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Insights into the molecular mechanisms in sepsis with microarray technology

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Abstract. – OBJECTIVE: Sepsis presents great threat to human health. Here we aimed to understand its pathogenesis and discover effective therapeutic targets.

MATERIALS AND METHODS: Gene expression data for sepsis samples was compared with healthy controls to identify differentially expressed genes (DEGs), then we constructed PPI network to detect the network clustering. Gene Ontology (GO) analysis was also done to identify over-represented biological pathways.

RESULTS: KEGG pathway analysis revealed that PI3K-AKT signaling pathway, chemokine signaling pathway and MAPK signaling pathway were significantly over-represented in these DEGs. Given proteins work together to exert certain biological functions, network analysis was done to identify genes closely associated with these DEGs. Using Fisher's exact test, 8 genes such as NEDD8, CUL1 and CUL3 were screened out.

CONCLUSIONS: Overall, our findings not only supplement the knowledge about sepsis, but also provide a number of potential biomarkers for diagnosis and treatment.

Key Words:
Sepsis, Microarray data, Functional enrichment analysis, Network analysis.

Abbreviations

DEGs = Differentially expressed genes; PPI = Protein-protein interaction; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of genes and genomes; PI3K-AKT = Phosphoinositide 3-kinase-protein kinase B; SIRS = Systemic inflammatory response syndrome; MAPK = Mitogen-activated protein kinase; CUL1 = Cullin 1; GEO = Gene Expression Omnibus; DAVID = Database for Annotation, Visualization and Integrated Discovery; Biogrid = The Biological General Repository for Interaction Datasets; MCL = The Markov Cluster Algorithm; STAT3 = Signal transducer and activator of transcription 3; CAND1 = Cullin-associated and neddylation-dissociated 1; NEDD8 = Neural precursor cell expressed, developmentally down-regulated 8; UBC = ubiquitin C; PSME2 = Proteasome (prosome, macropain) activator subunit 2; PSMB4 = Proteasome (prosome, macropain) subunit, beta type 4; KIAAOIOI = PCNA (Proliferating cell nuclear antigen) is encoded in human by KIAAOIOI gene; TNF-α = Tumor necrosis factor-α; LPS = Lipopolysaccharide; siRNA = silencing RNA; Mkp = MAP kinase phosphatase; ERK = Extracellular signal-regulated kinases; ILK = Integrin-linked kinase; ZIPK = Zipper-interacting protein kinase; CPI17 = Protein kinase C-dependent phosphatase inhibitor of 17-KD; MLC20 = Myosin light chain; JNK = c-JunN-terminal kinases; TLRS = Toll-like receptors; MyD88 = Mycloid differentiation primary response gene (88); TRIF = TIR domain containing adapter-inducing interferon-β; (TIR = Terminal Inverted Repeats); Nrf2 = Nuclear factor (erythroid-derived 2)-like 2; HO-1 = heme oxygenase-1; FOX = Forkhead box; CIFs = Cycle inhibiting factors; FDR = False Discovery Rate.

Introduction

Sepsis is a potentially deadly medical condition featured by a whole-body inflammatory state (called a systemic inflammatory response syndrome or SIRS) caused by severe infection[1]. It's caused by the immune system's excessive response to an infection, most commonly bacteria, but also fungi, viruses, and parasites in the blood, urinary tract, lungs, skin, or other tissues[2]. In US, there was an annualized increase in the incidence of sepsis of 8.7 percent between 1979 and 2000[3].

The need for more effective treatments never stops. By now, a number of therapies have been developed. Anti-inflammation is a key strategy to treat sepsis[4,5]. Recombinant human activated protein C, which presents antithrombotic, anti-inflammatory, and profibrinolytic properties, has been introduced into clinical application[6]. Toll-like receptor 4, the receptor for Gram-negative bacteria outer membrane lipopolysaccharide or endotoxin, also becomes the target to modulate inflammatory response[7]. Ichim et al[8] indicate that ascorbic acid can be used to prevent and treat

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cancer-associated sepsis as an adjuvant. Immunotherapy is now widely considered as a promising method[9,10]. Anti-apoptosis is another attractive way to cure sepsis[11,12]. IL-15 can be used to prevent apoptosis and improve survival in sepsis[13].

