Original Paper

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2011;27:299-304

Accepted: March 07, 2011

Whole Genome Network Analysis of Ion Channels and Connexins in Myocardial Infarction

Rui Zhou[1,2,*], Pengzhou Hang[1,3,*], Wenliang Zhu[1,2], Zhe Su[1,2], Haihai Liang[2,4] and Zhimin Du[1,2,3]

[1]Institute of Clinical Pharmacology of the Second Hospital, Harbin Medical University, Harbin, [2]College of Pharmacy, Harbin Medical University, Harbin, [3]Key Laboratory of Drug Research, Harbin Medical University, Heilongjiang Higher Education Institutions, [4]Department of Pharmacology, Harbin Medical University, Harbin, [*]The authors made equal contributions to this study

Key Words

Ion channel • Connexins • Myocardial infarction • miRNA

Abstract

It has been well documented that ion channels and intercellular gap junctional proteins participated in the pathophysiological processes of myocardial infarction (MI) which resulted in lethal arrhythmias and sudden cardiac death. miRNA expression is dynamically regulated during MI and altered miRNA expression can induce deregulation of ion channel genes at the post-transcriptional level. We conducted a rationally designed bioinformatics analysis combined with experimental approaches to screen key therapeutic members in the IUPHAR database and Wikipedia, a whole genome protein interaction network was established here and comprehensive topological assessment was applied to confirm the individual network status and to reflect their biological significance. Meanwhile, the number of validated and confidently predicted miRNAs regulating each gene encoding ion channel or gap junction protein was counted. miRNA analysis indicated that connexin 43 was under more intensive miRNA regulation compared with the other ion channel and gap junction proteins. Furthermore, the topological network analysis highlighted the important role of connexin 43 in MI and also identified the important biological roles of TRPV4, SCN5A, CACNA1C and TRPC6. The abnormal expression of TRPC6 was experimentally validated in 1 month MI model of rat, which implied its potential therapeutic target for MI. Our work suggested that network systems approach could gain valuable insight into the pathological mechanism of MI which has not been uncovered by previous experimental studies.

Introduction

Electrophysiological remodeling and functional abnormalities have been described in myocardial infarction (MI) following by lethal arrhythmias and sudden cardiac death. As ion channels and gap junctions participate in cardiac electrophysiological activities, their dysfunction is closely related to the pathology of MI [1]. It has been

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015-8987/11/0274-0299$38.00/0

Accessible online at:
www.karger.com/cpb

Dr. Zhimin Du
Institute of Clin Pharmacol of the Second Hospital, Harbin Medical University
Baojian Road 148, Nangang District, Harbin
Heilongjiang Province150086 (People's Republic of China)
Tel. +86 451 86605353, Fax +86 451 86665559, E-Mail dzm1956@126.com

shown that β-blockers could reduce sudden cardiac death. However, when β-blockers therapy is not effective, the potassium channel blockers, amiodarone can be used monitoring for side effects. Therefore, it is believed that ion channels and connexins form an underexploited therapeutic area, although current drugs targeting ion channels fail to prevent serious consequences such as sudden death in patients with MI [2]. Meanwhile, by selectively targeting connexin 43, the ischemia-induced conduction slowing and ventricular fibrillation could be successfully reversed [3]. Given the long list of ion channel and gap junction proteins in IUPHAR [4] and Wikipedia, the above evidences strongly suggest that potential therapeutic targets can be likely found if novel approach is applied. Unfortunately currently available techniques may not permit thorough characterization of ion channel and gap junction proteins, thus computational prediction remain the only approach to rapidly and comprehensively identifying an important protein in silico. Hence, we proposed a protein interaction network based systems biology method in order to comprehensively assess all the currently known 162 ion channel and gap junction proteins for therapeutic intervention.

We established a whole genome protein interaction network applying Bisogenet [5] and quantitatively described the status of all the studied proteins in the network by introducing two topological parameters degree and neighborhood connectivity [6]. Furthermore, many studies have proved that miRNAs play an important role in regulating ion channel genes expressions in posttranscriptional level [7], the number of miRNAs regulating each ion channel or connexin protein gene was counted from current available databases. Two integrative databases (miRSel and miRecords) were used to search for the experimentally validated and confidently predicted miRNA-gene interactions [8, 9].

