Pathway-related modules involved in the application of sevoflurane or propofol in off-pump coronary artery bypass graft surgery

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Abstract. Off-pump coronary artery bypass graft (CABG) surgery has recently emerged as a means to avoid the sequelae of extracorporeal circulation, including the whole-body inflammatory response, coagulation disorders and multiple organ dysfunction. At present, gas anesthesia, sevoflurane and intravenous anesthesia and propofol have been widely used during the CABG. To further understand the underlying mechanisms of these anesthetics on the gene level, the present study conducted pathway-related module analysis based on a co-expression network. This was performed in order to identify significant pathways in coronary artery disease patients who had undergone off-pump CABG surgery before and after applying sevoflurane or propofol. A total of 269 and 129 differentially expressed genes were obtained in the sevoflurane and propofol groups, respectively. In total, eight and seven pathways (P<0.05) in the sevoflurane and propofol groups were separately obtained via Kyoto Encyclopedia of Genes and Genome pathway analysis. Finally, eight and seven pathway-related modules in the sevoflurane and propofol groups were obtained, respectively. Furthermore, the mean degree of complement and coagulation cascades pathway-related module in both of the groups was the highest. It was predicted that during the CABG, the anesthetics might activate the complement and coagulation systems in order to possess some cardioprotective properties.

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Introduction

Cardiovascular diseases (CVDs) account for >17 million cases of mortality globally and annually (30% of all mortality), 80% of which occur in low- and middle-income countries, and this figure is expected to grow to 23.6 million by 2030 (1). Coronary artery disease (CAD) is the largest contributor to CVDs (2). Off-pump coronary artery bypass graft (CABG) surgery emerged in recent years as a means to avoid the sequelae of extracorporeal circulation such as whole-body inflammatory response, coagulation disorders, and multiple organ dysfunction (3). At present, gas anesthesia-sevoflurane and intravenous anesthesia-propofol have been widely used during the CABG (4).

Propofol and sevoflurane both possess certain, although different, cardioprotective properties. Sevoflurane appeared to be superior to propofol in patients with little or no ischemic heart disease, including CABG surgery without severe preoperative ischemia (5). However, propofol appeared superior in patients with cardiovascular instability, severe ischemia or acute/urgent surgery (6). Over the past decades, numerous experimental strategies (association studies, genome-wide linkage scan, proteomics and global microarray gene expression analysis amongst others and large efforts have been applied onto the studies (7,8). Lucchinetti *et al* (9) performed direct comparisons between anesthetic gases and intravenous anesthetics in human hearts at the gene expression level. These results indicated that anesthetic-induced and constitutive gene regulatory control of myocardial substrate metabolism predicts postoperative cardiac function in patients undergoing off-pump CABG surgery. However, the underlying mechanisms of these anesthetics on the gene level remain unclear.

Previous studies have established a constructed gene co-expression network, which contained genes that exhibited similar expression patterns across different organisms (10,11). It has also been demonstrated that functionally related genes were frequently co-expressed across organisms constituting conserved transcription modules (12). By constructing a co-expression network, the underlying regulatory relationships under different conditions may be estimated (13). In order to

define the adjacency matrix, one makes use of an adjacency function, which transforms the co-expression similarities into connection strengths (14). The node dissimilarity measure is used as input of a clustering method to define network modules (clusters of nodes) (15). Furthermore, modules are groups of genes whose expression profiles are highly correlated across the samples (16). Network modules implement the hypothesis that a network can be divided into functional modules (17). In this case, significant interactions, such as key genes in significant pathways can be tested. Therefore, in the present study modules from the co-expression network based on genes enriched in significant pathways were identified, and these modules were defined as pathway-related modules.

The present study aimed to identify changed pathway-related modules in CAD patients undergoing CABG under sevoflurane or propofol anesthesia based on network topological centralities. In order to achieve this, recruitment and preprocessing of the gene expression profile was initially conducted, and differentially expressed genes (DEGs) in CAD patients were identified before and after applying sevoflurane or propofol, respectively. Next, pathway analysis of the DEGs was performed using the Kyoto Encyclopedia of Genes and Genomes database. A co-expression network was constructed by weighted gene co-expression network analysis (WGCNA), and pathway-related modules were mined. Finally, significant pathway-related modules were identified by conducting analysis on the topological centralities of the co-expression network, in order to further understand the underlying mechanisms of these anesthetics on the CAD patients during the CABG process according to systematically analyzing the pathway-related modules of the co-expression network.

