumatoid arthritis	17	0.007
rno04913	Ovarian steroidogenesis	12	0.012
rno04514	Cell adhesion molecules (CAMs)	26	0.018
rno04010	MAPK signaling pathway	36	0.02
rno05150	Staphylococcus aureus infection	11	0.02
rno05204	Chemical carcinogenesis	15	0.027
rno05322	Systemic lupus erythematosus	20	0.030
rno05310	Asthma	7	0.035
rno05332	Graft-versus-host disease	12	0.036
rno04940	Type I diabetes mellitus	13	0.040
rno05410	Hypertrophic cardiomyopathy (HCM)	14	0.043
rno04668	TNF signaling pathway	17	0.044
rno00270	Cysteine and methionine metabolism	8	0.045
rno05030	Cocaine addiction	9	0.048

of death or recurrent myocardial infarction, suggesting CCL7 may be new therapeutic targets for acute myocardial infarction [23]. By DNA microarray data for four acute coronary syndrome patients' samples and four normal samples, Zhang et al. [24] reported that ten up-regulated genes belonging to chemokine family (CCL2, CCR1, CXCL3, CXCL2, CCL8, CXCL11, CCL7, IL-10, CCL22 and CCL20) were related to inflammatory response, speculating that these ge-nes might be related to acute coronary syndrome. Kimura et al. indicated that

Table 9. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways enriched for the differentially expressed genes (DEGs) involved in the protein-protein interaction (PPI) network constructed by PANTHER database

Term	Gene number	*P*-value	Gene symbol
P06587: Nicotine pharmacodynamics pathway	4	0.042	rno:54239, Chrnb2, 54239:down| rno:24318, Drd2, 24318:down| rno:304081, Clic6, 304081:down| rno:29238, Drd3, 29238:down
P00028: Heterotrimeric G-protein signaling pathway-rod outer segment phototransduction	5	0.045	rno:245986, Gng8, 245986:down| rno:25539, Sag, 25539:down| rno:363143, Gnat1, 363143:down| rno:81760, Grk1, 81760:down| rno:25343, Pdc, 25343:down

macrophage-derived chemokine (CCL22) was involved centrally in the development of human atherosclerotic lesions and was a characteristic of the monocytes/macrophages migrating into atherosclerotic lesions [25].

The results from function and pathway analysis indicated that the differentially expressed genes were most significantly associated with inflammation mediated by chemokine and cytokine signaling pathway, e.g. CXCR1, GLP1R, CCL21, GRK1. Opfermann et al. [26] reported a pilot study on a CXCR1/2 antagonist reparixin to assess safety and efficacy in attenuating I/R injury and inflammation after on-pump coronary artery bypass graft surgery, and fou-nd that administration of reparixin in CABG patients appeared to be feasible and safe. Leonard et al. [27] indicated that the expression of the interleukin-8 receptors CXCR1 and CXCR2 in peripheral blood cells were increased in obstructive coronary artery disease and decreased in patients with improved perfusion, suggesting that these genes may serve as markers of disease severity and progression. Xu et al. showed that overexpression of CXCR1/CXCR2 on mesenchymal stromal cells might be an effective treatment for acute myocardial infarction [28]. A study of Basalay et al. suggested that glucagon-like peptide-1 (GLP1) mediated cardioprotection by remote ischemic conditioning [29]. DeNicola et al. demonstrated that stimulation of glucagon-like peptide-1 receptor through exendin-4 preserved myocardial performance and prevented cardiac remodeling in infarcted myocardium, suggesting that GLP-1R serves as a novel approach to eliciting cardioprotection and mitigating oxidative stress-induced injury. Cervia et al. [30] reported the modulation of the neuronal response to ischemia by somatostatin analogues acting at G protein-coupled receptor kinase 1 (GRK1). Nihei et al. examined whether Rho-kinase activity in circulating leukocytes, and found that Rho-kinase activity exhibited distinct circadian variation associated with alterations in coronary vasomotor responses and autonomic activity in VSA patients [31].

Overall, the gene expression profiles of cardiac ischemia/reperfusion injury were provided and differentially expressed genes were screened out. These results will be beneficial for understanding the mechanism and potential drug targets of cardiac ischemia/reperfusion injury.

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Disclosure of conflict of interest

None.

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