Research Article

Xu Li, Dao-Kang Xiang*, Yi-Zhu Shu, Cheng-Hui Feng

Dysregulated pathways for off-pump coronary artery bypass grafting

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Abstract: *Background:* The objective of this paper was to identify dysregulated myocardial pathways with off-pump coronary artery bypass grafting (OPCABG) based on pathway interaction network (PIN). *Methodology:* To achieve this goal, firstly, gene expression profiles, protein-protein interactions (PPIs) and pathway data were collected. Secondly, we constructed a PIN by integrating these data and Pearson correlation coefficient (PCC) algorithm. Next, for every pathway in the PIN, its activity was counted dependent on the principal component analysis (PCA) method to select the seed pathway. Ultimately, a minimum pathway set (MPS) was extracted from the PIN on the basis of the seed pathway and the area under the receiver operating characteristics curve (AUROC) index, and pathways in the MPS were denoted as dysregulated pathways. *Results:* The PIN had 1,189 nodes and 22,756 interactions, of which mitochondrial translation termination was the seed pathway. Starting with mitochondrial translation termination, a MPS (AUROC = 0.983) with 7 nodes and 26 edges was obtained. The 7 pathways were regarded as dysregulated myocardial pathways with OPCABG. *Conclusion:* The findings might provide potential biomarkers to diagnose early, serve as the evidence to perform the OPCABG and predict inflammatory response and myocardial reperfusion injury after OPCABG in the future.

Keywords: myocardial, off-pump, data, seed pathway, pathway interaction network, dysregulated pathways

1 Introduction

Coronary artery bypass grafting (CABG) can decrease the mortality of extensive coronary artery patients, and even has been widely applied in cardiopulmonary bypass [1,2]. Recently, with an attempt to reduce the side complications after operations, off-pump CABG (OPCABG) has been proposed by researchers [3,4]. Besides, it has been demonstrated that abnormal regulations or expressions during the OPCABG procedure are correlated to inflammatory response and myocardial reperfusion injury [5]. However, there is rare research focus on these aspects for off-pump CABG (OPCABG) surgery. Hence detecting biomarkers related to OPCABG is an urgent task.

Generally, target biomarkers for a disease are often found by exploring differentially expressed genes (DEGs) compared with normal controls [6]. But studies show that DEGs identified from different reports are often inconsistent to one specific tumor [7]. In order to solve this problem, a network approach is produced to detect DEGs [8], such as protein-protein interaction (PPI) network. Meanwhile, pathway enrichment analysis could not only decrease the complexity to reveal gene set regulations but also increase the explanatory confidence of the study [9]. Hence we integrated pathway analysis and PPI network to construct a pathway interaction network (PIN) [10].

In the current study, we proposed to investigate dysregulated myocardial pathways with OPCABG in this work. The process was divided into four parts: collection of gene expression data, PPIs and pathway data; construction of a PIN by Pearson correlation coefficient (PCC) method; selection of seed pathway based on principal component analysis (PCA) method; and extraction of a minimum pathway set (MPS) by integrating seed pathway and the area under the receiver operating characteristics curve (AUROC) index. Pathways in the MPS were denoted as dysregulated pathways. These pathways might provide potential biomarkers for predicting inflammatory response or myocardial reperfusion injury in OPCABG.

*Corresponding author: Dao-Kang Xiang, Department of Cardiac Surgery, The People's Hospital of Guizhou, No.1 on Baoshan South Road, Guiyang, 550001, Guizhou Province, China, E-mail: daokangxiang2016@126.com

Xu Li, Yi-Zhu Shu, Cheng-Hui Feng, Department of Cardiac Surgery, Guizhou Provincial People's Hospital, Guiyang, 550001, Guizhou Province, China

2 Methods

2.1 Collecting data

Three kinds of data were prepared for this work, including gene expression data downloaded from ArrayExpress database, PPI data dependent on the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database and gene expression data, and pathway data by integrating Reactome database and gene expression data.

2.1.1 Gene expression data

In this paper, a gene expression dataset (E-GEOD-12486 [11]) for myocardial patients, which is deposited on A-AFFY-44 - Affymetrix GeneChip Human Genome U133 Plus 2.0 [HG-U133_Plus_2] Platform, was recruited from the ArrayExpress (<http://www.ebi.ac.uk/arrayexpress/>) database. E-GEOD-12486 collected myocardial samples, prior to and after grafting, from patients undergoing OPCABG with cardiopulmonary bypass and cardiac arrest. In detail, 5 samples prior to grafting were attributed to the control group, while 5 samples conducting OPCABG were defined as the experimental group or OPCABG group. After conducting standard pre-treatments [12,13], 20,545 genes were gained in total.