The family of ion channels and connexins represents an important category of potential drug targets as their dysfunction is intensively implicated in MI [10, 11]. Systems biology empowers us to understand the whole organism or system by individual connections at both the gene and protein levels [12]. In the current study, a novel network systems approach was proposed by placing the ion channel and gap junction proteins into a whole genome protein interaction network. This allowed us to comparatively investigate their different biological status. Assisted by miRNA regulation analysis, comprehensive assessment of ion channel and gap junction proteins could be attained. Our results will contribute to the rational drug target identification in this underexploited area.

Materials and Methods

Whole genome protein interaction network and topological parameter calculation

By combining the protein encoding genes list retrieved from HGNC [13] and the IUPHAR and Wikipedia recorded ion channel and gap junction genes list, a Cytoscape [14] plug-in BisoGenet was applied to establish the whole genome protein interaction network with interaction type of protein-protein interaction and distance of 3 selected. Another plug-in NetworkAnalyzer then calculated the two topological parameters degree and neighborhood connectivity for each node. All nodes representing proteins were classified into three categories according to their degree values. To a given node with a degree value of more than 100, it was regarded as a hub. If the degree value of a node was between 50 and 100 or less than 50, it should be defined as a sub-hub or common node (including ion channels and connexins) [15]. Finally, the degree and neighborhood connectivity values of each node were normalized with average parameter values of all the nodes in the network as baseline. Notably, for NetworkAnalyzer application, protein interaction direction should be ignored and self-loops should be also deleted in advance.

Establishment of MI model

Male wistar rats (250~270 g) were randomly divided into two groups: control, MI for 1 month. The animals were anaesthetized with chloral hydrate (300 mg/kg). A left thoracotomy was performed, and the left anterior descending coronary artery was ligated as described previously for 1 month [16]. Remote tissues of ischemic areas in the left ventricles were quickly dissected for subsequent analysis. The control group of rats was handled in the same manner except the ligation of coronary artery.

Western Blot

The total amount of protein was extracted from the left ventricular peri-infarct region of rats for immunoblotting analysis, by use of procedures essentially the same as described in detail elsewhere [17]. Proteins were separated by electrophoresis on 10% SDS–polyacrylamide gels and transferred moist to polyvinylidene difluoride membranes. Membranes were blocked by 5% nonfat dry milk in PBS for 2 h at 4 °C. Membranes were washed with PBS containing 0.5% Tween 20 (PBS-T) and then probed with TRPC6 antibody (Alomone, Jerusalem, Israel) overnight at 4°C. Membranes were washed three times, 15 min each time with PBS-T and incubated with secondary antibody (Alexa Fluor) for 1 h. Western blot bands were captured on the Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA) and quantified with Odyssey v1.2 software by measuring the band intensity (area × OD) in each group and normalizing to the internal control (GAPDH, Kangcheng, Shanghai, China).

miRNA regulation analysis

By retrieving the miRNA-gene interactions from miRecords and miRSel, we counted the summed number of the

