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Screening of differentially expressed genes related to severe sepsis induced by multiple trauma with DNA microarray

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Abstract. – OBJECTIVES: Severe sepsis after trauma still associated with a high mortality rate in intensive care units (ICU). In this study we aimed to identify genes related to multiple trauma complicated by severe sepsis.

MATERIALS AND METHODS: The gene expression profile dataset GSE12624 including 36 samples of traumatic patients not complicated by sepsis and 34 traumatic patients complicated by sepsis was downloaded from GEO (Gene Expression Omnibus) database. The limma package in R was applied to identify differentially expressed genes (DEGs) between these two groups of samples. All the DEGs were divided into up- and down-regulation groups according to the changes of their expression value, which were then subjected to GO enrichment analysis. Two genes with largest changes among the up- and down-regulation groups were selected. Interaction networks based on these two genes were constructed using HitPredict software and then pathway enrichment analysis for the networks were performed by WebGestalt software.

RESULTS: A total of 21 up-regulated genes and 37 down-regulated genes were obtained, which were mainly related to GO terms "endopeptidase inhibitor activity" and "response to wounding", respectively. The |logFC| of genes PLAU (urokinase-type plasminogen activator) and MMP8 (matrix metalloproteinase-8) ranked first in down-regulated or up-regulated list. There were 18 genes which can interact with PLAU at a high degree of confidence while there were 5 genes with MMP8. Further analysis showed that PLAU was closely associated with the pathway "complement and coagulation cascades".

CONCLUSIONS: PLAU and MMP8 may act as potential targets for diagnosis and therapy of trauma complicated by sepsis.

Key Words:

Trauma complicated by sepsis, Differentially expressed gene, Interaction network, GO enrichment analysis, Pathway analysis.

Introduction

Trauma is still one of the leading causes of death among the population worldwide. In addition to the injury severity or premorbid health status, trauma triggers a complex cascade of post-traumatic events that are closely correlated with the outcomes of victims. Severe sepsis, defined as sepsis with one or more organ system dysfunctions, is one of the complications induced by trauma and the development of severe sepsis after trauma is associated with a high mortality rate in intensive care units (ICU).Recent studies suggest a mortality rate of 17%-23% in patients who develop post-traumatic sepsis during their hospital stays[1-4].

Massive injury in the patients with trauma drives the activation of immune system and the early inflammatory response followed by an anti-inflammatory response,which can result in immune suppression with high risk of infection and sepsis[5]. In the past, sepsis was described as loss of control of inflammation; however, immunosuppression is observed in the later stages of sepsis recently[6]. Whatever, the process of sepsis is extremely complex involving many cell types and molecules[7-8]. Evidence revealed that TLR4 (Toll-like receptor 4) and complement components such as C5a are involved in the initiation of inflammation in sepsis[9]. HMGB1 (High Mobility Group 1) is released systemically during sepsis and suppression the secretion of HMGB1 by activation of cholinergic anti-inflammatory pathway can improve survival[10]. Besides, procalcitonin (ProCT), which is overexpressed up to thousands of fold in patients with sepsis compared to normal, has been a well-established biomarker for the diagnosis of sepsis[11]. Recent researches show that soluble TREM (soluble triggering receptor expressed on myeloid cells-1)[12], LBP (lipoprotein

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binding protein)[13] and pro-ANP (pro-atrial natriuretic peptide)[14] may be used as biomarkers of sepsis and have potential clinical use.

In spite of the more and more researches on sepsis, current knowledge about the molecular mechanisms of the development of sepsis is limited and the early diagnosis is still difficult which may results in a delayed therapy.Thus identification of new biomarkers of sepsis is urgently needed, especially when considering the its unspecific clinical signs and laboratory findings.

In this work, we analyzed the differentially expressed genes (DEGs) between the traumatic patients complicated and not complicated with sepsis using a computational bioinformatics analysis of gene expression to identify molecular biomarkers for post-traumatic sepsis.