Although considerable achievements have been made, more studies are required to deepen the knowledge about the pathogenesis of sepsis and discover good biomarkers for diagnosis or treatment. Therefore, in present study, microarray data provided by Tang et al[14,15] was analyzed with a variety of bioinformatic tools to identify key genes associated with sepsis.

Materials and Methods

Microarray Data

Microarray data set GSE5772 and GSE6535 were downloaded from Gene Expression Omnibus (GEO). GSE5772 contained 71 sepsis samples and 23 controls while GSE6535 included 55 sepsis samples and 17 controls. GenBank accession was converted into gene name with DAVID[16].

Screening of DEGs

Genes with missing values no more than 10% of the total number of samples were retained. Differential analysis was conducted with *t* test. *p* value < 0.05 was set as the cut-off.

Gene functional Annotation and Pathway Analysis

Gene Ontology (GO) analysis was done with Database for Annotation, Visualization, and Integrated Discovery (DAVID) to identify over-represented biological pathways. FDR < 0.005 was set as the threshold. Then Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis was performed with KEGG-mapper[17].

Construction of Protein-Protein Interaction (PPI) Network

PPI information was downloaded from Biogrid[18] and those regarding the DEGs was retained. The PPI network was visualized with cytoscape[19] and sub-networks were detected with MCL network clustering[20].

Calculation of PPI p-value

PPI p-value was calculated for each gene with the method from Tuller et al[21]. Genes not detected in microarray were removed from the assembly. For each gene, interactors were retrieved as a set and then its intersection with DEGs was determined. The significance of the intersection was tested with Fisher's exact test, i.e. PPI *p*-value. The Bonferroni correction was applied and (0.05/number of *p*-values) was set as the cut-off.

Results

DEGs and Their Biological Functions

498 probes were up-regulated in sepsis and 151 were down-regulated, which corresponded to 460 and 125 genes. Metabolisms like oxidative phosphorylation and cell cycle were over-represented in up-regulated genes while cell death was over-represented in down-regulated genes (Table I). According to KEGG pathway analysis, top 3 terms were PI3K-AKT1 signaling pathway (Figure 1), chemokine signaling pathway (Figure 2) and MAPK signaling pathway (Figure 3).

PPI network Analysis Result

The PPI network was constructed for all the DEGs, and then sub-networks were identified with MCL network clustering. The top 5 were ubiquitin ligase activity, protein kinase B signal coupling, STAT3 tyrosine phosphorylation, redox processes and glucocorticoid response (Figure 4).

Screening of Key Genes Associated with DEGs

9993 genes were analyzed and *p* value < 0.05/9993 (0.000005)(Figure 5) was set as the cut-off. A total of 8 genes met the threshold: Cullin-3 (CUL3), Cullin-1 (CUL1), cullin-associated and neddylation-dissociated 1 (CAND1), neural precursor cell expressed, developmentally down-regulated 8 (NEDD8), ubiquitin C (UBC), proteasome (prosome, macropain) activator subunit 2 (PSME2), proteasome (prosome, macropain) subunit, beta type, 4 (PSMB4) and KIAA0101. Those genes were not differentially expressed in sepsis compared with healthy control. Cul1 and Cul3 belong to cullins protein family while NEDD8 can bind to their conservative lysine residue, which is so-called neddylation.

Discussion

Microarray technology is a powerful tool to explore the global changes in the incidence and development of cancer[22]. A number of DEGs

Table I. Function enrichment of differentially expressed genes.

