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Screening of differentially expressed genes between multiple trauma patients with and without sepsis

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ABSTRACT. The purpose of this study was to identify critical genes associated with septic multiple trauma by comparing peripheral whole blood samples from multiple trauma patients with and without sepsis. A microarray data set was downloaded from the Gene Expression Omnibus (GEO) database. This data set included 70 samples, 36 from multiple trauma patients with sepsis and 34 from multiple trauma patients without sepsis (as a control set). The data were preprocessed, and differentially expressed genes (DEGs) were then screened for using packages of the R language. Functional analysis of DEGs was performed with DAVID. Interaction networks were then established for the most up- and down-regulated genes using HitPredict. Pathway-enrichment analysis was conducted for genes in the networks using WebGestalt. Fifty-eight DEGs were identified. The expression levels of *PLAU* (down-regulated) and *MMP8* (up-regulated) presented the largest fold-changes, and interaction networks were established for these genes. Further analysis revealed that PLAT (plasminogen activator, tissue) and SERPINF2 (serpin peptidase inhibitor, clade F, member 2), which interact with PLAU, play important roles in the pathway of the component and coagulation cascade. We

hypothesize that PLAU is a major regulator of the component and coagulation cascade, and down-regulation of *PLAU* results in dysfunction of the pathway, causing sepsis.

Key words: Sepsis; Multiple trauma; Differentially expressed genes; Interaction network; Functional enrichment analysis; Pathway analysis

INTRODUCTION

Multiple trauma occurs when traumas simultaneously occur in 2 or more anatomical sites and when at least 1 trauma is life threatening. Sepsis is a common complication of severe traumas, burns, shocks, major surgeries, and infections (Rice and Bernard, 2005). If sepsis is not effectively controlled at an early stage, septic shock and multiple organ dysfunction syndrome will occur, causing a high mortality rate (Taylor et al., 1991). Statistical data show that 70 million people suffer from sepsis in the United States each year, of which 21 million people die, corresponding to a mortality rate of 30%. This number is greater in China, approximately 45% (Riedemann et al., 2003). Wafaisade et al. (2011) showed that although the incidence of sepsis has decreased in Germany, there has been no significant decrease in mortality in the subgroup of septic trauma patients. Considering the danger and mortality of sepsis, it is urgent to characterize the deteriorative process and then develop effective treatments.

Researchers have adopted various methods to uncover different aspects of the underlying mechanisms of sepsis. Studies (Chen et al., 2011; Zhao et al., 2012) have linked polymorphisms in the Toll-like receptor 9-encoding gene to sepsis and multiple organ dysfunction. Polymorphisms of the tumor necrosis factor-encoding gene (Majetschak et al., 2002; O'Keefe et al., 2002) and interferon-gamma-encoding gene (Stassen et al., 2002) have also been investigated. Gouel-Cheron et al. (2012) found that a high interleukin-6 (IL-6) concentration and a persistent decrease in monocytic human leukocyte antigen DR (mHLA-DR) expression are indicators for development of sepsis. Microarray technology allows a widely used gene expression profile method for exploring molecular mechanisms. Using this method, Paunel-Gorgulu et al. (2012) explored the delayed apoptosis in neutrophils from multiple trauma patients with and without sepsis.

Considerable advancements have been made in the study sepsis, especially elucidating potential biomarkers, such as c-type natriuretic peptide (Suttner and Boldt, 2010), soluble Fas (Paunel-Gorgulu et al., 2011), procalcitonin (Svoboda et al., 2007), and IL-6 (Haasper et al., 2010). Several therapies have also been proposed, e.g., estrogen treatment (Raju and Chaudry, 2008; Kawasaki and Chaudry, 2012). However, knowledge about sepsis is inadequate for achieving effectively clinical control. Therefore, we compared multiple trauma patients with and without sepsis by microarray technology to identify key genes implicated in the process, which have the potential to be used as the targets to combat the disease.

MATERIAL AND METHODS

Microarray data

Data set GSE12624 was downloaded from the Gene Expression Omnibus (GEO) database, comprising 70 peripheral blood samples, 36 from multiple trauma patients with sepsis

and 34 from multiple trauma patients without sepsis. The microarray data had been collected using a GPL4204 GE Healthcare/Amersham Biosciences CodeLinkUniSet Human I Bioarray instrument. Raw data were downloaded along with the probe annotation files.