Materials and Methods

Affymetrix Microarray Data

The gene expression profile dataset GSE12624which is based on the platform GPL4024 (GE Healthcare/Amersham Biosciences CodeLinkUniSet Human I Bioarray) was obtained from the GEO (Gene Expression Omnibus, http://www.ncbi.nlm.nih.gov/geo/) database. The whole blood samples of 70 traumatized patients from intensive care units (ICU) were tested, including 36 patients not complicated by sepsis and 34 patients complicated by sepsis. All the original files and the files regarding the probe annotation information were downloaded.

Data Preprocessing and Differentially Expressed Gene Analysis

The original expression dataset in the form of txt were downloaded and the missing data were imputed[15]. For each sample, the expression values of all probes for a given gene were reduced to a single value by taking the average expression value. Then the data were standardized using the median method[16]. The limma package[17] in R was used to identify differentially expressed genes (DEGs) between traumatized patients not complicated by sepsis and patients complicated by sepsis. To circumvent the multi-test problem which might induce too much false positive results, the Benjamini Hochbery method[18] was used to adjust the raw P-values into false discovery rate (FDR). The FDR <0.05 and |logFC| >1 were selected as the cut-off criterion.

GO Functional Enrichment Analysis

The DEGs were divided into up-regulated group and down-regulated group. Then we used EASE (Expressing Analysis Systematic Explorer)[19] to identify over-represented GO categories among the up- and down-regulated genes. The Fisher exact test was applied to assess the significance and FDR less than 0.05 was selected as the cut-off criterion.

Construction of the Interaction Networks

Genes with FDR less than 0.05, and logFC ranked first in either up-regulated or down-regulated list were selected to construct the protein-protein interaction (PPI) networks using HitPredict[20] based on the dataset with high degree of confidence (obtained from experiment and likelihood ratio > 1).

Pathway Enrichment Analysis

We inputted each group of genes in the interaction networks into the WebGestalt (WEB-based GEneSeTAnaLysis Toolkit, http://genereg.ornl.gov/webgestalt/)[21,22] to perform the pathways enrichment analysis based on the hyper-geometric distribution algorithm test. Adjust p value less than 0.05 was considered as significant for pathway.

Results

Identification of Differentially Expressed Genes

Based on the normalized data, total 58 genes with FDR < 0.05 and |logFC| >1 were selected which were differentially expressed between traumatized patients not complicated by sepsis and patients complicated by sepsis. Among them, there were 21 up-regulated genes and 37 down-regulated genes.

GO Enrichment Analysis of the DEGs

Through the functional enrichment analysis by EASE, 21 up-regulated and 37 down-regulated genes were found to be enriched in 9 and 4 GO categories, respectively (Table I). The most over-represented GO term among the down-regulated genes was response to wounding and there four genes SERPINB2 (plasminogen activator inhibitor-2), PLA2G7 (platelet-activating factor acetylhydrolase), PLAU (urokinase-type plasminogen activator), SPP1 (Secreted Phosphoprotein 1) were associated with this term. The up-regulated genes were most strongly related to the GO term of endopeptidase inhibitor activity.

Table I. GO functional enrichment analysis for the up- and down-regulated genes.

Term	*p* Value	Genes
a) Down-regulated genes		
GO:0009611~response to wounding	0.000114771	SERPINB2, PLA2G7, PLAU, SPP1
GO:0007566~embryo implantation	0.000191978	PLAU, SPP1
GO:0008233~peptidase activity	0.00034444	RPS6KA5, SERPINB2, MME, CPA3, GZMH, PLAU
GO:0004175~endopeptidase activity	0.008399553	SERPINB2, MME, GZMH, PLAU
GO:0004252~serine-type endopeptidase activity	0.013264395	SERPINB2, GZMH, PLAU
GO:0008236~serine-type peptidase activity	0.017455779	SERPINB2, GZMH, PLAU
GO:0017171~serine hydrolase activity	0.017827658	SERPINB2, GZMH, PLAU
GO:0008237~metallopeptidase activity	0.018391852	RPS6KA5, MME, CPA3
GO:0006508~proteolysis	0.046500307	RPS6KA5, MME, CPA3, GZMH, PLAU
b)		
GO:0004866~endopeptidase inhibitor activity	0.036235741	WFDC1, BIRC5, PROS1
GO:0000228~nuclear chromosome	0.039583162	BAZ1A, BIRC5, SYCP2
GO:0030414~peptidase inhibitor activity	0.039952973	WFDC1, BIRC5, PROS1
GO:0051087~chaperone binding	0.046792097	PCSK1, BIRC5