Materials and methods

Data recruitment and preprocessing. The gene expression profile of E-GEOD-4386 was obtained from the ArrayExpress database (http://www.ebi.ac.uk/arrayexpress/). E-GEOD-4386 existed on the A-AFFY-44-Affymetrix GeneChip Human Genome U133 Plus 2.0 Platform. The data were obtained from patients that had undergone off-pump CABG surgery, and they were allocated either to receive the anesthetic gas sevoflurane or the intravenous anesthetic propofol. The samples were then divided into two groups: Baseline sevoflurane (n=10)-sevoflurane (n=10) and baseline propofol (n=10)-propofol (n=10) (9). Furthermore, the microarray data and annotation files were downloaded for further analysis.

Background-corrected signal intensities were determined using the Micro Array Suite 5.0 (MAS 5.0) software (Affymetrix, Inc., Santa Clara, CA, USA) (18). The normalization of datasets obtained from the ArrayExpress database was performed using a robust multichip average method (19) and quantile based algorithm (20). Meanwhile, the gene expression value was transformed to a comparable level. Additionally, a gene-filter package was used to screen the data. Each probe was mapped to one gene, and the probe was discarded if it did not match any genes. Furthermore, the expression value averaged over probes was used as the gene expression value if the gene had multiple probes, and 20,102 genes were obtained overall.

Identification of DEGs. The linear model for microarray data (LIMMA) package, a core component of Bioconductor, is an R-based open-source software development project in statistical genomics (21). A core capability is the use of linear models to assess differential expression in the context of multi-factor designed experiments. In the present study, for genes with scores greater than an adjustable threshold, empirical Bayes method that was implemented in the package (22) was used to identify DEGs in the sevoflurane and propofol groups, respectively. Furthermore, the false discovery rate was used to proofread the P-values. Values of [log Fold Change (FC)] >2.0 and P<0.01 were selected as the cut-off criteria.

Functional enrichment analysis of DEGs. KEGG pathway database is a recognized and comprehensive database including all types of biochemical pathways (23). In the present study, the KEGG database was applied to investigate the enrichment analysis of the nodes in order to find the biochemical pathways of DEGs that were involved in patients that had undergone off-pump CABG surgery before and after applying sevoflurane or propofol. The Database for Annotation, Visualization and Integrated Discovery (DAVID) (24) was used to perform the KEGG pathway enrichment analysis with the P<0.05 and gene count >5.

Co-expression network analysis
Identifying differential co-expression by WGCNA. Gene co-expression networks, which represent a major application of correlation network methodology, are instrumental for describing the pair-wise relationships among gene transcripts and facilitate the understanding of their function and identification of their key players (25,26). WGCNA, as a statistical approach based on correlations, has been widely used to analyze transcriptional profiles, and has proved to be an informative approach for the functional annotation of uncharacterized genes (27). A coefficient of variation (CV=μ/σ) filtering was applied to remove genes that were constitutively expressed, unexpressed or vary only modestly across experimental treatments or conditions (28). In this study, a CV cutoff value of 0.6 was selected to obtain co-expression interactions.

Co-expression network construction. Cytoscape provides an environment for the visualization and analysis of networks and associated annotations (29). The primary audience for Cytoscape is the biological community, and it supports a number of standard use cases for analyzing and visualizing biological data (30). In the present study, the co-expression network was constructed using Cytoscape version 3.1.0. Meanwhile, the expression values of each node were mapped to the co-expression network, where different colors represent the differences in the expression value of the nodes.

Pathway-related module mining and topological analysis. In the present study, pathway-related modules were extracted from a co-expression network in order to investigate significant genes and modules that played key roles in patients undergoing off-pump CABG surgery before and after applying sevoflurane or propofol. To achieve this, firstly, genes in each significant pathway of the two groups were explored and mapped into the co-expression network. Next, pathway genes in the network

Table I. KEGG pathway of DEGs in the sevoflurane group.

ID	Term	Count	P-value
hsa04621	NOD-like receptor signaling pathway	11	7.39×10^{-8}
hsa04060	Cytokine-cytokine receptor interaction	17	7.92×10^{-6}
hsa04610	Complement and coagulation cascades	9	2.06×10^{-5}
hsa05219	Bladder cancer	5	5.68×10^{-3}
hsa04010	MAPK signaling pathway	12	5.92×10^{-3}
hsa04115	p53 signaling pathway	6	6.13×10^{-3}
hsa04062	Chemokine signaling pathway	9	1.50×10^{-2}
hsa04630	Jak-STAT signaling pathway	8	1.72×10^{-2}

KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially expressed genes; NOD, nucleotide-binding oligomerization domain; MAPK, mitogen-activated protein kinase; STAT, Janus kinase/signal transducers and activators of transcription.

Table II. KEGG pathway of DEGs in the propofol group.

