Differential gene sets profiling in gram-negative and gram-positive sepsis

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Abstract

Introduction: The host response to bacterial sepsis is reported to be nonspecific regardless of the causative pathogen. However, newer paradigms indicated that host response of Gram-negative sepsis may be different from Gram-positive sepsis and the difference has not been clearly clarified. The current study aimed to explore the difference by identifying the differential gene sets using genome-wide technique.

Methods: The training dataset GSE6535 and the validation dataset GSE13015 were used for bioinformatics analysis. The distinct gene sets of sepsis with different infections were screened using Gene set variation analysis (GSVA) and Gene set enrichment analysis (GSEA). The intersection gene sets based on the two algorithms were confirmed through Venn analysis. Finally, the common gene sets between GSE6535 and GSE13015 were determined by GSVA.

Results: Two immunologic gene sets in GSE6535 were identified based on GSVA, which could be used to discriminate sepsis caused by Gram-positive, Gram-negative or mixed infection. A total of 19 gene sets were obtained in GSE6535 through Venn analysis, revealed the heterogeneity of sepsis between gram-negative bacteria and gram-positive bacteria at the molecular level. The result was also verified by analysis the validation set GSE13015, 31 gene sets were identified by GSVA and GSEA. Furthermore, 10 common differential gene sets were finally confirmed based on GSVA for dataset GSE6535 and GSE13015.

Conclusions: Our data indicated that host response may differ dramatically depending on the inciting organism. The findings offer new insight to investigate the pathophysiology of bacterial sepsis.

Introduction

Sepsis is a potentially life-threatening condition caused mainly by bacterial infection, with high morbidity and mortality. It is now defined as infection accompanied with organ dysfunction resulting from dysregulated host responses [1]. The early phase of sepsis is characterized by systemic excessive inflammation followed by a prolonged period of sepsis-induced immunosuppression [2]. However, the pathophysiological mechanisms and host responses to sepsis have not been clearly elucidated, which hindered the development of new therapeutic approaches.

Although organs damaged by gram-positive sepsis are clinically no different from gram-negative sepsis, there is increasing evidence that differences exist in the host response [3]. The primary cause of infection with Gram-negative bacteria is endotoxin while Gram-positive bacteria rely on the production of exotoxin [4]. Gram-negative sepsis differs from Gram-positive sepsis in that the organisms often arise from enteric or genitourinary sources rather than skin, wounds and catheter sites [5]. In addition, Gram-positive pathogens require a highly orchestrated host response with intracellular killing by neutrophils and macrophages. This is different for Gram-negative, which may be readily killed in the extracellular space by antibody and complement [6]. It is gradually realized that the major difference between Gram-positive sepsis and gram-negative sepsis is the way in which they initiate disease [7]. Thus, exploring the difference in host response between gram-negative sepsis and gram-positive sepsis becomes increasingly important.

Microarray technology provides a powerful tool to examine genome-wide expression profiles. Although a great deal of information has become available on the molecular signatures of sepsis [8-10], few reports have compared the difference between Gram-negative sepsis and Gram-positive sepsis. After analysis the gene expression profiling of circulating neutrophils, Tang et al verified that there was no difference in the expression profile. Gram-positive sepsis and Gram-negative sepsis share a common host response at a transcriptome level [11]. However, the plasma IL-1β, IL-6, and IL-18 concentrations were significantly higher in gram-positive sepsis patients even though the host inflammatory responses to gram negative and gram positive stimuli share some common response elements [12].

The different mechanisms of sepsis caused by Gram-positive bacteria and Gram-negative bacteria was also illustrated previously [13-15]. It was also reported that NDUFB2, NDUFB8 and UQCRH may be associated with Gram-negative bacterial sepsis while LATS2 may contribute to the progression of Gram-negative bacterial sepsis [16]. Since sepsis was an overwhelming inflammatory response, it is really different to distinguish the difference at molecular levels with several differentially expressed genes. To further elucidate the effect of sepsis on host response, we undertook gene sets comparison analysis based on gene set variation analysis (GSVA) and Gene set enrichment analysis (GSEA) in this study. By screening differentially expressed gene sets (cellular pathways), we want to provide a novel approach to gain important biological insights into the cellular response of sepsis.