Construction of the Interaction Networks

The logFC of genes PLAU (logFC = –1.32793955) and MMP8 (matrix metalloproteinase-8, logFC = 1.74640401) ranked first in down-regulated or up-regulated list, respectively, which were selected to construct the interaction networks. The data showed that there were 18 genes which can interact with PLAU at a high degree of confidence while there were 5 genes with MMP8 (Figure 1).

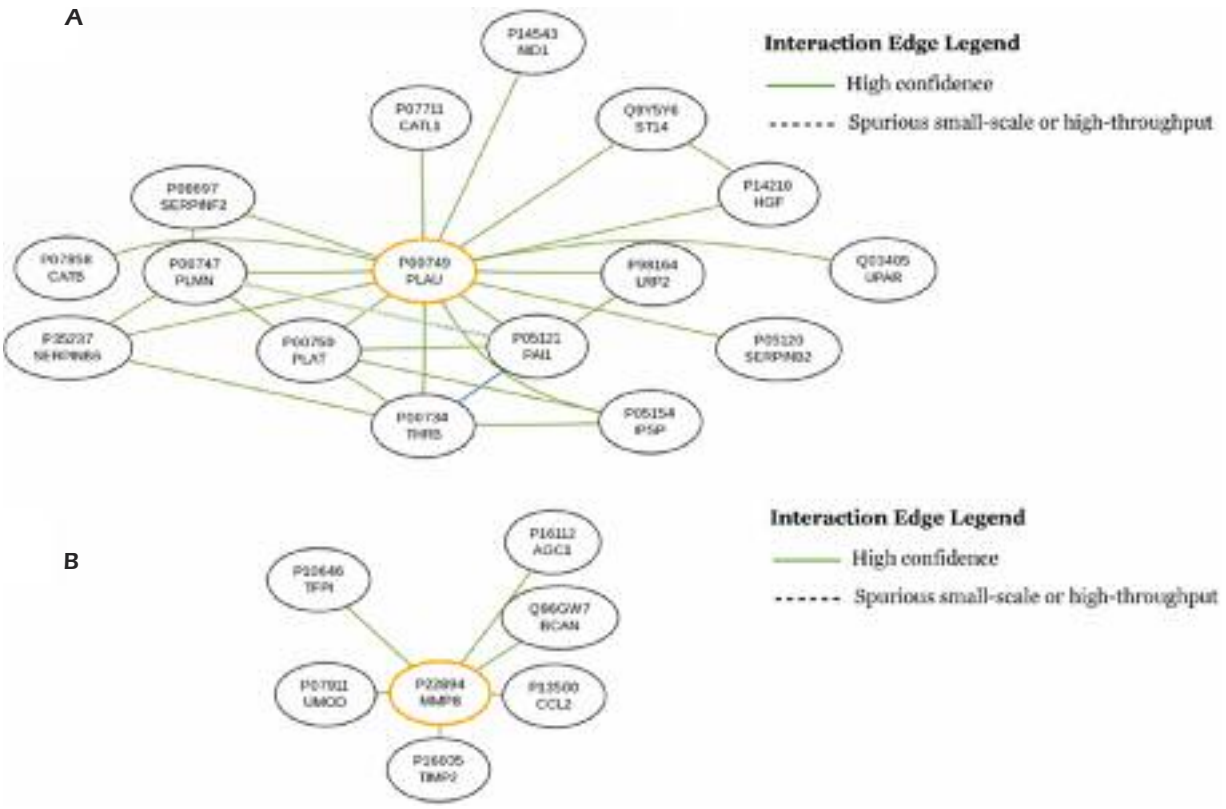

Figure 1. The interaction network constructed based on PLAU ***(A)*** and MMP8 ***(B)***.