ID	Term	Count	P-value
hsa04621	NOD-like receptor signaling pathway	7	4.68×10^{-5}
hsa04060	Cytokine-cytokine receptor interaction	11	4.14×10^{-4}
hsa04010	MAPK signaling pathway	10	2.00×10^{-3}
hsa04115	p53 signaling pathway	5	5.98×10^{-3}
hsa05219	Bladder cancer	4	1.02×10^{-2}
hsa05120	Epithelial cell signaling in *Helicobacter pylori* infection	4	3.67×10^{-2}
hsa04610	Complement and coagulation cascades	4	3.80×10^{-2}

NOD, nucleotide-binding oligomerization domain; MAPK, mitogen-activated protein kinase.

and their adjacent genes were captured to form a sub-network, which were also called pathway-related modules. Finally, module topological analysis (the mean degree centrality of genes in the corresponding module) was conducted to evaluate significant pathway-related modules.

Results

Identifying DEGs. After having preprocessed the profile, the empirical Bayes method (F test) that was implemented in the LIMMA package was used to identify DEGs in the sevoflurane and propofol group. Under this condition, when the threshold values of [log(FC)]>2.0 and P<0.01 were set, a total of 269 DEGs were obtained in the sevoflurane group and a total of 129 DEGs in the propofol group.

KEGG pathway analysis of the DEGs. Based on human genomes, DAVID for KEGG pathway enrichment analysis was performed to further investigate the biological functions of the DEGs. When the threshold of P-value was set to 0.05, eight significant pathways in the sevoflurane (Table I) and seven in the propofol (Table II) groups were obtained.

It was evident that nucleotide-binding oligomerization domain (NOD)-like receptor signaling pathway, cytokine-cytokine receptor interaction, complement and coagulation cascades, mitogen-activated protein kinase

and p53 signaling pathways were enriched in both groups. While the chemokine and Janus kinase/signal transducers and activators of transcription signaling pathways were only enriched in the sevoflurane group, epithelial cell signaling in *Helicobacter pylori* infection was only enriched in the propofol group.

Co-expression network construction and topological analysis. After having identified the DEGs, co-expression analysis was conducted on these 269 DEGs in the sevoflurane group and 129 DEGs in the propofol group using the WGCNA method. By setting a threshold CV cutoff value of 0.6, 813 (180 DEGs) and 1,216 (119 DEGs) co-expression interactions were obtained in two groups. Furthermore, two co-expression networks were obtained via a conducting network with the co-expression interactions in Cytoscape Version 3.1.0. Additionally, as we mapped the expression values of each node to the co-expression network separately, two networks with expression values were obtained (Figs. 1 and 2). It was evident that all of the pathway genes were in green ([log(FC)]>2.0), which meant that these genes were all upregulated.