Gene	Term	Count	*p*-value	FDR
Upregulated	GO:0006119~oxidative phosphorylation	23	1.55E-14	2.70E-11
	GO:0006091~generation of precursor metabolites and energy	38	5.15E-14	8.96E-11
	GO:0045333~cellular respiration	22	1.35E-13	2.34E-10
	GO:0042775~mitochondrial ATP synthesis coupled electron transport	17	1.14E-12	1.98E-09
	GO:0042773~ATP synthesis coupled electron transport	17	1.14E-12	1.98E-09
	GO:0015980~energy derivation by oxidation of organic compounds	24	7.78E-12	1.35E-08
	GO:0022904~respiratory electron transport chain	17	1.09E-11	1.90E-08
	GO:0022900~electron transport chain	21	3.18E-11	5.53E-08
	GO:0006120~mitochondrial electron transport, NADH to ubiquinone	14	5.01E-11	8.71E-08
	GO:0000278~mitotic cell cycle	31	1.12E-07	1.95E-04
	GO:0007049~cell cycle	47	6.19E-07	0.001076396
	GO:0044092~negative regulation of molecular function	27	1.74E-06	0.003020484
	GO:0022402~cell cycle process	37	2.23E-06	0.003873826
	GO:0055114~oxidation reduction	40	2.36E-06	0.004112101
Downregulated	GO:0008219~cell death	25	1.22E-10	1.95E-07
	GO:0016265~death	25	1.40E-10	2.25E-07
	GO:0006915~apoptosis	23	1.52E-10	2.43E-07
	GO:0012501~programmed cell death	23	2.02E-10	3.23E-07
	GO:0010942~positive regulation of cell death	16	3.85E-07	6.16E-04
	GO:0043065~positive regulation of apoptosis	15	1.93E-06	0.003087164
	GO:0043068~positive regulation of programmed cell death	15	2.09E-06	0.003349341

Only biological processes with a false discovery rate (FDR) less than 0.005 were shown in the list. Count is the number of genes annotated by the corresponding term. The *p*-values associated with each terms inside the clusters is *p*-values by the Fisher Exact Test which represent the "degree of enrichment" of the annotation term with the input gene list. Benjamin FDR *q*-value is the correction for multiple comparison.

were obtained in present study. Functional enrichment analysis revealed that oxidative phosphorylation and cell cycle were enriched in up-regulated genes while apoptosis was enriched in down-regulated genes. According to KEGG pathway analysis, MAPK signaling pathway, chemokine signaling pathway and PI3K-AKT signaling pathway were significantly over-represented.

MAPKs are important signal transducers mediating inflammation. Wang et al[23] report that p38 MAPK mediates myocardial proinflammatory cytokine (TNF-) production. Frazier et al[24] investigate the interaction of MAPK and G-protein with a sepsis model and find that LPS stimulated proinflammatory cytokine production is markedly exacerbated by siRNA knockdown of the MAPK negative regulator Mkp-1. Vasoconstriction plays an important role in diseases, such as septic shock. Yang et al[25] find that MAPKs participate in the regulation of vascular reactivity during shock, and ERK and p38 MAPK is mainly through ILK, ZIPK, and CPI17-mediated MLC20 phosphorylation-dependent pathway, while JNK may be involved in the regulation of vascular reactivity by other mechanisms. Given their roles in inflammatory response, they can serve as molecular targets for anti-inflammatory therapy[26]. The study by O'Sullivan et al[27] show that inhibition of p38 MAPK may be a good way to treat sepsis. Of course, details in the regulatory mechanism remain to be answered and DEGs identified in present study are worthy of future researches.

Mitochondrial recovery during inflammatory processes such as sepsis is associated with cell survival. Akt/PKB is a serine/threonine protein kinase that plays an important role in the regulation of inflammatory responses, protein synthesis, and controlled inflammation in response to TLRs. Bauerfeld et al[28] find that TLR4-mediated Akt activation is MyD88/TRIF dependent and critical for induction of oxidative phosphorylation and mitochondrial transcription factor A in murine macrophages. MacGarvey et al[29] identify an inducible Nrf2/HO-1 regulatory cycle for mitochondrial biogenesis that is prosurvival and counter-inflammatory in sepsis. Crossland et al[30]