Pre-processed data and differentially expressed genes (DEGs) identification

Data were pre-processed using Affy (Troyanskaya et al., 2001; Fujita et al., 2006) and part of the R language. Differential expression analysis was performed using Limma package (Smyth, 2005). The Bayesian method (Benjamini and Hochberg, 1995) was employed for multiple testing corrections. A false-discovery rate (FDR) <0.05 and |logFC| >1 were the cutoffs to filter DEGs. Genes with the maximum fold-changes (FC) in expression levels were targeted for deep mining.

Functional analysis of all DEGs

Functional analysis was performed for all of the DEGs using the Expressing Analysis Systematic Explorer (EASE) (Hosack et al., 2003). FDR <0.05 was regarded as significant.

Establishing protein-protein interaction networks

Because most proteins function in concert or in complexes to exerting their biological functions, building interaction networks is beneficial for understanding of protein functions. HitPredict was utilized to establish the interaction networks for protein products of the most up- and down-regulated genes.

HitPredict includes protein-protein interactions from the IntAct, BIOGRID, and HPRD databases; information was originally obtained from high-throughput or small-scale experiments. HitPredict also contains predicted interactions based on scores that integrate information such as sequence, structure, and function using a Bayesian algorithm (Patil et al., 2011). Interactions obtained from experiments or with a score >1 are likely of significance (Patil and Nakamura, 2005) and form a basis for our interaction networks.

Pathway-enrichment analysis of network

WebGestalt (Zhang et al., 2005; Duncan et al., 2010) was employed to analyze the genes in the networks, using a hypergeometric distribution algorithm to uncover the functions, and adj. $P < 0.05$ was set as the cutoff.

RESULTS

Differentially expressed genes

Microarray data were preprocessed and normalized (Figure 1). Fifty-eight DEGs were identified according to the criteria ($P < 0.05$ and |logFC| >1): 21 down-regulated and 37 up-regulated DEGs (Table 1). *PLAU* (logFC = -1.32793955) was the most down-regulated gene, while *MMP8* (logFC = 1.74640401) was the most up-regulated gene (Figure 2). These two genes were selected for further analysis.

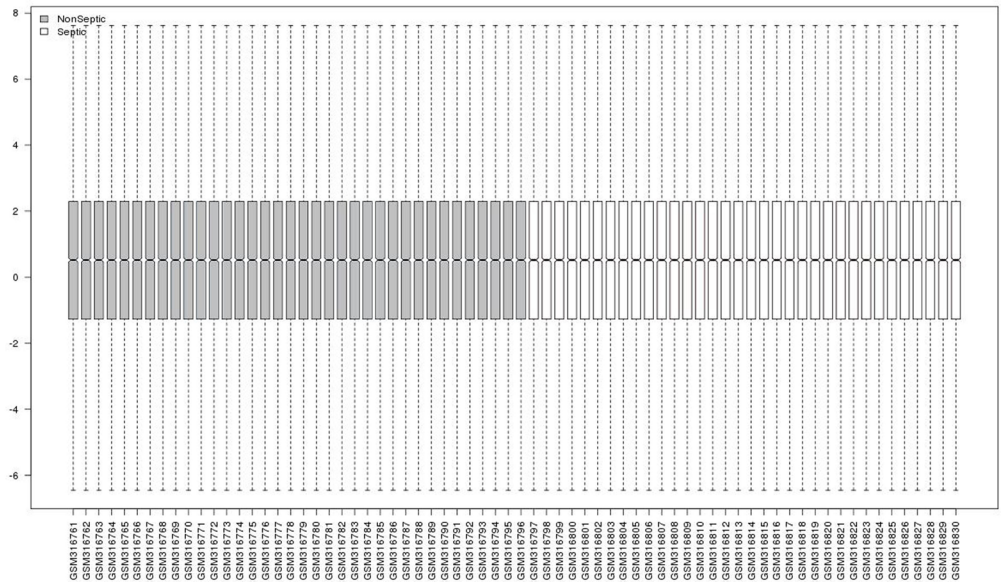

Figure 1. Box plot for normalized gene expression data, 34 gray boxes for non-sepsis-induced multiple trauma samples and 36 white boxes for sepsis-induced multiple trauma samples. Black line in the box represents the median of data set. The black lines are almost on the same line, indicating good effectiveness of normalization.

Table 1. List for differentially expressed genes.