Pathway-related module mining and topological analysis. After having separately investigated genes in each significant pathway of the two groups, and having mapped them into the co-expression network, eight and seven pathway-related
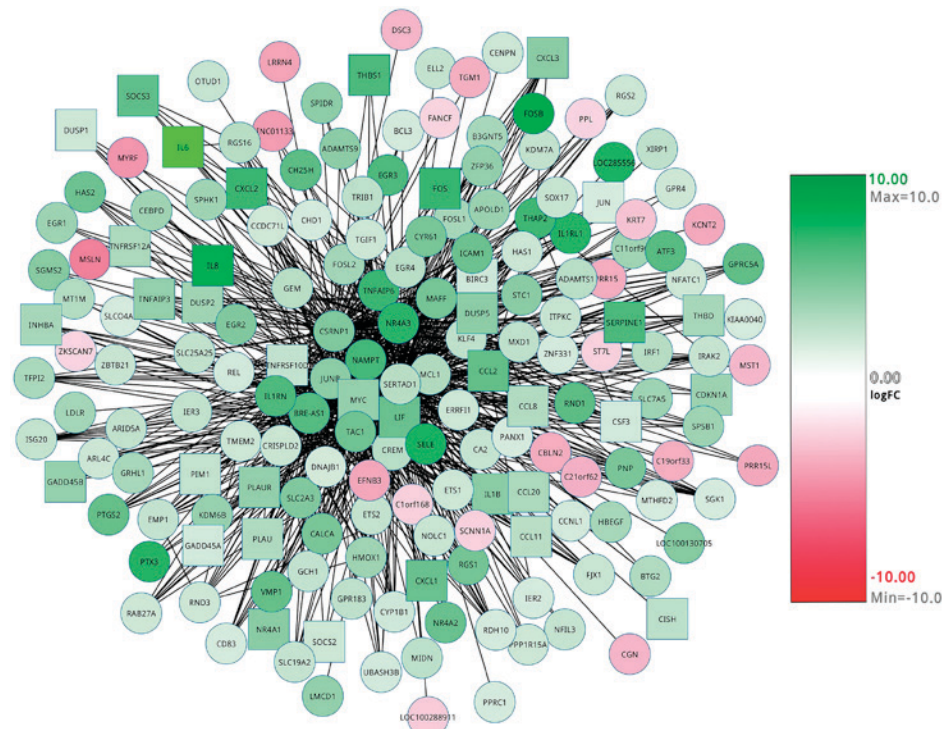
Figure 1. Co-expression network of DEGs in the sevoflurane group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The round nodes only consisted of DEGs whereas the square nodes consisted of DEGs and also other genes present in the pathways (P<0.05). DEG, differentially expressed genes; FC, fold change.
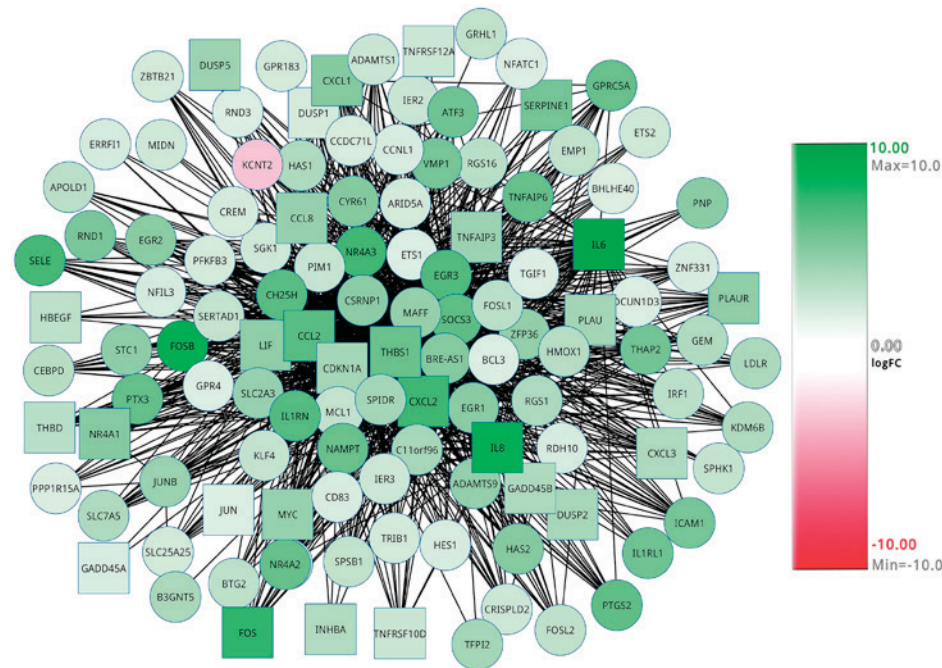

Figure 2. Co-expression network of DEGs in the propofol group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The round nodes only consisted of DEGs whereas the square nodes consisted of DEGs and also other genes present in the pathways (P<0.05). DEG, differentially expressed genes; FC, fold change.