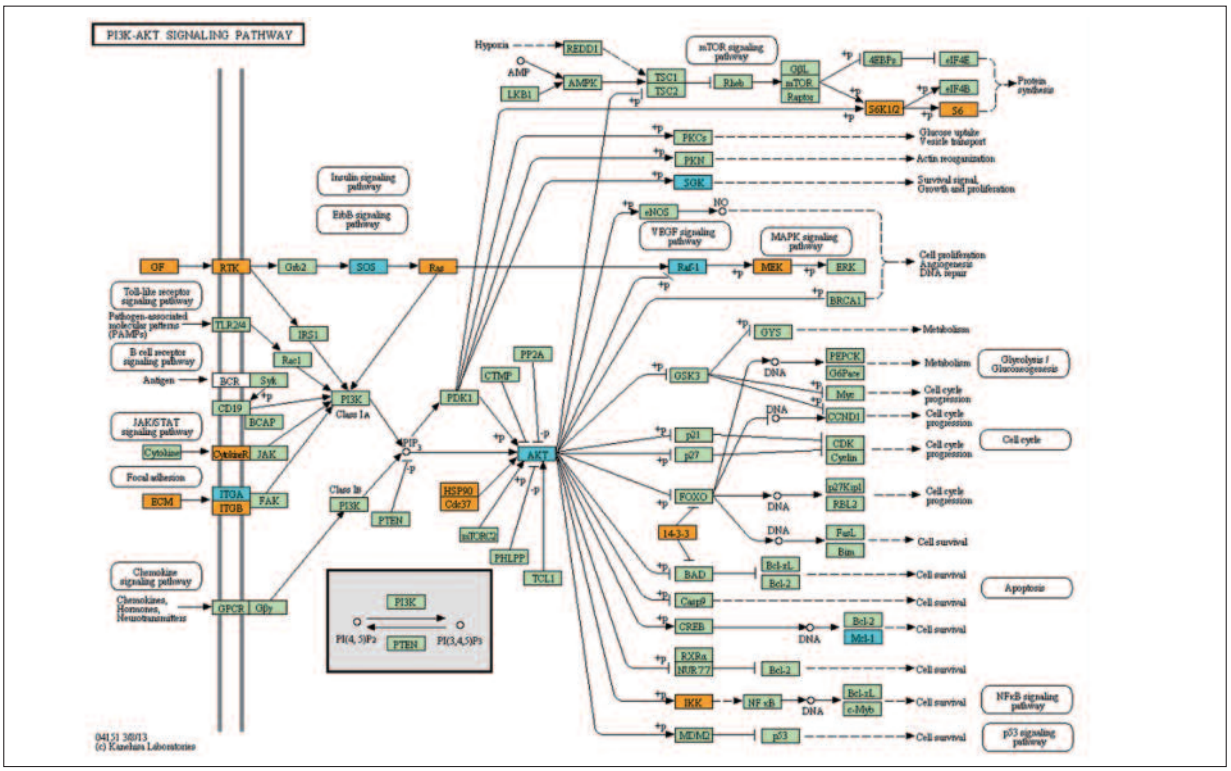

Figure 1. Differentially expressed genes in the PI3K-AKT signaling pathway. Up-regulated genes are in orange while down-regulated in blue.

