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Screening of genes related to acute coronary syndrome and identification of functional modules in the PPI network

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Abstract. – BACKGROUND: Acute coronary syndrome (ACS), referring to any group of symptoms attributed to obstruction of the coronary arteries, affects millions of patients annually and requires immediate diagnosis and therapy.

OBJECTIVE: The purpose of this study was to find more biomarkers through identifying the genes which are related to ACS with samples from normal and diseased blood vessel.

MATERIALS AND METHODS: We downloaded the gene expression profile of GSE19339 from GEO (Gene Expression Omnibus) database, including 4 samples for normal and 4 for ACS. Then, the preprocessing of the data and analysis of differentially expressed genes (DEGs) were conducted with R language. WebGestalt was used to analyze the functions of the DEGs and STRING was applied to build the protein-protein interaction (PPI) network. At last, we used two plugins of Cytoscape to map the functional modules on the base of the PPI network.

RESULTS: A total of 480 genes were identified as DEGs between normal and disease samples, which were most significantly related to blood vessel development. After the partition of the PPI network, we got two functional modules, and CD, CXCL, CCL and ICAM1 genes were found served as the nodes of the two modules. These genes were all related to the immune response system.

CONCLUSIONS: Our present findings suggest that CD, CXCL, CCL and IL genes may be used as biomarkers in the research of ACS and the immune response system may also play an important role in the pathogenesis of ACS. However, further experiments are still needed to confirm our result.

Key Words:

Differentially expressed genes, ACS, Functional analysis, Protein-protein interaction network.

Introduction

Acute coronary syndrome (ACS) encompasses a range of conditions ranging from non–ST-segment elevation myocardial infarction (NSTEMI, ST segment elevation generally absent) to ST-segment elevation myocardial infarction (STEMI, persistent ST segment elevation usually present) and unstable angina. Although with diverse clinical syndromes, it is mainly caused by reduced blood flow to the heart due to an arterial blockage[1].

In recent years, some biomarkers of ACS have been identified. Falcone et al[2] have reported that low levels of eotaxin-3 can be used as an independent predictor of patients with ACS and may be useful for risk stratification. Hayashidani et al[3] report that the inhibition of MCP-1 signaling can improve the survival of ACS. AREG (amphiregulin) is found significantly differently expressed between ACS and healthy persons by Silbiger et al[4]. However, these several articles only concerned single genes with abnormal expression, then got results lacking of enough evidence about its effects in the whole regulatory network. Meanwhile the microarray data were also not been analyzed carefully. Thus, more experiments and high throughput data analysis of differentially expressed genes (DEGs) are still needed.

As a fast, large-scale and economical way, genome-wide microarray studies of pooled DNA samples have been applied to identify candidate DEGs associated to a phenotype[5]. In our study, we found that the modules built on the base of the protein-protein interaction (PPI) network were strongly related to the function of immune response system. The genes appeared as the central nodes of the modules may be used as the potential biomarkers. It is believed that our report will shed new light on the molecular mechanism of ACS.

Materials and Methods

Affymetrix Microarray Data

The transcription profile of GSE19339 was obtained from Gene Expression Omnibus (GEO)

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database (http://www.ncbi.nlm.nih.gov/geo/) which is based on the Affymetrix Human Genome U133 Plus 2.0 Array platform. Thrombi of ACS patients were harvested from the site of coronary occlusion. Leukocytes were isolated by Ficoll centrifugation. Peripheral blood leukocytes (PBL) were treated in a similar fashion and mRNA was extracted from both cells. A total of 8 chips including 4 chips of normal tissue and 4 chips of ACS tissue were used for analysis.

Identification of DEGs

GSE19339 was preprocessed using Affy package in R language[6,7]. The original expression datasets from all conditions were extracted into expression estimates, and then the linear model was constructed. Then significance of differential expression was analyzed by R package-limma[8] and adjusted by multiple testing with the Benjamini and Hochberg (BH) method[9]. In this work, genes only with the false discovery rate (FDR) less than 0.05 and |log fold change (FC)| larger than 1 were selected as DEGs.

Functional Enrichment Analysis of DEGs

WebGestalt is a "WEB-based GEne SeT AnaLysis Toolkit", designed for functional genomic, proteomic and large-scale genetic studies[10]. WebGestalt was used to perform gene ontology (GO) enrichment analysis. FDR less than 0.05 was set as the threshold.