modules were obtained in the sevoflurane group (Figs. 3 and 4) and in the propofol group (Figs. 5 and 6), respectively. To further investigate the biological functions of these modules, degree centrality analysis was performed, the details of which are shown in Table III (sevoflurane group) and Table IV (propofol group). The mean degree of modules of complement and coagulation cascades related, p53 signaling related, NOD-like receptor signaling related and cytokine-cytokine receptor interaction related were >20 in both of the groups. Moreover, the complement and coagulation cascades pathway-related
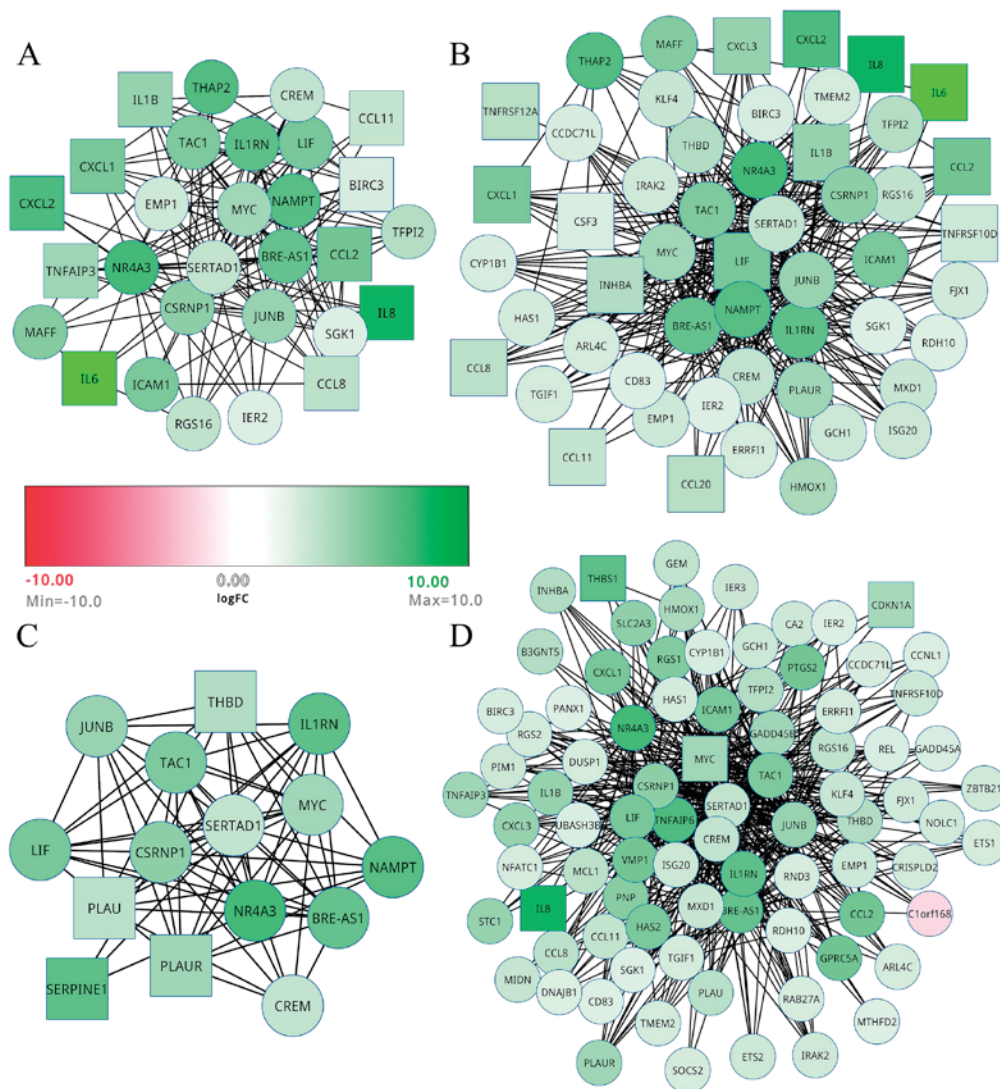
Figure 3. Pathway-related modules in the sevoflurane group. (A-D) Respectively represent the pathway-related modules of pathways 1-4 in the sevoflurane group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The square nodes represented pathway genes. FC, fold change.

module revealed the highest mean degree in both groups, which in the sevoflurane group had a mean degree of 56.67, and in the propofol group a mean degree of 71.88. However, the chemokine signaling pathway-related module only existed in the sevoflurane group with a mean degree of 36.92, and epithelial cell signaling in *H. pylori* infection pathway-related module only existed in the propofol group with a mean degree of 35.98.

By conducting analysis on the frequency of genes contained in the pathway-related modules, it was identified that there were several genes that presented in more than one module in both of the groups, as presented in Table V (sevoflurane group) and Table VI (propofol group). Furthermore, it was evident that in the sevoflurane group, genes IL8, CXCL2, CCL2, IL6, IL1B, CXCL1, CCL11 and MYC had a frequency >3 and in the propofol group, genes IL8 and CXCL1 had a frequency >3. In addition, genes IL8, CXCL2, CCL2, CXCL1 and CCL11 were all enriched in the chemokine signaling pathway-related module of the sevoflurane group, and genes IL8, CXCL1 were all enriched in epithelial cell signaling in