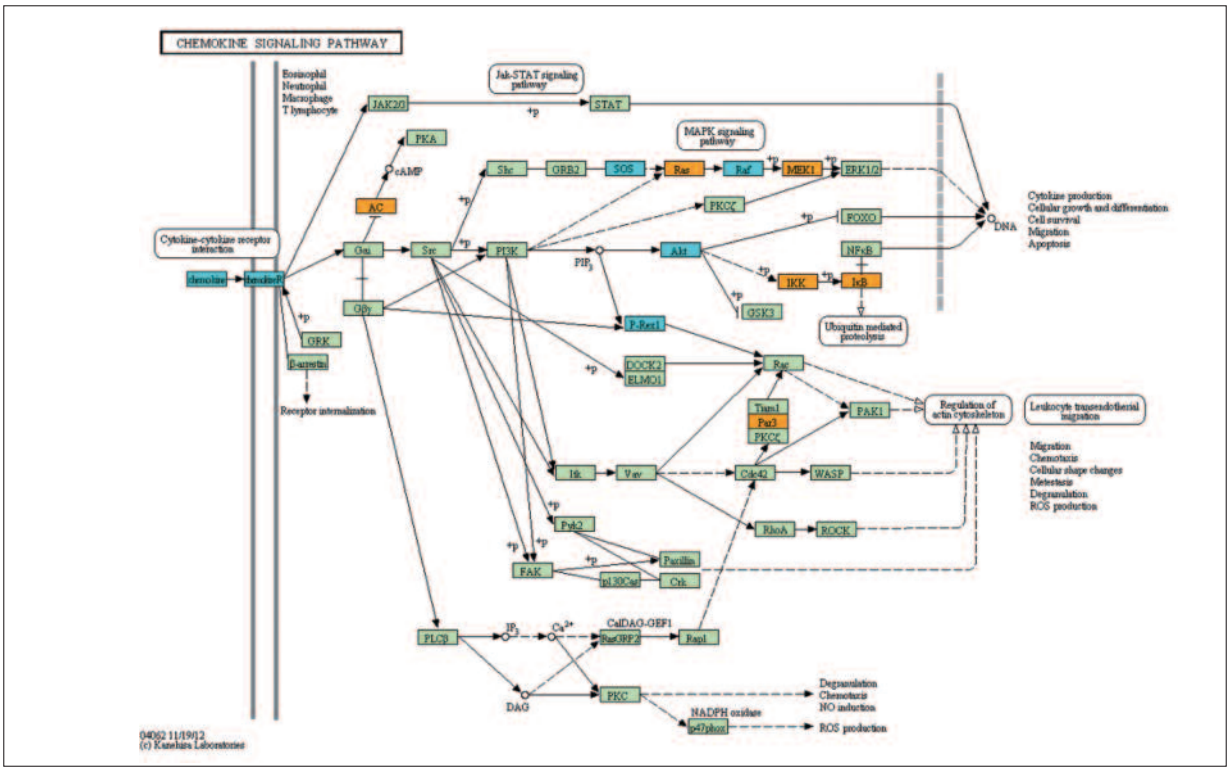

Figure 2. Differentially expressed genes in the chemokine signaling pathway. Up-regulated genes are in orange while down-regulated in blue.