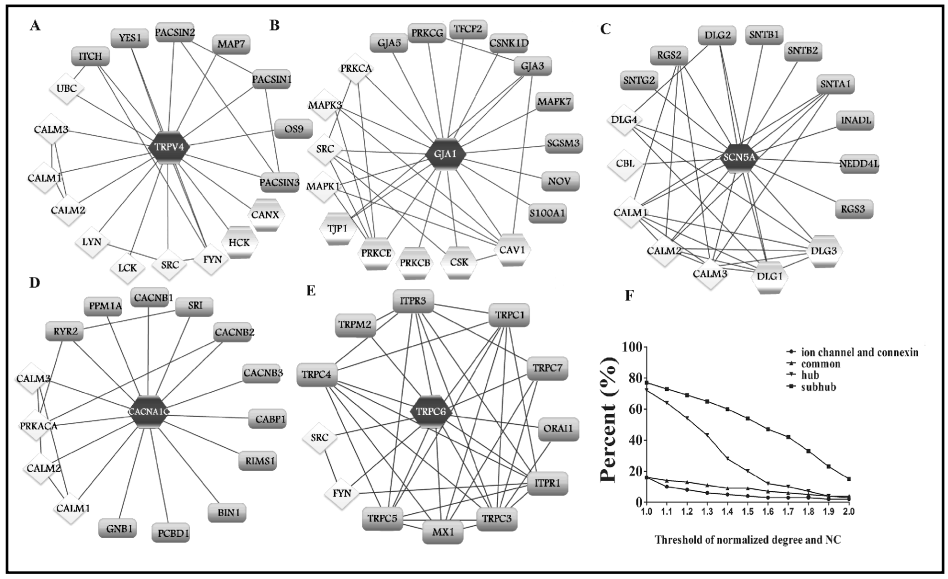

Fig. 1. Results of topological network analysis. (A-E) The proteins TRPV4, GJA1, SCN5A, CACNA1C and TRPC6 with their directly interacted proteins respectively. Nodes of round rectangle: common nodes; nodes of hexagon: subhubs; nodes of diamond: hubs. (F) Distribution of normalized degree and neighborhood connectivity.

miRNAs and MI dysregulated miRNAs [18] interacting with individual ion channel or gap junction protein genes and those encoding hubs genes in the whole genome protein interaction network. Then we compare the number of miRNAs between hub group and ion channel and connexin group in the whole genome and in MI. Notably, although miRecords also provided the experimentally validated miRNA-gene interactions in human being, miRSel was alternatively chosen as the validated interaction source for the timely update of data [8]. MiRecords was only used for obtaining the comparatively confidently predicted miRNA-gene interactions which were simultaneously identified by at least 4 prediction methods.

Softwares and databases

Cytoscape 2.6.3 software was applied for free and with two plugins, which are Bisogenet and NetworkAnalyzer. HUGO Gene Nomenclature Committee (HGNC), IUPHAR, miRecords and miRSel databases are provided online.

Statistical analysis

All data are expressed as mean ± SEM. Statistical analysis was performed using the Student's t test. Differences were considered to be statistically significant when $p < 0.05$.

Results

Network Analyzer analysis

There were totally 19363 protein-encoding genes retrieved from HGNC, and 162 ion channel and gap junction genes were recorded by IUPHAR and Wikipedia. BisoGenet created a whole genome protein interaction network which contained 19539 nodes and 64594 edges. Among the 19539 nodes, 120 were named as hubs and 349 as sub-hubs based upon the calculation result of NetworkAnalyzer. None node representing ion channel or gap junction protein was classified into the category of

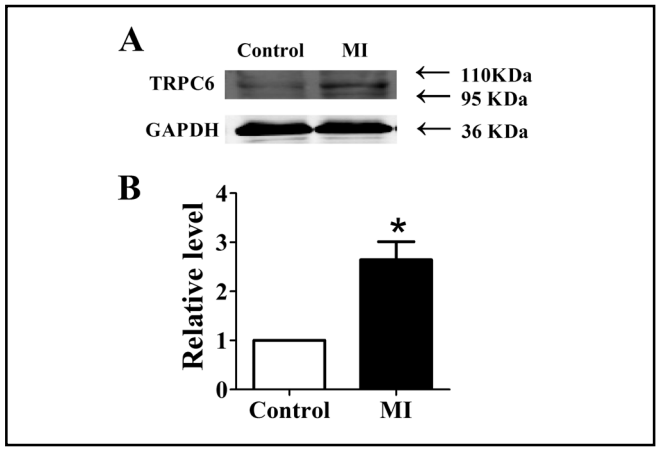

Fig. 2. Protein expression of TRPC6 in myocardial infarction (MI) rat heart. (A) Western blot result for TRPC expression in the left ventricles. The expression of TRPC6 was markedly increased in MI heart. (B) Values are given normalized to band intensity of GAPDH used as internal control. All values are expressed as mean ± SEM (n = 3 in each group).

hub or sub-hub. We calculated the percent part of hubs, sub-hubs, common nodes and especially the nodes representing ion channel or gap junction proteins at different thresholds of normalized degree and neighborhood connectivity (Fig. 1F). Figure 1F showed that sub-hubs would more likely connect with nodes of comparatively higher degree than hubs and common nodes. There were only approximately 5% of common nodes left at the threshold of 1.8. Among them, one connexin and four ion channel proteins were found. They were connexin 43, TRPV4 (transient receptor potential cation channel, subfamily V, member 4), SCN5A (sodium channel, voltage-gated, type V, alpha subunit), CACNA1C (calcium channel, voltage-dependent, L type, alpha 1C subunit) and TRPC6 (transient receptor potential cation

channel, subfamily C, member 6). Higher degree and neighborhood connectivity values indicated their more important biological status compared with other ion channel and gap junction proteins (Fig. 1A-E).