H. pylori infection pathway-related module of the propofol group.

Discussion

In the present study, an analysis on the gene profiles of patients who had undergone off-pump CABG surgery before and after applying sevoflurane or propofol was conducted based on a pathway-related module associated co-expression network. Since the co-expression network that separately mapped all of the genes present in the significant pathways was analyzed, it was shown that the mean degree of several modules (complement and coagulation cascades pathway-related module, p53 signaling pathway-related module, NOD-like receptor signaling pathway-related module and cytokine-cytokine receptor interaction pathway-related module) were >20 in both of the groups, and the mean degree of complement and coagulation cascades pathway-related module in both of the groups were the highest. However, a chemokine signaling pathway-related module only existed in the sevoflurane group with a mean degree of 36.92, and
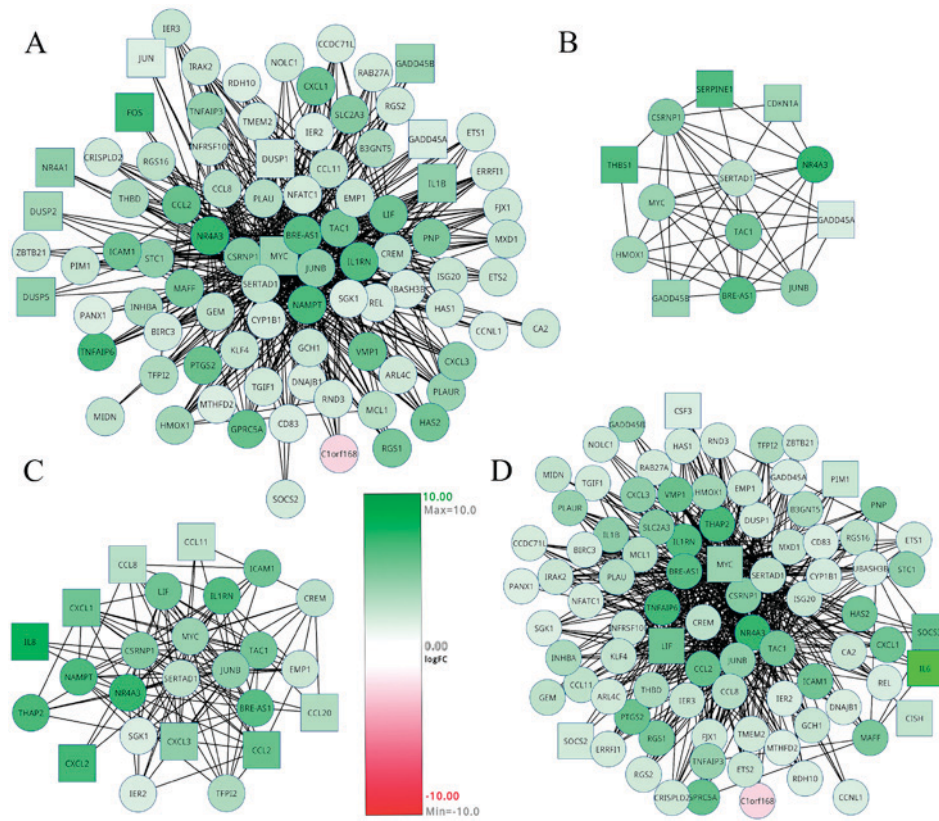
Figure 4. Pathway-related modules in the sevoflurane group. (A-D) Respectively represent the pathway-related modules of pathway 5-8 in the sevoflurane group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The square nodes represented the pathway genes. FC, fold change.
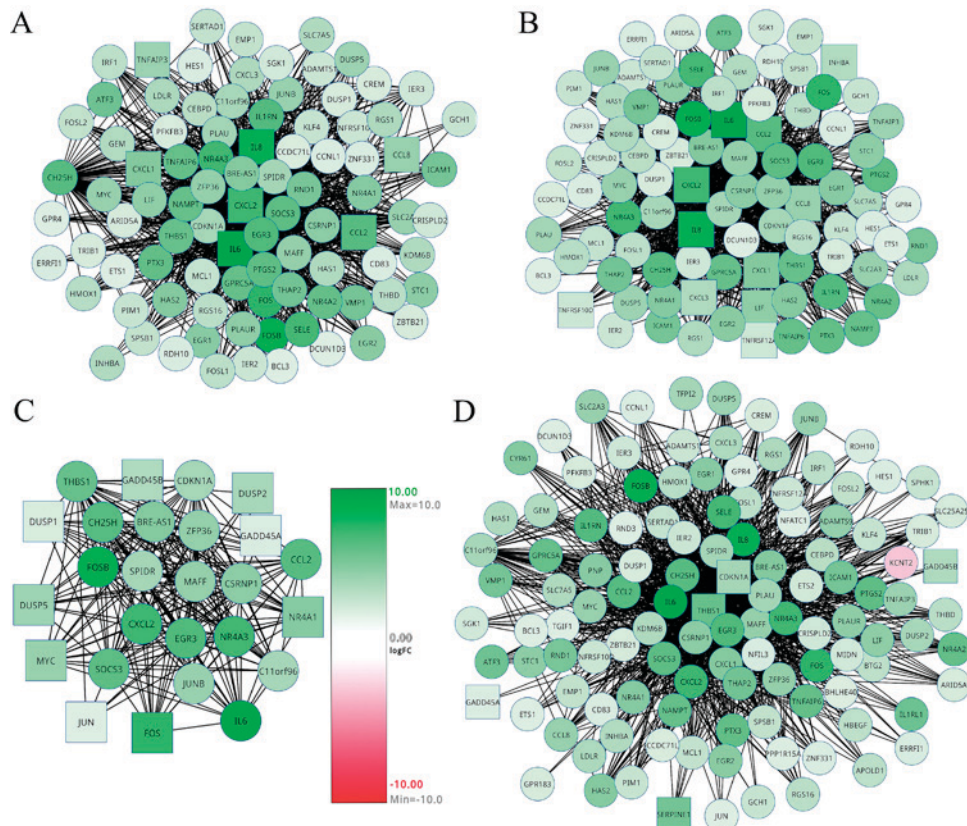