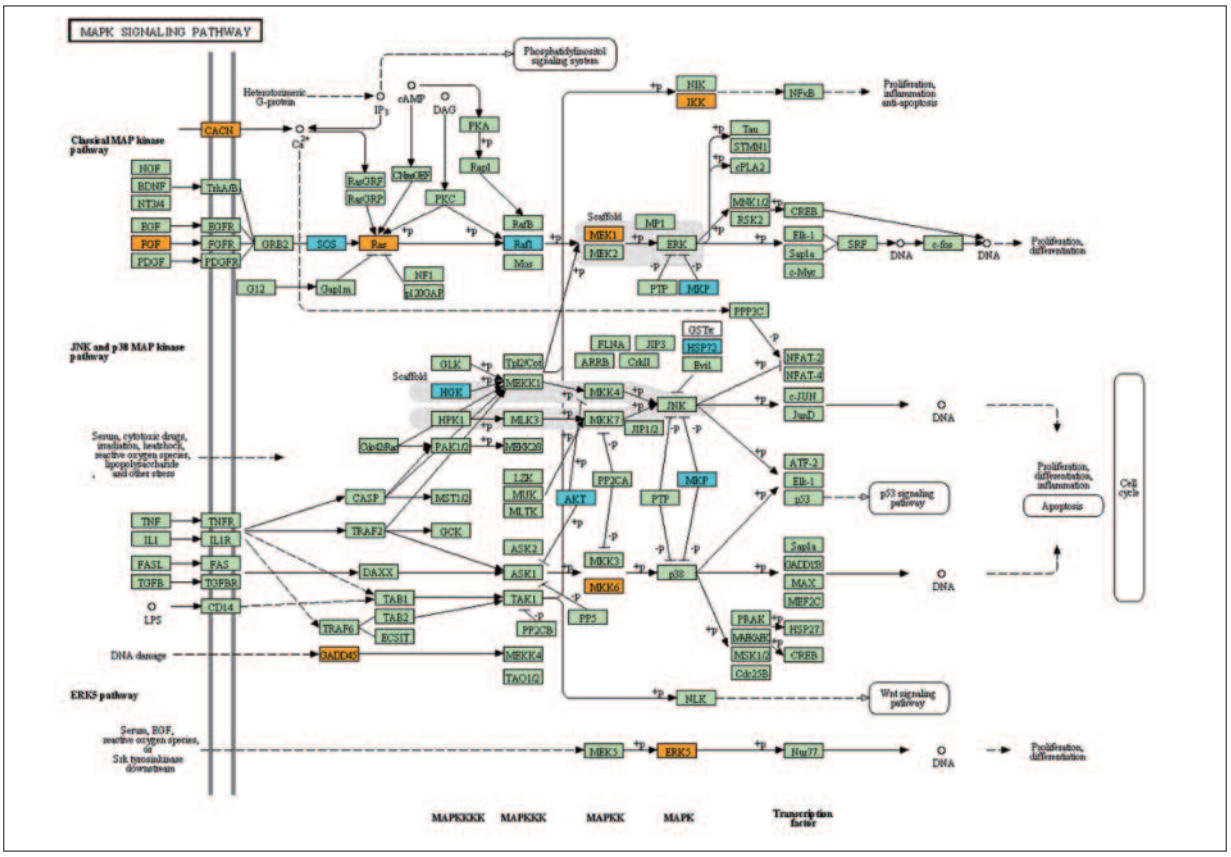

Figure 3. Differentially expressed genes in the MAPK signaling pathway. Up-regulated genes are in orange while down-regulated in blue.

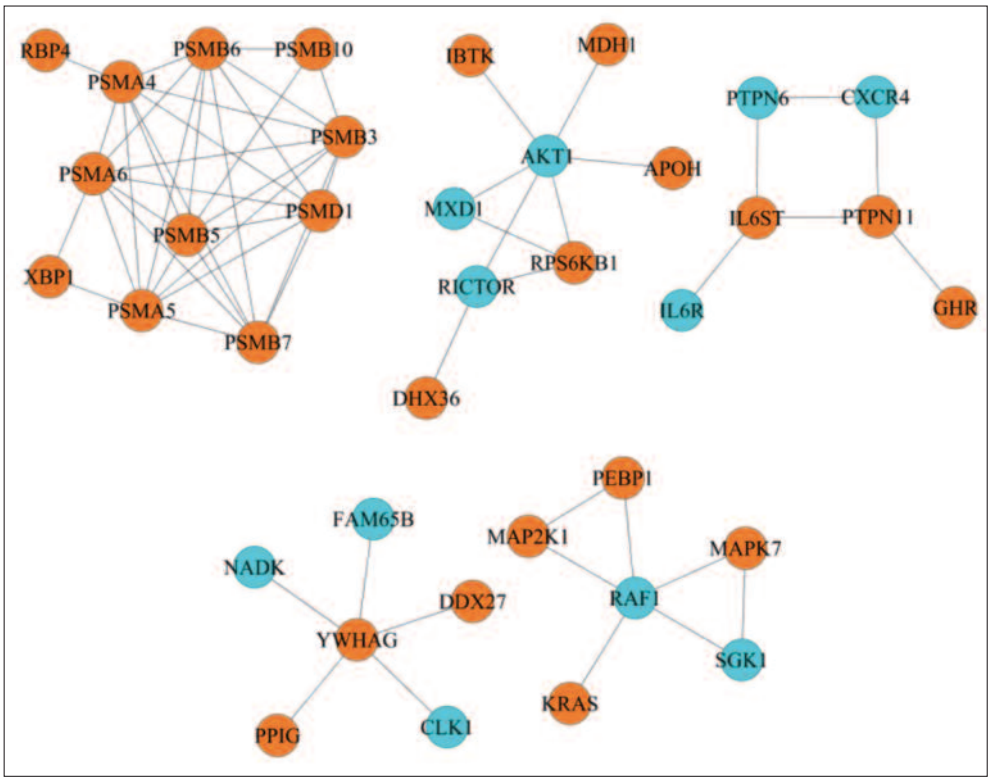

Figure 4. Top 5 sub-networks identified from the PPI network by MCL clustering. Up-regulated genes are in orange while down-regulated in blue.