2.1.2 PPI data

The STRING (<https://string-db.org/>) database was utilized for acquiring all human PPIs [14]. A total of 16,730 genes and 787,896 interactions were obtained. For purpose of building correlations between these PPIs and myocardial patients, we removed interactions of score < 0.2. The retained interactions were interacted with the gene data; as a result, 449,833 interactions covering 14,917 genes were gained, named as PPI data.

2.1.3 Pathway data

In this paper, all human pathways were captured from the Reactome pathway (<http://www.reactome.org/>) database [15], from which we obtained 1,675 pathways. Next, the number of common genes between every pathway and the gene expression data was counted. Only pathways with the intersected amount distributed in the section of 5 ~ 100 were left, because too small or large sizes were inconvenient for researchers [16]. Finally, we explored 1,189 pathways, termed pathway data for the current work.

2.2 Constructing PIN

Utilizing gene expression profiles, PPIs and pathway data, we constructed a PIN for the OPCABG group, where a node was a pathway, and an edge represented the interaction between a pair of pathways. Importantly, there were two distinct and strict conditions for one edge, one condition requested that the pair of pathways must have at least one common gene, and further at least one of the common genes must belong to DEGs between the OPCABG group and control group. DEGs were identified using unpaired two-tailed Student's *t*-test, and the threshold was $P < 0.05$. Subsequently, the other condition was that genes from the two pathways based on PPIs were highly co-expressed ($|PCC| > 0.8$). To our knowledge, PCC is a widely utilized manner to count the correlation strength for two variables [17].

2.3 Selecting seed pathway

Before determining the seed pathway, the PCA method was employed to compute the activity score for every pathway in the PIN [18]. Specifically, all data were assembled to a matrix X with j samples ($j = 1, 2, \dots, J$) and k pathways ($k = 1, 2, \dots, K$). Hence each single parameter of X was referred to as x_{jk} and was assigned all vectors in the J -dimensional space, x_{jk} . The activity score S_{kj} of pathway k in sample j was counted according to the followed formula:

$$S_{kj} = w_{1jk}x_{1jk} + w_{2jk}x_{2jk} \cdots + w_{ijk}x_{ijk}$$

Where x_{ijk} represented the standardized expression value of gene i from pathway k in sample j , and w_{ijk} was the weight of x_{ijk} . In the present study, the pathway whose activity score had the greatest difference across OPCABG samples and controls was regarded as the seed pathway for OPCABG.

2.4 Investigating dysregulated pathways

Starting with the seed pathway, the minimum pathway set (MPS) was extracted from the PIN of OPCABG. In detail, the research process was repeatedly collecting pathways to increase the predicted accuracy maximally, and would be stopped if the accuracy decreased. The predicted accuracy was detected by AUROC implemented in support vector machines (SVM) [19]. High AUROC suggested good classification performance between the OPCABG group and control group. For purpose of achieving stable

outcomes and increasing the confidence of our results, all AUROC values were calculated 100 times. Finally, we took the average AUROC value as the final result.

Ethical approval: The conducted research is not related to either human or animals use.

3 Results

3.1 Data

In the present study, there were 20,545 genes, 449,833 interactions and 1,189 pathways in the gene expression data, PPI data and pathway data, respectively. In particular, the PPI data were the intersections between gene expression data and STRING PPI data, and the pathway data were extracted based on the Reactome pathway database and the gene expression data.

3.2 PIN

Utilizing Student's *t*-test, a total of 296 DEGs with $P < 0.05$ between the OPCABG group and control group were detected from the gene expression data. The top 100 DEGs in ascending order of *P* values are displayed in Table 1; especially *ADAMTS1* ($P = 8.26E-05$), *EGR1* ($P = 8.84E-05$), *CSRNP1* ($P = 1.93E-04$), *ZFP36* ($P = 2.72E-04$) and *ATF3* ($P = 2.80E-04$), the genes with the lowest *P* values. DEGs were prepared for choosing the interactions for constructing PIN, since only interactions in the PPI data that satisfied at least one of the two conditions were retained to construct the PIN. Additionally, genes in the two pathways were highly co-expressed ($|PCC| > 0.8$).