Pathway Enrichment Analysis of the Genes in the Networks

To gain great insights into the function of genes in the interaction network, we used the WebGestalt to identify the significant pathways related with them. Finally, we got the pathway "complement and coagulation cascades" which PLAU participated in (Figure 2). Besides, PLAT (tissue type plasminogen activator) and SERPINF2 in the network were also enriched in this pathway.

Discussion

In the present study, we analyzed the gene expression patterns in a set of traumatic patients with sepsis and without sepsis by the computational methods and found 21 up-regulated genes and 37 down-regulated genes in the patients with sepsis. GO functional enrichment analysis revealed that they were mainly related to the "endopeptidase inhibitor activity" and "response to

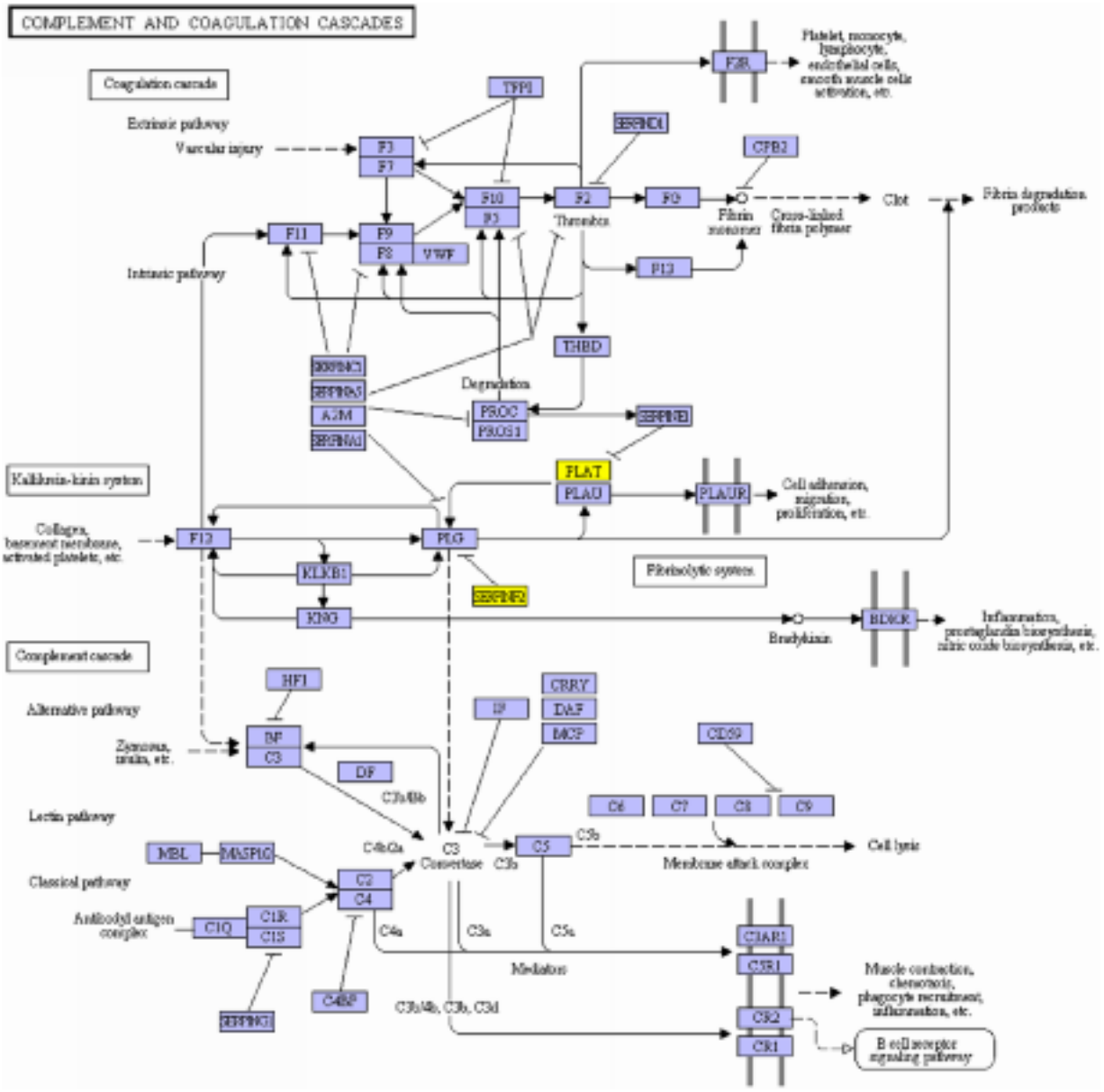

Figure 2. Pathway of complement and coagulation cascades. The yellow nodes represent genes which can interact with PLAU.

wounding", respectively. The expression value of PLAU and MMP8 underwent largest changes among the down- and up-regulated genes. Further analysis found that PLAU participate in the pathway of complement and coagulation cascade.

MMP8,one member of the MMPs, is a neutrophil-derived collagenase which is involved in the degradation of type I collagen[23]. The family of MMPs acts widely in inflammation to regulate barrier function, inflammatory cytokine and chemokine activity, and the generation of chemokine gradients[24]. The exacerbated inflammation has been found in MMP8 deficient mice[25,26]. In concordant with our result, current study demonstrated the up-regulation of MMP8 in the serum of critically ill sepsis patients and the serum MMP-8 levels among non-survivors were significantly higher than among survivors[27]. Furthermore, Solan et al[28] found the correlation of increased MMP-8 expression and activity in septic shock with decreased survival and increased organ failure in pediatric patients. All those suggest that MMP8 can be used as potential target for diagnosis or therapy of sepsis.