Experimental validation

The expression of TRPC6 was markedly increased in MI group compared with that in control group ($p < 0.05$) (Fig. 2). This experimental result was consistent with our previous computational prediction.

miRNA regulation analysis

By applying the miRecords and miRSel databases, we counted the experimentally validated and confidently predicted miRNA-gene interactions of the hub genes and ion channel and connexin genes (Fig. 3E). Consistent with the previous study [7], we found that hub genes would be more likely under intensive miRNA regulation than ion channel and connexin genes that belonged to common nodes ($p < 0.0001$). Correspondingly, significantly altered miRNA regulation focused on the hub genes could be found in MI (Fig. 3E). By categorizing the ion channel proteins into different subfamilies according to the ion selectivity, we conducted the distribution statistics of the topological parameters and miRNA regulation intensity among different subfamilies (Fig. 4A-D). Fig. 4A and 4B showed that calcium ion channel proteins were generally assigned with higher neighborhood connectivity than the other proteins, while there was no difference in the degree distribution in the whole as different categories were compared. The sodium ion channels were under intensive miRNA regulation that tended to be deregulated in MI (Fig. 4C and 4D).

Discussion

The main finding of the present work is that: (1) we successfully established a whole genome network to study ion channel and gap junction proteins in MI; (2) we confirmed that connexin 43 et al is putative protein in MI which is consistent with previous studies; (3) we also predicted and validated TRPC6 as a novel potential targets in MI.

Currently, most of the drugs for MI therapy are proved failing to protect cardiac physiological function and reverse pathological conditions of MI [2]. Because dysregulation of ion channel proteins and connexins was intensively associated with the lethal arrhythmias, markers of an elevated risk of sudden death from MI, we proposed

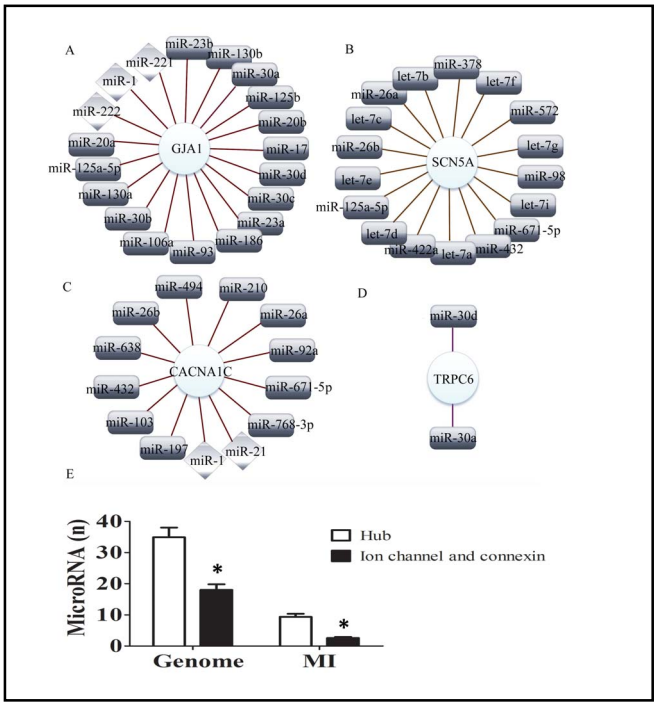

Fig. 3. Results of miRNA regulation analysis. (A-D) The genes GJA1, SCN5A, CACNA1C and TRPC6 with their interacted miRNAs, respectively. Nodes of round rectangle: predicted miRNAs; nodes of diamond: experimentally validated miRNAs. (E) The number of miRNAs in whole genome and MI dysregulated miRNAs regulating hub genes and ion channel and gap junction proteins.