Ultimately, 455,124 pathway-pathway interactions were gained in total. Because the large scale of these interactions brought troubles and inconveniences for researchers, we removed the interactions with low $|PCC|$ scores, and adopted the top 5% of all for further study. The network made up of the top 5% was defined as the PIN for

Table 1. Top 100 differentially expressed genes (DEGs)

No.	DEG	No.	DEG	No.	DEG	No.	DEG
1	<i>ADAMTS1</i>	26	<i>BTG2</i>	51	<i>RBBP6</i>	76	<i>DNAJB1</i>
2	<i>EGR1</i>	27	<i>SLC2A3</i>	52	<i>CH25H</i>	77	<i>OTUD1</i>
3	<i>CSRNP1</i>	28	<i>MYC</i>	53	<i>ACVR1C</i>	78	<i>IL1RN</i>
4	<i>ZFP36</i>	29	<i>RASD1</i>	54	<i>HES1</i>	79	<i>PF4V1</i>
5	<i>ATF3</i>	30	<i>CXCR4</i>	55	<i>PTGS2</i>	80	<i>YRDC</i>
6	<i>MAFF</i>	31	<i>DUSP2</i>	56	<i>KLF6</i>	81	<i>MEGF10</i>
7	<i>CEBPD</i>	32	<i>CCL2</i>	57	<i>S100A9</i>	82	<i>GJA4</i>
8	<i>EGR2</i>	33	<i>ZNF331</i>	58	<i>SEMG2</i>	83	<i>THEMIS2</i>
9	<i>S100A8</i>	34	<i>S100P</i>	59	<i>SELE</i>	84	<i>KCNJ10</i>
10	<i>NR4A3</i>	35	<i>JUN</i>	60	<i>RGS16</i>	85	<i>ZKSCAN1</i>
11	<i>IER5</i>	36	<i>NR4A2</i>	61	<i>GADD45B</i>	86	<i>PMAIP1</i>
12	<i>SOCS3</i>	37	<i>TTC30A</i>	62	<i>FOXF1</i>	87	<i>FOSB</i>
13	<i>CXCL3</i>	38	<i>SGK1</i>	63	<i>FOS</i>	88	<i>FPR2</i>
14	<i>NR4A1</i>	39	<i>S100A12</i>	64	<i>STC1</i>	89	<i>LRRC32</i>
15	<i>CCL4</i>	40	<i>CXCL8</i>	65	<i>GPR183</i>	90	<i>CCDC42</i>
16	<i>SERTAD1</i>	41	<i>USP27X</i>	66	<i>C11orf96</i>	91	<i>IBA57-AS1</i>
17	<i>KLF4</i>	42	<i>CCNL1</i>	67	<i>HAS1</i>	92	<i>DDIT3</i>
18	<i>FOSL2</i>	43	<i>APOLD1</i>	68	<i>NFKBIZ</i>	93	<i>CD69</i>
19	<i>NEDD9</i>	44	<i>CD83</i>	69	<i>CHST2</i>	94	<i>NEBL</i>
20	<i>BHLHE40</i>	45	<i>DUSP1</i>	70	<i>GAS5</i>	95	<i>PIGS</i>
21	<i>CYR61</i>	46	<i>PLAUR</i>	71	<i>CDKN1A</i>	96	<i>LINC01538</i>
22	<i>JUNB</i>	47	<i>CCL8</i>	72	<i>CREG2</i>	97	<i>TMEM208</i>
23	<i>EGR3</i>	48	<i>UNC119B</i>	73	<i>MYH13</i>	98	<i>LOC100507468</i>
24	<i>IER2</i>	49	<i>TTL11-IT1</i>	74	<i>LINC00958</i>	99	<i>PLIN1</i>
25	<i>SFT2D3</i>	50	<i>IL6</i>	75	<i>MMP9</i>	100	<i>RNASEH2C</i>

OPCABG myocardial samples, as shown in Figure 1. The PIN possessed 22,756 interactions and 1,189 pathways. Besides, we could find that pathways connected with each other, but the strengths were different due to the differences of their weights. The weight value for a pathway-pathway interaction was defined as its total |PCC| scores of all genes, and interaction of higher weight value might be more significant for OPCABG group than the others. Figure 2 illustrates the specific weight distributions among 22,756 interactions. It uncovered that the weights of 18,997 interactions ranged from 50 to 150, whereas only 402 interactions had weights > 350. Hence we may infer that the function and property of a pathway was greatly different from those of others although they occurred in the same network.

Figure 1. Pathway interaction network (PIN) for off-pump coronary artery bypass grafting (OPCABG). Nodes represent pathways, and edges are the interaction among any two pathways.

Figure 2. Distributions of weights in pathway interaction network (PIN) [inset: zoom into (350, 650)].

3.3 Seed pathway

Since clear distinctions existed for pathways in the PIN, how to evaluate the significance of each node and select the significant node in the PIN became another challenge. We assigned an activity score to every pathway based on the PCA method to assess its significance. The pathway whose activity score had the greatest change between OPCABG samples and controls was regarded as the seed pathway. In this research, the seed pathway was mitochondrial translation termination.