Figure 5. Pathway-related modules in the propofol group. (A-D) Respectively represent the pathway-related modules of pathway 1-4 in the propofol group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The square nodes represented pathway genes. FC, fold change.
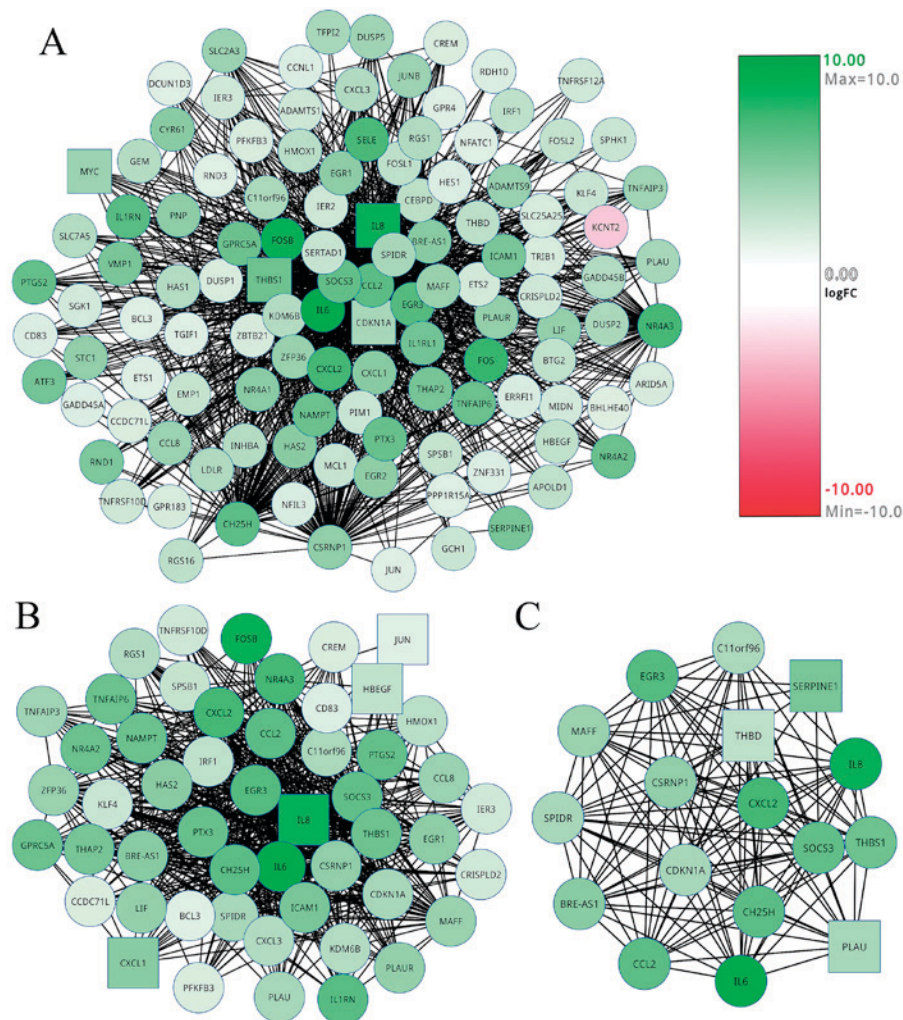
Figure 6. Pathway-related modules in the propofol group. (A-C) Respectively represents the pathway-related modules of pathway 5-7 in the propofol group. The color represented the expression value of the genes, where dark red represented the minimum (logFC=-10) and dark green the maximum (logFC=10). The square nodes represented pathway genes. FC, fold change.

epithelial cell signaling in *H. pylori* infection pathway-related module only existed in the propofol group with a mean degree of 35.98.

The complement and coagulation systems were described as separate cascades and as descendants of a common ancestral pathway. Both proteolytic cascades were composed of serine proteases with common structural characteristics, including highly conserved catalytic sites of histidine, aspartate and serine (31,32). Furthermore, both systems belonged to a complex inflammatory network (33) and exhibited some similar characteristics with regard to the specialized functions of their inhibitors and activators (34). It was indicated that cardiac surgery with cardiopulmonary bypass gives rise to a systemic inflammatory reaction, caused by the extracorporeal circuit and surgical trauma (35), which generated activation of the complement, fibrinolytic, kallikrein and coagulation cascades, activation of leukocytes and endothelial cells with expression of adhesion molecules and the release of inflammatory mediators, such as cytokines (36). Pathways of cytokine-cytokine receptor interaction were at the top of an enriched pathway list in an CAD gene KEGG pathway analysis (37).