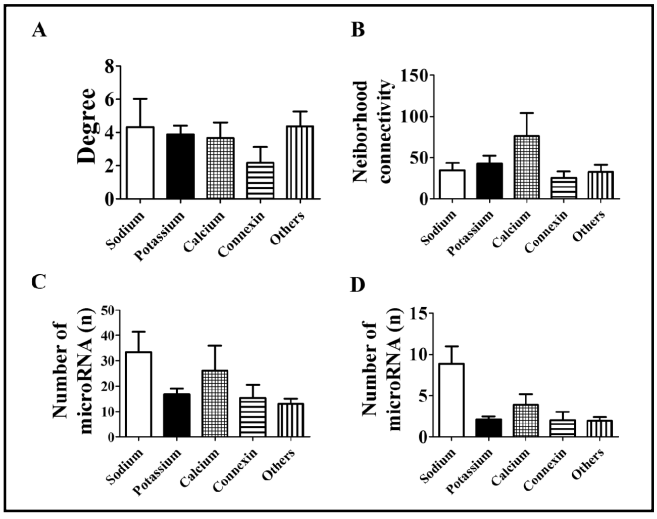

Fig. 4. Statistics of topological parameters and miRNA regulation intensity. (A) Distribution of degree. (B) Distribution of neighborhood connectivity. (C) Distribution of the number of miRNAs in the whole genome. (D) Distribution of the number of miRNAs dysregulated in MI. Sodium: selectively sodium ion channel proteins; Potassium: selectively potassium ion channel proteins; Calcium: selectively calcium ion channel proteins; Connexin: gap junctional proteins; Other: proteins assembling CatSper and Two-Pore Channels, Cyclic Nucleotide-Regulated Channels or Transient Receptor Potential Channels.

Gene Name	IUPHAR Name	Official Full Name	Expression in MI
GJA1	Cx43	gap junction protein, alpha 1, 43kDa	changed [25-27]
SCN5A	$Na_v1.5$	sodium channel, voltage-gated, type V, alpha subunit	changed [28]
CACNA1C	$Ca_v1.2$	calcium channel, voltage-dependent, L type, alpha 1C subunit	changed [28, 29]
TRPC6	TRPC6	transient receptor potential cation channel, subfamily C, member 6	changed (in this study)
TRPV4	TRPV4	transient receptor potential cation channel, subfamily V, member 4	Not reported

Table 1. The genes encoding cardiac ion channel proteins and connexins screened by our study.

a novel system biology approach to investigate which could be the potential therapeutic targets among these proteins. Depending upon the whole genome protein interaction network by the Cytoscape plug-in BisoGenet, the Cytoscape plug-in NetworkAnalyzer allowed us to comprehensively assess the inherent biological status of these proteins (Fig. 1A-E). Two topological parameters were introduced here to quantitatively describe the network status of each protein. Higher degree and neighborhood connectivity empower a protein to play more important roles in regulating cellular functions [19, 20]. Connexin 43 and four ion channel proteins were assigned with higher degree and neighborhood connectivity, implying their important biological roles (Fig. 1A-E). Except TRPV4, connexin 43 and other three ion channel proteins were experimentally validated to be involved into the pathology of MI (Table 1 and Fig. 2). Recently, Du et al. reported that inhibition of TRPC6 degradation could protect against cerebral ischemia [21]. In the current study, we experimentally verified its deregulation in MI (Fig. 2). Further biological experiments were definitely needed to detect its underlying mechanisms in MI. In addition, several studies have focused on the relationship between TRPV4 and ischemia in other tissues [22]. Therefore, it should be noted that TRPV4 also triggers our interesting to study based on both our analysis and previous relevant studies.

As miRNAs play important roles in gene expression, at posttranscriptional regulation, they have been proved as indispensable participators in the pathological process of cardiac arrhythmia induced by MI [23]. We counted the miRNAs which potentially interacted with the 162 ion channel and gap junction proteins in the whole genome range and especially at the pathological state of MI, with the hub proteins as reference (Fig. 3E). Although connexin 43 was only under moderate miRNA regulation from the whole genome perspective (58 miRNA-gene interactions found), the most intensive miRNA regulation focusing on it was discovered in MI (Fig. 3A), compared with the other connexins and ion channel proteins. This result indicated that on the one hand the normal function of connexin 43 was under the fine-tuning of numerous miRNAs in the heart and essential to cardiac health; on the other hand this precise and complex regulation was very vulnerable during deterioration of intracellular environment.