3.4 Dysregulated pathways

In the current study, we obtained one MPS with AUROC = 0.983, which indicated that the MPS had a good prediction accuracy and classification performance between the OPCABG group and control group. Pathways in the MPS were denoted as dysregulated pathways whose network was described in Figure 3. A total of 7 dysregulated pathways including the seed pathway were obtained for OPCABG myocardial patients, and interacted with each other forming 26 interactions. The 7 dysregulated pathways were mitochondrial translation termination (number of genes = 82), mitochondrial translation (number of genes = 88), cyclin A:Cdk2-associated events at S phase entry (number of genes = 64), dectin-1 mediated noncanonical NF- κ B signaling (number of genes = 58), insulin receptor signaling cascade (number of genes = 92), IRS-related events (number of genes = 88), and MyD88-independent TLR3/TLR4 cascade (number of genes = 95) (Table 2). In detail, mitochondrial translation termination was the seed pathway, and MyD88-independent TLR3/TLR4 cascade was composed of the most genes.

4 Discussion

Present pathway researches generally pay attention to abnormal activities of a single pathway, and ignore that there might exist interactions among them [20]. Hence we proposed to construct a PIN for the correlated pathways. Normally, the huge amount of genes and edges in the global network would make explaining them a challenge, and thus identifying sub-networks of the complex network is a good choice to reveal molecular mechanisms of one disease [21,22]. Hence detecting the MPS from the PIN was regarded as the optimal manner to classify OPCABG group from control group.

Figure 3. Dysregulated pathways interaction network for off-pump coronary artery bypass grafting (OPCABG). Nodes represent pathways, and edges are the interaction among any two pathways. The red node was the seed pathway.

Table 2. Dysregulated pathways

No.	Pathway	Number of genes
1	Mitochondrial translation termination	82
2	Mitochondrial translation	88
3	Cyclin A:Cdk2-associated events at S phase entry	64
4	Dectin-1 mediated noncanonical NF-kB signaling	58
5	Insulin receptor signalling cascade	92
6	IRS-related events	88
7	MyD88-independent TLR3/TLR4 cascade	95

In this paper, we obtained a PIN with 1,189 nodes and 22,756 edges by integrating gene expression data, PPIs, pathway data and PCC related analyses. After counting the activity score for each pathway based on the PCA method, mitochondrial translation termination was selected as the seed pathway. Finally, a MPS (AUROC = 0.983) with 7 dysregulated pathways and 26 interactions was gained. The 7 dysregulated pathways, such as mitochondrial translation termination, mitochondrial translation and cyclin A:Cdk2-associated events at S phase entry, might play more significant roles in OPCABG group and be potential biomarkers for the progression. Among the 7 dysregulated pathways in the MPS, 2 (mitochondrial translation termination and mitochondrial translation) were correlated with mitochondrial functions and activities. Interestingly, 2 (dectin-1 mediated noncanonical

NF-kB signaling and insulin receptor signaling cascade) of the 7 dysregulated pathways belonged to signaling pathways. Taking mitochondrial translation termination and mitochondrial translation as examples, mitochondrial translation is a ribosome-mediated process where the information of mRNA is applied to display the sequence of amino acids in the protein [23]. When the mitochondrial release factor (mtRF1a) recognizes the stop codon and binds to the mitoribosome, mitochondrial translation termination is conducted [24]. Previous studies suggested inhibition or defects of mitochondrial translation were correlated to acute myeloid leukemia [25] and hypertrophic cardiomyopathy [26]. Meanwhile, dysregulations of mitochondria are usually correlated to multiple malignant diseases [27]. For instance, Zhang et al. demonstrated

that mitochondrial damage played a significant role in mediating cardiac injury and decreased EF-Tumt expression mediates oxidative damage in postburn myocardium [28]. Therefore, we inferred that abnormal expression of mitochondrial translation termination and mitochondrial translation might lead to the abnormal occurrences in the OPCABG group.

In conclusion, we have investigated 7 dysregulated myocardial pathways (such as mitochondrial translation termination, mitochondrial translation and cyclin A:Cdk2-associated events at S phase entry) with OPCABG utilizing PIN. These dysregulated pathways could be regarded as potential biomarkers to diagnose earlier and predict inflammatory response and myocardial reperfusion injury after OPCABG. However, the specific co-operated genes among dysregulated pathways still remain unclear, and thus further investigations are indispensable. Particularly, the present study only covers pure bioinformatics analyses, and lacks experimental or clinical validations. Hence, in the future, great efforts should be made to convert the theoretical results into clinical practice.

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