Materials And Methods

Microarray data

The training dataset of GSE6535 and validation dataset GSE13015 were obtained from gene expression omnibus database (www.ncbi.nlm.nih.gov/geo). The original study was approved by the ethics committee of each institution, and written informed consent was provided by the patients or their families. There were totally 72 critically ill patients in GSE6535, 17 of whom were served as control. Based on the results of clinical features and microbiological culture, 18 patients were diagnosed as Gram-positive sepsis, 25 were confirmed as Gram-negative sepsis while 12 were identified as mixed sepsis. The neutrophil RNA was isolated within 24 hours of admission and microarray experiments were then performed. Whole blood of 63 patients with sepsis was used to generate genome-wide transcriptional profiles in GSE13015. All patients were diagnosed as sepsis based on blood culture, including 46 patients with gram-negative bacteria (mainly *Burkholderia pseudomallei*) and 17 patients with gram-positive sepsis.

Gene set variation analysis

Probe IDs were firstly converted into their corresponding gene symbols. GSVA package in R platform (4.0.3) was used to calculate the enrichment score of the pathways in each sample, which was then visualized in a heatmap by the pheatmap package. The reference gene sets were the hallmark gene sets, C2 gene

sets, C7 gene sets and the threshold was $P<0.05$ using T test. Subsequently, the common gene sets between Gram-positive and Gram-negative samples, Gram-positive and mixed samples as well as Gram-negative and mixed samples were identified with the VennDiagram in R.

Protein-protein interaction network analysis

Protein-protein interaction (PPI) network was analyzed with the online database Search Tool for the Retrieval of Interacting Genes (STRING 11.0, http://stringdb.org). The distinct gene sets-encoded proteins were employed to build the PPI network with the default threshold value (a combined score ≥0.4). Then, the PPI network was constructed by means of Cytoscape software (version 3.8.0) and the plug-in of Molecular Complex Detection (MCODE) and cytoHubba were applied for further analyzed. The criteria for selection was that MCODE scores >5.

Gene set enrichment analysis

Gene set enrichment analysis (GSEA) was performed to explore the biological pathways of different groups. The annotated gene sets, "c2.all.v7.2.symbols.gmt", "c7.all.v7.1.symbols.gmt" and "h.all.v7.1.symbols.gmt", downloaded from the Molecular Signature Database (MSigDB,) were considered as the reference gene sets. The number of permutations was 1,000 and other parameters were set to default. A significant difference at P-value<0.05 was defined as the cutoff criteria after 1000-time permutations.

GO and KEGG enrichment analysis

Gene Ontology (GO) and Kyoto encyclopedia of Genes and Genomes (KEGG) were used to elucidate the potential gene functional annotation and pathway enrichment. Both GO and KEGG analysis were performed by R package "cluster Profiler" and adjusted P value<0.05 were regarded as statistically significant. GO analysis were comprised of biological process (BP), cellular component (CC) and molecular function (MF), describe the facilities of genes in three distinct biological aspects. The Bioconductor package in R platform was used to supply enrichment result visualization.

Results

Identify the distinct gene sets based on GSVA

The flowchart of this study is illustrated in Figure 1. All patients in GSE6535 were grouped according to the infection status and analyzed through GSVA. The variation of the activity for gene sets was estimated and the matrix containing enrichment scores was depicted in a heatmap (Fig. 2). Next, the enrichment score (ES) of gene sets between Gram-positive sepsis patients and Gram-negative sepsis patients was compared. There were totally 373 differential gene sets were confirmed. The ES heat map showed that the ES patterns may distinguish Gram-positive sepsis patients from Gram-negative sepsis patients easily (Fig. 3a). In addition, we also screened 640 differential gene sets between Gram- negative sepsis patients and mixed infection patients, 682 differential gene sets between Gram-positive sepsis patients and mixed infection patients, which were also displayed in heatmap (Fig. 3b, c). The top ten representative differential gene sets between different groups were also listed (Additional file 1: table S1). After intersection analysis, two distinct immunologic gene sets"GSE13522_CTRL_VS_T_CRUZI_Y_STRAIN_INF_SKIN_129_MOUSE_UP" , GSE23308_WT_VS_MINERALCORTICOID_REC_KO_MACROPHAGE_CORTICOSTERONE_TREATED_DN" were identified (Fig. 3d). The detailed expression of each infected patient is also described in the heatmap, which Gram-positive sepsis patients exhibit the relatively highest expression in gene set "GSE13522" and the lowest expression in gene set "GSE23308" (Fig. 3e).