The statistics of the topological parameters degree and neighborhood connectivity implied that the normal operation of calcium channels was more necessary for important physiological functions than the channels assembled by the proteins in the other categories (Fig. 4A and 4B). It is probably because involvement of calcium ion is universal in various physiological processes. Surprisingly, we found that sodium channel proteins but not calcium channel proteins would be more likely under intensive miRNA regulation that tended to be seriously imbalanced in the MI state (Fig. 4C and 4D). Loss of control and the prominent role of I_{Na} in cardiac electrophysiology [24] well explain the arrhythmia followed by MI.

In conclusion, connexin 43 was the best therapeutic target evaluated by both NetworkAnalyzer and miRNA regulation analyses, which plays important role in intercellular conductance in cardiovascular system. The dysfunction of intercellular conductance is responsible for ischemia-induced arrhythmia in MI. Experimental results demonstrated that the connexin 43 expression was significantly decreased in rat model of MI [25]. According to our hypothesis mentioned before, drugs target for connexin 43 could enhance intercellular conductance and prevent arrhythmia induced by MI. This can be well proved by the anti-arrhythmic peptide (AAP10) which targets connexin 43 and successfully prevent ischemia-induced arrhythmia under the clinical trials [3].

However, it should be noted that the present computational study could not replace experimental studies for understanding the role of ion channels in MI. This theoretical analysis like all other in silico studies needs to be eventually verified and should not be considered original data, which may be the commonness of those studies [30]. Nevertheless, due to few experimental data published and the difficulties to get complete experimental data, this study can well serve as important information to guide our future studies. Another limitation of the present study is that our study may underestimate few proteins because of the stringent criterion. Yet, these above limitations

should eventually be solved with deepened and broadened understanding of ion channels interaction and miRNA regulation.

Acknowledgements

This work was supported by grants from the National Natural Science Foundation of China (No. 30873065, No. 81072639).

References

1 Lu Y, Zhang Y, Shan H, Pan Z, Li X, Li B, Xu C, Zhang B, Zhang F, Dong D, Song W, Qiao G, Yang B: MicroRNA-1 downregulation by propranolol in a rat model of myocardial infarction: a new mechanism for ischaemic cardioprotection. Cardiovasc Res 2009;84:434-441.

2 Camerino DC, Desaphy JF, Tricarico D, Pierno S, Liantonio A: Therapeutic approaches to ion channel diseases. Adv Genet 2008;64:81-145.

3 Hagen A, Dietze A, Dhein S: Human cardiac gap-junction coupling: effects of antiarrhythmic peptide AAP10. Cardiovasc Res 2009;83:405-415.

4 Harmar AJ, Hills RA, Rosser EM, Jones M, Buneman OP, Dunbar DR, Greenhill SD, Hale VA, Sharman JL, Bonner TI, Catterall WA, Davenport AP, Delagrange P, Dollery CT, Foord SM, Gutman GA, Laudet V, Neubig RR, Ohlstein EH, Olsen RW, Peters J, Pin JP, Ruffolo RR, Searls DB, Wright MW, Spedding M: IUPHAR-DB: the IUPHAR database of G protein-coupled receptors and ion channels. Nucleic Acids Res 2009;37:D680-685.

5 Martin A, Ochagavia ME, Rabasa LC, Miranda J, Fernandez-de-Cossio J, Bringas R: BisoGenet: a new tool for gene network building, visualization and analysis. BMC Bioinformatics 2010;11:91.

6 Assenov Y, Ramírez F, Schelhorn SE, Lengauer T, Albrecht M: Computing topological parameters of biological networks. Bioinformatics 2008;24:282-284.

7 Liang H, Li WH: MicroRNA regulation of human protein protein interaction network. RNA 2007;13:1402-1408.

8 Naeem H, Küffner R, Csaba G, Zimmer R: miRSel: automated extraction of associations between microRNAs and genes from the biomedical literature. BMC Bioinformatics 2010;11:135.

9 Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T: miRecords: an integrated resource for microRNA-target interactions. Nucleic Acids Res 2009;37:D105-110.

10 Catalucci D, Gallo P, Condorelli G: MicroRNAs in cardiovascular biology and heart disease. Circ Cardiovasc Genet 2009;2:402-408.