Construction of PPI Network

It is thought that life is the result of complex interactions among molecules, such as protein-protein, protein-DNA or protein-RNA. PPI in various organisms are increasingly becoming the focus of study in the identification of cellular functions of proteins[11]. We constructed an PPI network by integrating the proteins expressed by the DEGs using STRING[8].

Functional Modules Analysis of Network

The genes existed in the same module interacted with each other to drive a complete biological process[12]. We used mcode[13], a Cytoscape plugin to analyze and visualize the clusters, and clusters with node degree larger than 2 were recorded. Bingo[14] (Biological Networks Gene Ontology) was used to annotate the functions of modules containing DEGs based on the hypergeometric distribution with FDR less than 0.05.

Results

Identification of DEGs

Figure 1 shows the gene expression value after normalization. We can judge the standardization level of database through the position of the black line in the plot. As shown in Figure 1, the black lines located almost in the same level, suggesting good standardization.

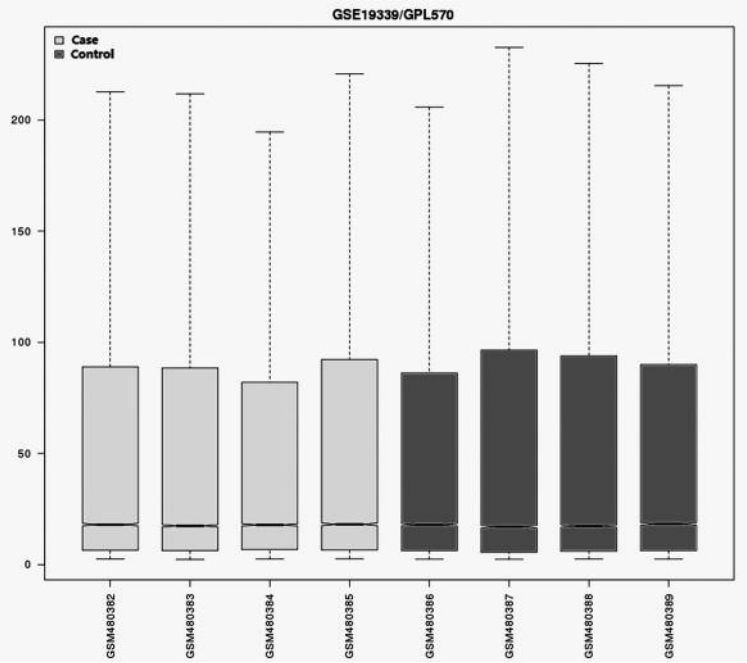

Figure 1. The box-whisker plot of gene expression value after normalization. The gray (black) box stands for disease (normal) tissue samples. The black line in each box stands for the median for each set of database.

Table I. GO terms list of DEGs.

	Term	Count	FDR
GO:0001568	Blood vessel development	25	2.36E-05
GO:0001944	Vasculature development	25	3.79E-05
GO:0001525	Angiogenesis	19	5.91E-05
GO:0048514	Blood vessel morphogenesis	22	1.48E-04
GO:0009611	Response to wounding	33	0.007546272
GO:0006952	Defense response	36	0.009938959
GO:0016477	Cell migration	22	0.012045837
GO:0001569	Patterning of blood vessels	7	0.016832533
GO:0048870	Cell motility	23	0.018956935
GO:0051674	Localization of cell	23	0.018956935
GO:0007626	Locomotory behavior	21	0.036623154
GO:0051094	Positive regulation of developmental process	21	0.045080254
GO:0006954	Inflammatory response	23	0.045825754
GO:0006928	Cell motion	29	0.04761842

To get the DEGs between normal tissues and ACS tissues, we obtained publicly available microarray dataset GSE19339 from GEO. Based on normalized expression data after data processing, a total of 480 genes were identified as DEGs in this study with a FDR less than 0.05, containing 206 up-regulated genes and 274 down-regulated genes.

Functional Enrichment Analysis of DEGs

We used WebGestalt to biologically characterize these selected DEGs, and 14 significant GO terms were enriched (as shown in Table I). From Table I, we can see that these DEGs were remarkably related to defense response, wounding response, cell motion, blood vessel development and vasculature development.

PPI Network

STRING was used to mine the proteins expressed by the DEGs which can interact with others. Then we constructed a network using Cytoscape (Figure 2).