The gene PLAU (urokinase-type plasminogen activator) encodes a serine protease that catalyzes the conversion of plasminogen to plasmin. Urokinase generated plasmin has been reported to be a significant activator of pro-MMPs[29], which play important roles in inflammatory response as mentioned above. Thus PLAU may act as a mediator to regulate the inflammation. Indeed, evidence showed that PLAU can enhance the release of pro-inflammatory mediators, such as IL-1β, TNF-α and MIP-2 (macrophage-inflammatory protein-2)[30]. In addition, activation of the plasmin/plasminogen activator cascade occurs frequently in severe, life-threatening infections including sepsis. Elevation of PLAU antigen level has been found in the plasmid of severe sepsis patients and PLAU activity was detected in the severe sepsis patients but not in healthy persons[31]. Besides the up-regulation of PLAU found in our study, we predicted that PLAU and its interaction protein SERPINF2 both were associated with the pathway of "complement and coagulation cascades". SERPINF2 encodes a member of the serpin family of serine protease inhibitors which is a major inhibitor of plasmin. Notably, both complement and coagulation systems are proteolytic cascades that are consisted of a series of serine proteases with common structural characteristics and similar activating stimuli[32,33]. During sepsis, the activated coagulation pathway induced thrombosis and disseminated intravascular coagulation (DIC), which further aggravate the excessive inflammatory response and complement activation[32]. Therefore, we inferred that PLAU may play important roles in sepsis through the complement and coagulation pathway.

Conclusions

We showed the abnormal expression of genes in the traumatic patients complicated with sepsis. The candidate genes, PLAU and MMP8, which were most abnormally expressed in traumatic patients complicated with sepsis, may be potentially used as the targets for diagnosis and therapy of sepsis. However, the potential application of them in clinic need further study to support.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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