Gene symbol	FDR	logFC	Gene symbol	FDR	logFC
PLAU	2.65E-05	-1.32794	ITGA7	0.003925	1.047575
ABP1	0.000767	-1.31794	SAMSN1	0.000543	1.054721
KIAA0484	0.002364	-1.30972	KLHL2	0.002387	1.07438
CPA3	0.018433	-1.29321	IPO11	6.1E-07	1.075049
SPP1	0.009126	-1.25878	HAT1	2.65E-05	1.093273
HSD17B3	0.000799	-1.24959	TPST1	0.014875	1.10131
RPS6KA5	0.000549	-1.22299	CLEC2B	0.000934	1.102343
ETV7	0.028783	-1.22112	C9orf103	0.000129	1.106543
SPON2	0.000672	-1.20473	DHRS9	0.000864	1.159279
MPZL2	0.000119	-1.20289	BIRC5	0.00049	1.161581
EXOSC5	0.028183	-1.18981	CPNE5	0.000919	1.163124
SLC45A4	0.008763	-1.13275	VSIG4	0.010235	1.164657
GNLY	6.44E-05	-1.1292	UQCRB	0.000515	1.168334
PLA2G7	0.003009	-1.0861	PROS1	2.65E-05	1.177566
ZNF12	0.002486	-1.04606	GUCY1B3	3.31E-05	1.218599
SERPINB2	0.017122	-1.04274	TNFRSF17	7.9E-05	1.239881
MME	0.00056	-1.03697	PRUNE2	2.65E-05	1.273587
APCDD1	0.005063	-1.02828	OLAH	0.000396	1.319922
GZMH	0.000209	-1.0275	MED18	0.001043	1.326346
XAF1	0.008767	-1.02284	CWF19L1	0.009126	1.334516
EPHB4	6.51E-05	-1.0209	ARG1	0.000228	1.392289
PDZD8	0.0002	1.005948	EDG1	2.39E-06	1.416898
ERLIN1	0.005074	1.009205	IGJ	0.000767	1.420944
BAZ1A	0.004154	1.015047	TACSTD1	0.048965	1.455898
LEPROT	0.00032	1.018187	PCOLCE2	2.65E-05	1.475443
PCSK1	0.006772	1.0192	C5orf30	0.003405	1.557862
SYCP2	6.51E-05	1.030233	WFDC1	0.01506	1.618033
RNASE1	0.016689	1.036442	HPGD	0.001807	1.732723
SMPDL3A	0.012126	1.038862	MMP8	0.008898	1.746404

FDR = false-discovery rate; FC = fold-changes.

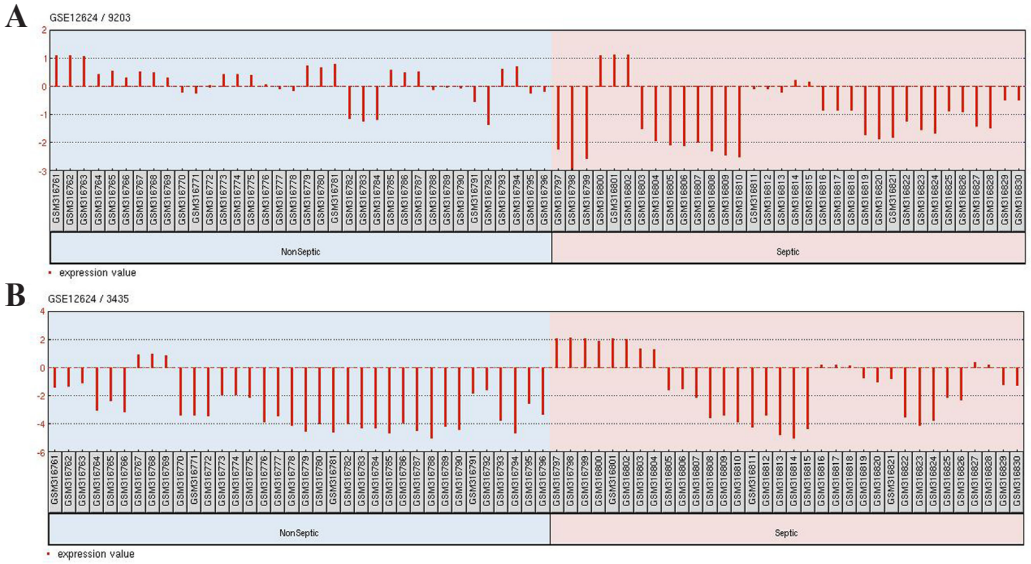

Figure 2. Expression data of down-regulated *PLAU* (**A**) and up-regulated *MMP8* (**B**) for all the samples.

Functional analysis of all DEGs

Functional analysis of the DEGs revealed 3 significantly enriched terms (Table 2), among which GO: 0009611 - response to wounding - was the most significant term. Eight DEGs were associated with this function, *TPST1*, *PCSK1*, *SERPINB2*, PLA2G7, *VSIG4*, *PROS1*, *PLAU*, and *SPP1*. The term of proteolysis was also enriched.