11 Minamino T: Gap junctions mediate the spread of ischemia-reperfusion injury. Circ J 2009;73:1591-1592.

12 Diez D, Wheelock AM, Goto S, Haeggström JZ, Paulsson-Berne G, Hansson GK, Hedin U, Gabrielsen A, Wheelock CE: The use of network analyses for elucidating mechanisms in cardiovascular disease. Mol Biosyst 2010;6:289-304.

13 HUGO Gene Nomenclature Committee (HGNC) 2010 [http://www.gene-names.org].

14 Cline MS, Smoot M, Cerami E, Kuchinsky A, Landys N, Workman C, Christmas R, Avila-Campilo I, Creech M, Gross B, Hanspers K, Isserlin R, Kelley R, Killcoyne S, Lotia S, Maere S, Morris J, Ono K, Pavlovic V, Pico AR, Vailaya A, Wang PL, Adler A, Conklin BR, Hood L, Kuiper M, Sander C, Schmulevich I, Schwikowski B, Warner GJ, Ideker T, Bader GD: Integration of biological networks and gene expression data using Cytoscape. Nat Protoc 2007;2:2366-2382.

15 Camargo A, Azuaje F: Linking gene expression and functional network data in human heart failure. PLoS One 2007;2:e1347.

16 Yang B, Lin H, Xu C, Liu Y, Wang H, Han H, Wang Z: Choline produces cytoprotective effects against ischemic myocardial injuries: evidence for the role of cardiac m3 subtype muscarinic acetylcholine receptors. Cell Physiol Biochem 2005;16:163-174.

17 Zhao N, Sun Z, Mao Y, Hang P, Jiang X, Sun L, Zhao J, Du Z: Myocardial iron metabolism in the regulation of cardiovascular diseases in rats. Cell Physiol Biochem 2010;25:587-594.

18 Bostjancic E, Zidar N, Glavac D: MicroRNA microarray expression profiling in human myocardial infarction. Dis Markers 2009;27:255-268.

19 Maslov S, Sneppen K: Specificity and stability in topology of protein networks. Science 2002;296:910-913.

20 Brandes U: A faster algorithm for betweenness centrality. J Math Sociol 2001;25:163-177.

21 Du W, Huang J, Yao H, Zhou K, Duan B, Wang Y: Inhibition of TRPC6 degradation suppresses ischemic brain damage in rats. J Clin Invest 2010;120:3480-3492.

22 Lipski J, Park TI, Li D, Lee SC, Trevarton AJ, Chung KK, Freestone PS, Bai JZ: Involvement of TRP-like channels in the acute ischemic response of hippocampal CA1 neurons in brain slices. Brain Res 2006;1077:187-199.

23 Frost RJ, van Rooij E: miRNAs as therapeutic targets in ischemic heart disease. J Cardiovasc Transl Res 2010;3:280-289.

24 Makielski JC, Farley AL: Na^+ current in human ventricle: implications for sodium loading and homeostasis. J Cardiovasc Electrophysiol 2006;1:S15-S20.

25 Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G, Wang Z: The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. Nat Med 2007;13:486-491.

26 Yue P, Zhang Y, Du Z, Xiao J, Pan Z, Wang N, Yu H, Ma W, Qin H, Wang WH, Lin DH, Yang B: Ischemia impairs the association between connexin 43 and M3 subtype of acetylcholine muscarinic receptor (M3-mAChR) in ventricular myocytes. Cell Physiol Biochem 2006;17:129-136.

27 Zhang Y, Wang H, Kovacs A, Kanter EM, Yamada KA: Reduced expression of Cx43 attenuates ventricular remodeling after myocardial infarction via impaired TGF-beta signaling. Am J Physiol Heart Circ Physiol 2010;298:H477-487.

28 Decker KF, Rudy Y: Ionic mechanisms of electrophysiological heterogeneity and conduction block in the infarct border zone. Am J Physiol Heart Circ Physiol 2010;299:H1588-1597.

29 Liao P, Li G, Yu de J, Yong TF, Wang JJ, Wang J, Soong TW: Molecular alteration of Cav1.2 calcium channel in chronic myocardial infarction. Pflugers Arch 2009;458:701-711.

30 Luo X, Zhang H, Xiao J, Wang Z: Regulation of human cardiac ion channel genes by microRNAs: theoretical perspective and pathophysiological implications. Cell Physiol Biochem 2010;25:571-586.