Cytokines represent a diverse group of molecules that transmit intercellular signals. These signals may be paracrine or autocrine. Both of these situations could occur simultaneously (38). In addition, injured endothelial cells produce cytokines (including interleukin) that stimulate the expression of adhesion proteins selectins and cell adhesion molecules including vascular cell adhesion and intercellular adhesion molecules on the endothelial surface (39). It had been concluded that among apparently healthy women and men, elevated levels of IL8 are associated with an increased risk of CAD (40). There has also been increasing recognition that in various pathological conditions, CB1 receptor activation by endocannabinoids may promote activation of signaling pathways promoting cell death (41).

The chemokines are a family of low-molecular-weight proteins involved in leukocyte activation and migration (42). Significant advances have been made in understanding the role of chemokines and their receptors in cardiovascular diseases (43) tumor growth as well as metastasis (44) and inflammatory diseases (45). It has been indicated that the molecular mechanisms responsible for monocyte accumulation in plaque of atherosclerosis are likely to include chemokines and their receptors, as these molecules were major regulators of specific leukocyte trafficking (46). In the present study, the chemokine signaling pathway-related module only existed

Table III. Mean degree centrality of pathway-related modules in the sevoflurane group.

Pathway-term	Degree
Complement and coagulation cascades	56.67
p53 signaling pathway	55.69
Chemokine signaling pathway	36.92
NOD-like receptor signaling pathway	33.31
Cytokine-cytokine receptor interaction	22.29
MAPK signaling pathway	15.67
Bladder cancer	15.64
Jak-STAT signaling pathway	15.52

NOD, nucleotide-binding oligomerization domain; MAPK, mitogen-activated protein kinase; STAT, Janus kinase/signal transducers and activators of transcription.

Table IV. Mean degree centrality of pathway-related modules in the propofol group.

Pathway Term	Degree
Complement and coagulation cascades	71.88
MAPK signaling pathway	53.46
Epithelial cell signaling in *Helicobacter pylori* infection	35.98
NOD-like receptor signaling pathway	25.01
Cytokine-cytokine receptor interaction	24.79
p53 signaling pathway	20.77
Bladder cancer	20.76

MAPK, mitogen-activated protein kinase; NOD, nucleotide-binding oligomerization domain.

Table V. Frequency of genes that are in the pathway-related modulus of the sevoflurane group.

Symbol	No.
IL8	4
CXCL2	3
CCL2	3
IL6	3
IL1B	3
CXCL1	3
CCL11	3
MYC	3
CCL8	2
CXCL3	2
CCL20	2
CSF3	2
LIF	2
SERPINE1	2
CDKN1A	2
THBS1	2
GADD45A	2
GADD45B	2
BIRC3	1
TNFAIP3	1
INHBA	1
TNFRSF12A	1
TNFRSF10D	1
PLAUR	1
SOCS2	1
CISH	1
THBD	1
PLAU	1
DUSP1	1
NR4A1	1
FOS	1
DUSP2	1
JUN	1
DUSP5	1
CDKN1A	1
CXCL2	1
SOCS3	1
PIM1	1

in the sevoflurane group, which meant that this anesthetic might give the patients with more protection in hematopoiesis, angiogenesis, metastasis and tumor rejection or inflammatory diseases. However, it has also been reported that epithelial cell signaling in *H. pylori* infection was mainly associated with peptic ulceration, chronic gastritis and more rarely with gastric adenocarcinoma (47).

In conclusion, in the present study, complement and coagulation cascade related modules were successfully identified to be significant in both groups of sevoflurane and propofol, which meant that during the CABG, these anesthetics might activate the complement and coagulation systems so as to exert some cardioprotective properties. While the chemokine signaling pathway-related module only existed in the sevoflurane group, which meant that this anesthetic might provide the patients with more protection in hematopoiesis, angiogenesis, metastasis and tumor rejection or inflammatory diseases.

However, there remained certain limitations in the present study that must be taken into account. First of all, the sample size was not large enough. Additionally, an experimental verification analysis should be conducted in order to verify the

results obtained by the bioinformatics method used in the present study. Although disadvantages existed, it is believed that this method and the results offered investigators valuable resources for better understanding the underlying mechanisms of sevoflurane and propofol of the CAD patients who undergo CABG on the pathway level.

Table VI. Frequency of genes that are in the pathway-related modulus of the propofol group.

Symbol	No.
IL8	4
CXCL1	3
IL5	2
CXCL2	2
CCL2	2
CCL8	2
GADD45A	2
GADD45B	2
MYC	2
DUSP2	2
JUN	2
SERPINE1	2
THBS1	2
CDKN1A	2
TNFAIP3	1
LIF	1
TNFRSF10D	1
INHBA	1
TNFRSF12A	1
CXCL3	1
DUSP5	1
DUSP1	1
NR4A1	1
FOS	1
HBEGF	1
THBD	1
PLAU	1
PLAUR	1

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