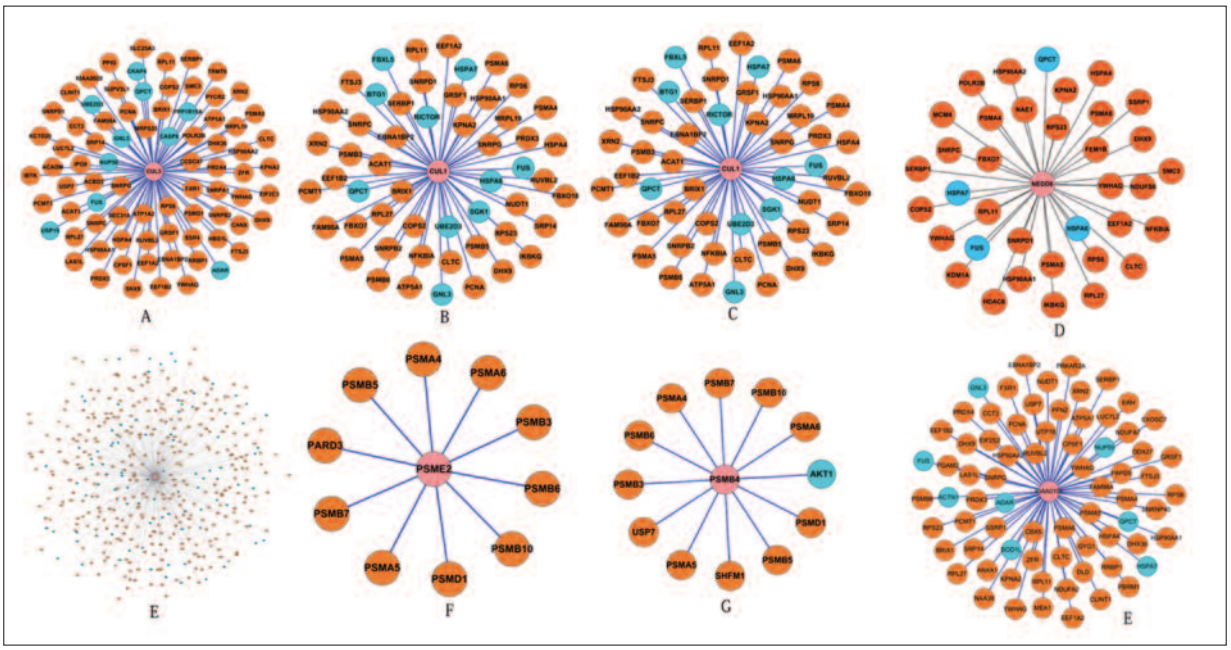

Figure 5. Eight genes closely associated with differentially expressed genes: CUL3 (A), CUL1 (B), CAND1 (C), NEDD8 (D), UBC (E), PSME2 (F), PSMB4 (G) and KIAA0101 (H). Up-regulated genes are in orange while down-regulated in blue.

examine the effects of LPS-induced sepsis on the expression of Akt, FOXO and its downstream targets and find that LPS infusion decreases Akt1 protein and cytosolic FOXO1 and FOXO3 phosphorylation. In present study, a variety of DEGs were associated with oxidative phosphorylation as well as generation of precursor metabolites and energy, modulation of the expression of these genes might be helpful in treatment of sepsis.

Genes closely connected with DEGs might undergo changes in different levels, such as post-transcription, and thus also take important parts in development of sepsis[21]. Therefore, network analysis was conducted and several genes were revealed, such as CUL3, CUL1 and NEDD8.

NEDD8 is a target gene of cycle inhibiting factors (CIFs)[31], which can be produced by pathogen and block the cell cycle. Accumulation of NEDD8-conjugated cullins is observed after infection. Cui et al[32] report that *Burkholderia pseudomallei* can inhibit the ubiquitination pathway of eukaryotic cells via deamidation of NEDD8. NEDD8 can bind to the conservative lysine residue in cullins protein, a process known as neddylation. Wu et al[33] indicate that neddylation and deneddylation can regulate Cul1 and Cul3 protein accumulation. Singleton et al[34] report that glutamine can inhibit NF-k Beta activation and cytokine expression following sepsis via Cullin-1 deneddylation, providing a possible mechanistic explanation for GLN's anti-inflammatory effects. UBC, PSME2 and PSMB4 are components of the ubiquitin system, which plays a key role in the immune system. Ponelies et al[35] indicate that the regulation of the cytosolic ubiquitin is significantly altered during sepsis. Wray et al[36] find that sepsis upregulates the gene expression of multiple ubiquitin ligases in skeletal muscle. Based upon these previous studies, it could be speculated that these genes were implicated in sepsis. Nevertheless, more works are needed to fully disclose the mechanisms, which may offer new therapeutic targets for sepsis.

Conclusions

Overall, a range of DEGs were obtained through comparing gene expression profiles of sepsis with those of healthy controls. Our findings could provide helpful directions for future researches.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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