Table 2. Functional analysis results for all of the differentially expressed genes.

Term	Adj. P	Genes
GO:0009611 - response to wounding	0.00148	TPST1, PCSK1, SERPINB2, PLA2G7, VSIG4, PROS1, PLAU, SPP1
GO:0006508 - proteolysis	0.018919	RPS6KA5, PCSK1, MMP8, MME, CPA3, ERLIN1, GZMH, VSIG4, PLAU
GO:0007565 - female pregnancy	0.0496789	HPGD, PLAU, SPP1

Further analysis of PLAU and MMP8

Interaction networks for the protein products of *PLAU* and *MMP8* were comprised of 19 and 7 proteins, respectively (Figure 3). Pathway-enrichment analysis revealed that proteins in the former network enriched in ko04610: complement and coagulation cascade (Figure 4). *PLAT* and *SERPINB2* were involved in this pathway.

DISCUSSION

We compared gene expression data from multiple trauma patients with and without sepsis and identified 58 DEGs. Functional analysis found 3 significant terms with apparent or unapparent associations with the disease. We further analyzed the most down-regulated gene

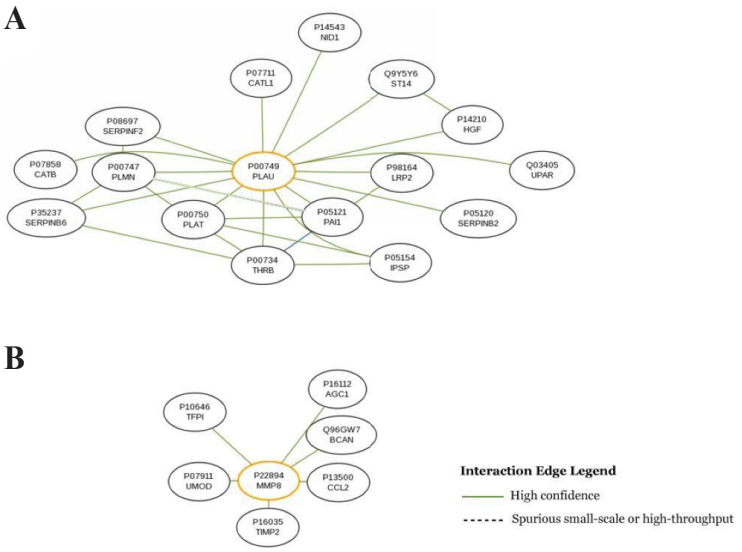

Figure 3. Interaction networks for PLAU (**A**) and MMP8 (**B**) generated by HitPredict. Green line represents a connection of high confidence (likelihood >1).

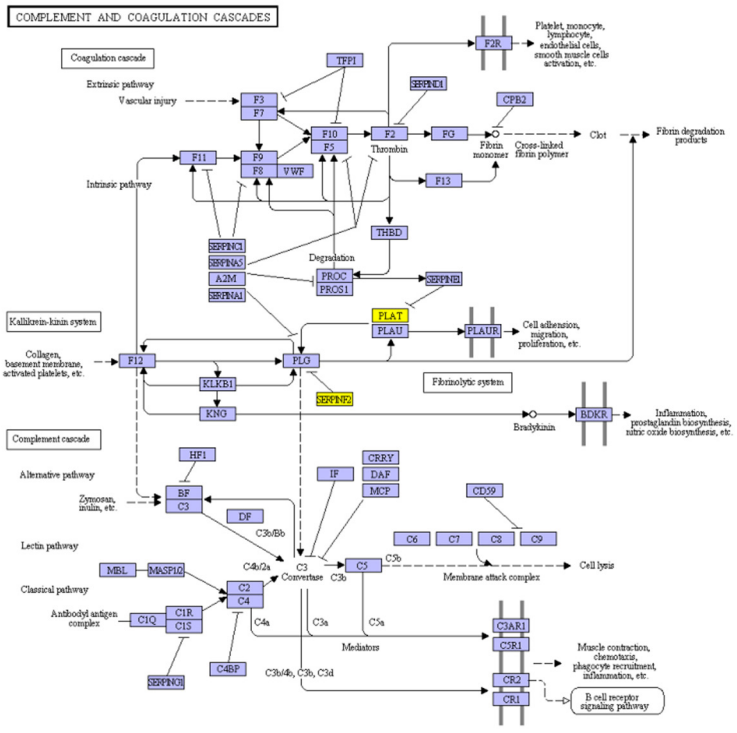

Figure 4. Schematic diagram for complement and coagulation cascades. The yellow box indicates protein that interacts with PLAU.