Functional Modules in the Network

As the PPI network was too huge and complex, we can hardly acquire useful information in it. So, we screened 2 functional modules, module A and module B with the help of Cytoscape (as shown in figure 3) in the network. Table II provides the function information of the two modules screened from the above network. Module A and module B contain 9 and 11 nodes, respectively. Both of the DEGs in these two modules were strongly associated with immune response. Among them, CD (CD molecule), CXCL (chemokine (C-X-C motif) ligand), CCL (chemokine (C-C motif) ligand) and ICAM1 (intercellular adhesion molecule 1) were mostly remarkable (the node genes in the network), which are chemotactic factor genes and interleukin genes.

Discussion

In this study, we identified 480 DEGs, most of which are significantly associated with the function of blood vessel development. We got two functional modules on the base of the PPI network, which were found to be associated with immune response function. The important genes located in the central nodes were CD, CXCL, CCL and ICAM1.

There are already some biomarkers reported to be involved in thrombogenesis and arterial blockage, such as inflammation, oxidative stress and platelet activation[15-19]. ACS happens when plaque narrows or blocks the arteries that supply blood to the heart. By enriching the functions of the DEGs, we confirmed that blood vessel development may attribute to ACS.

While the genes on the central nodes of the modules have the most interactions with other genes, we researched them respectively. The proteins encoded by gene CD80 and CD86 are a pair of co-stimulatory molecules. Binding of their proteins with cytotoxic T-lymphocyte-associated protein 4 negatively regulates T-cell activation and diminishes the immune response. Some previous studies have reported that their expression levels are improved in ACS patients[20,21].

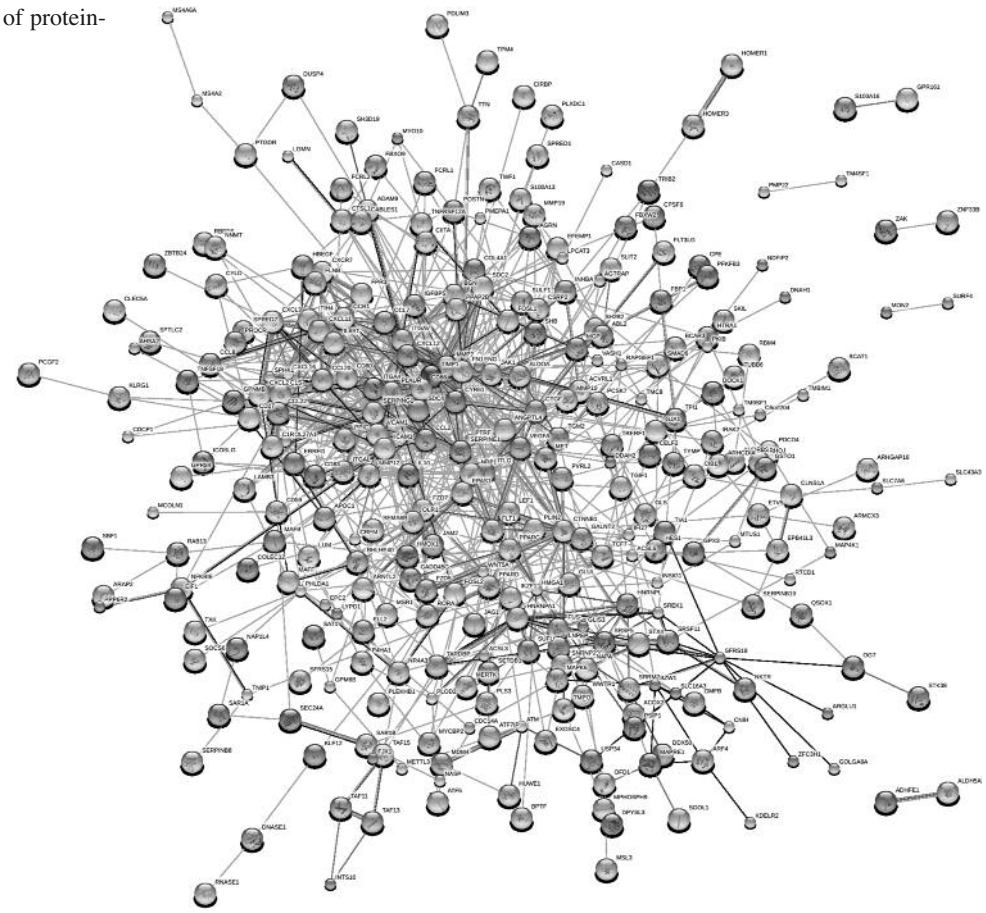

Figure 2. The network of protein-protein interaction (PPI).