PLAU and the most up-regulated gene *MMA8*. Eighteen and six interaction partners for *PLAU* and *MMA8*, respectively, were acquired using bioinformatic tools, and the functions of the interaction partners were explored (Table 3). The pathway of the complementary and coagulation cascade was identified for the former group of interaction partners.

Table 3. Interactors for PLAU (A) and MMP8 (B).

A

ID	Interactor	Likelihood score
160779	SERPINB6	999
160800	PLMN	999
160822	PAI1	999
160828	PLAT	999
160839	SERPINB2	999
160842	UPAR	999
161280	THRB	999
163183	LRP2	999
163185	IPSP	999
163186	ST14	999
169965	CATB	999
170178	CATL1	999
170456	SERPINF2	999
174248	HGF	999
179105	NID1	999
180745	MPRI	999
214893	SERPINE2	999
195271	BTK	3.37

B

ID	Interactor	Likelihood score
173399	CCL2	999
173402	TIMP2	999
173403	BCAN	999
173405	UMOD	999
176642	TFPI	999
176672	AGC1	999

The most significant function term was response to wounding, which was expected because multiple trauma had occurred to the patient. Eight proteins belong to this group. The changes in expression levels of several genes may provide an explanation for the incidence of sepsis. V-set and immunoglobulin domain containing 4 (VSIG4) functions as a negative regulator of T cell activation (Vogt et al., 2006) but VSIG4 is up-regulated in sepsis patients. In contrast, secreted phosphoprotein 1 (SPP1), a cytokine that up-regulates expression of interferon-gamma and IL-12, is down-regulated in sepsis patients. Plasminogen activator inhibitor-2 (SerpinB2), which inhibits the proteasome (Boncela et al., 2011), is down-regulated in our study, favoring protein degradation in accordance with the second function term, proteolysis. However, Schroder et al. (2010) reported that SerpinB2 could play a positive role in adaptive immunity. Further studies on SerpinB2 polymorphisms may be beneficial and interesting. In addition, protein S (alpha) (PROS1) is up-regulated and is a cofactor for the anticoagulant protease.

Because accelerated proteolysis of muscle is characteristic of patients with sepsis (Clowes Jr. et al., 1983), the term of proteolysis is also significantly enriched. Several DEGs associated with degradation of the extracellular matrix were included in this group, but these

DEGs presented different changes in the expression level. The protein encoded by the most up-regulated gene *MMA8* was included in this group. MMA8 is a member of the matrix metalloproteinase (MMP) family that is involved in the breakdown of the extracellular matrix. Plasminogen activator urokinase (PLAU) is a serine protease involved in degradation of the extracellular matrix and its gene is down-regulated in patients with sepsis. In addition, granzyme H (GZMH), which plays a role in innate immune response, is down-regulated at the gene expression level. This observation is in accordance with previous research on granzyme A (Accardo-Palumbo et al., 2010).

Our analysis also suggested that complement and coagulation cascades were implicated in the pathological process of sepsis. Previous studies have shown that an overactive complement system induced by sepsis is harmful (Ward et al., 2003; Albrecht and Ward, 2005) and may even cause multiple organ failure (Kansas, 1996; Flierl et al., 2006). Schefold et al. (2007) performed an interventional extracorporeal treatment that simultaneously reduced the levels of endotoxin, IL-6, and C5a via selective extracorporeal immunoadsorption, and they successfully restored monocytic responsiveness and improved organ function. Janssen et al. (2007) investigated the efficacy of complement inhibitor - compstatin - on complement component C3 and the treatment of sepsis using a baboon sepsis model. They find that compstatin could lower the activity of complement in plasma and tissue, reduce the accumulation of macrophages and platelets in organs, and decrease the clotting activity through down-regulation of coagulation factors I and plasminogen activator inhibitor-1. Therefore, inhibition of the undesired activity of the complement system can be a promising means of protecting organ function.

Because PLAU interacts with PLAT and SerpinB2, which are involved in the complement cascade, we speculate that PLAU is a regulator for normal function of the complement system, and the down-regulation of PLAU may contribute to the dysfunction of the system that subsequently causes multiple organ injury in patients.

In summary, we discovered several important DEGs likely involved in the pathological process of sepsis. These DEGs may represent potential drug targets for multiple trauma patients with sepsis. Nevertheless, more research is necessary to study the function and role of these DEGs in sepsis.

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