Table II. The list for function of modules.

GO-ID	corr p-value	count	Description
a) Module A			
2376	3.62E-07	10	Immune system process
42221	0.000011507	10	Response to chemical stimulus
23052	0.000096456	12	Signaling
50896	0.0021371	11	Response to stimulus
50794	0.0085427	13	Regulation of cellular process
50789	0.011188	13	Regulation of biological process
9987	0.013473	15	Cellular process
65007	0.015304	13	Biological regulation
32501	0.019089	10	Multicellular organismal process
b) Module B			
2376	1.7444E-07	9	Immune system process
6952	1.9778E-07	8	Defense response
9605	1.7543E-06	7	Response to external stimulus
42221	0.000002485	9	Response to chemical stimulus
6955	2.9328E-06	7	Immune response
7610	0.000010513	6	Behavior
6950	0.000086401	8	Response to stress
50896	0.001026	9	Response to stimulus
48522	0.0093445	6	Positive regulation of cellular process
48518	0.012519	6	Positive regulation of biological process
23052	0.04076	6	Signaling

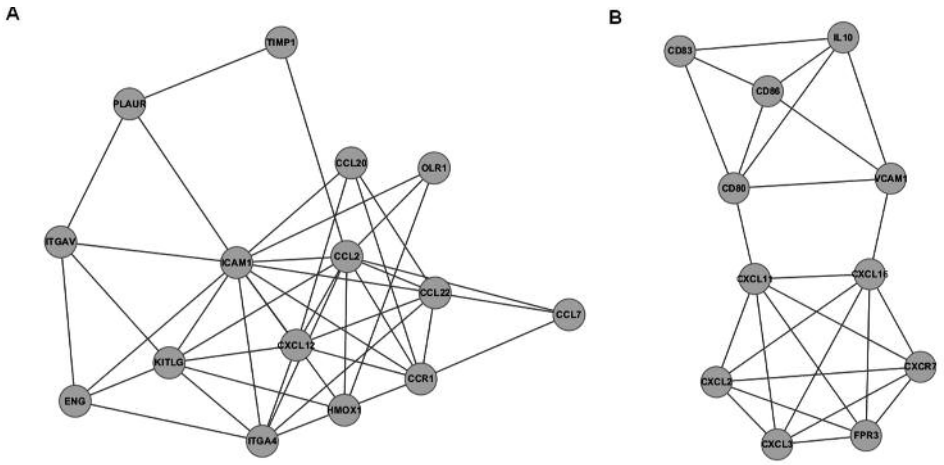

Figure 3. The modules screened from the network. The circles stand for DEGs, and the lines stand for their interactions. In module A, CCL, CXCL, and ICAM1 genes are the most important nodes, while in module B, they are CD, CXCL and ICAM1 genes.

Chemokines are a group of small (approximately 8 to 14 kD), mostly basic, structurally related molecules that regulate cell trafficking of various types of leukocytes through interactions with a subset of 7-transmembrane, G protein-coupled receptors. Chemokines play fundamental roles in the development, homeostasis, and function of the immune system, and they have effects on endothelial cells involved in angiogenesis. Chemokines are divided into 2 major subfamilies, CXC and CC. Two important nodes in our study, CXCL12 and CXCL11, belongs to the CXC subfamily, are implicated in the metastasis of some cancers such as breast cancer[22]. However, there are no reports about its effects on ACS. CCL2, CCL20 and CCL22, another three vital genes identified in our research, belong to the CC subfamily. Known as MCP1, CCL2 has been found to play an important role in ACS[23-25], while there are hardly any researches about CCL20 and CCL22.

ICAM1 encodes a cell surface glycoprotein which is typically expressed on endothelial cells and cells of the immune system. Previous studies have revealed that ICAM1 is associated with long-term mortality of ACS and provides prognostic information[26,27].

The results suggested that immune system play an important role in the development and progression of ACS. Similar with our result, Alfakry et al[28] report that the immune level increases in patients with ACS compared to normal controls. Therefore, harnessing the molecules and cells of the immune system could be helpful to treat ACS.

Conclusions

DNA microarray is used to identify DEGs that closely associated with ACS. Our findings suggest that immune system may strongly contribute to the development and progression of ACS. The present findings shed new light on the molecular mechanism of ACS and have implications for future research. More works are still needed to confirm the possible biomarkers.

Acknowledgements

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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