PPI network construction, module analysis and hub genes identification

Next, the PPI network of the two distinct gene sets (335 genes) was constructed from STRING. Based on the information from this public database, a total of 242 nodes and 479 protein pairs were obtained while the isolated genes without interaction were removed. To further investigate the hub genes, the plug-in app "cytoHubba" were used to parse the network and top 5 hub genes were identified according to the "Degree" algorithm (Fig. 4a), including SRC (degree =33), IL1B (degree =20), CD40 (degree =20), TLR6 (degree =16), and CCL2 (degree =16). After that, the Module analysis was performed by MCODE and three modules were screened. The module 1 was the most significant module, located in the center of the entire PPI network, included 8 genes and 24 edges (Fig. 4b). The module 2 and module 3 had 11 nodes (Fig. 4c) and 6 nodes (Fig. 4d) respectively, containing several hub genes such as IL1B, TLR6 and CCL2 (Fig. 4d).

Screening differential gene sets with GSEA and GSVA

To further elucidate the different pathway involved in Gram-positive sepsis and Gram-negative sepsis, GSEA was performed between the two groups in GSE6535. It evaluates the microarray data by performing unbiased global searches for genes that are coordinately regulated in the three predefined gene sets. The results showed significant differences in enrichment. The analysis of the hallmark gene sets revealed that there were four significantly enriched gene sets, HALLMARK_APICAL_JUNCTION, HALLMARK_NOTCH_SIGNALING, HALLMARK_KRAS_SIGNALING_DN, HALLMARK_INTERFERON_ALPHA_RESPONS. The enrichment of c2 indicated that there were 226 differential gene sets while the enrichment of c7 showed 199 differential gene sets. The representative plots of each gene sets were are shown in Figure 5. After that, the intersection gene sets based on the two algorithms, GSVA and GSEA, were finally confirmed through Venn analysis. A total of 19 gene sets were obtained (Table 1), most of which are related to immunity.

GO and KEGG enrichment analysis

To gain more biological insight of the screened gene sets, GO annotation and KEGG pathway enrichment analysis were conducted with the 19 gene sets. The top 10 enriched GO terms and KEGG pathways were identified and presented in Figure 6. GO analysis showed that the most enriched MF terms were actin binding, cadherin binding, cytokine receptor binding and protein-macromolecule adaptor activity (Fig. 6a). For GO CC analysis, the top five significantly

enriched terms were cell-substrate junction, focal adhesion, collagen-containing extracellular matrix, cell leading edge and membrane region (Fig. 6b). In the BP, the genes were mainly enriched in response to virus, defense response to virus, response to interferon-gamma, cellular response to interferon-gamma and NF-κB signaling ((Fig. 6c). KEGG pathway analysis demonstrated that genes were mainly enriched in MAPK signaling pathway, Pathogenic Escherichia coli infection, Salmonella infection, Epstein-Barr virus infection and Influenza A ((Figure 6d).

Differential gene sets verification with GSE13015

The differential gene sets between patients with Gram-positive Sepsis and Gram-negative Sepsis were further verified with dataset GSE13015. According to GSEA, there were 7 significantly enriched gene sets in the hallmark gene sets, 296 in c2 gene sets and 404 in c7 gene sets. Although there was no common gene set with dataset GSE6535, 31 differential gene sets were confirmed after Venn analysis based on GSVA and GSEA (Table 1), including 5 in c7 gene sets and 26 in c2 gene sets. The further analysis showed that there were 10 common differential gene sets between dataset GSE13015 and dataset GSE6535 based on GSVA (Fig. 7a). Next, the result of gene sets comparison and the corresponding *P* value was also shown (Fig. 7b). Compared with Gram-negative sepsis patients, the expression of most gene sets was increased in gram-positive sepsis patients (6 in GSE6535 and 7 in GSE13015).

Discussion

In the present study, the host response to different invading pathogens was assessed using gene expression patterns. The results from training set revealed that the expression profiling of neutrophils could reliably distinguish the molecular difference. Exploring the potential differences in sepsis is essential to further understand the mechanism. GSVA provides increased power to detect subtle pathway activity changes in an unsupervised manner [17]. After GSVA enrichment and intersection analysis, two distinct immunologic gene sets were confirmed, which can be used to discriminate the different types of sepsis. It also indicated that the host immune system is activated even in the early stage of sepsis, rather than at the classic anti-inflammatory phase [18].

The functional interaction between proteins was also analyzed in the current study. Three densely connected regions and several hub genes were identified, which revealed important biological insights into the host response mediated by neutrophils. SRC belongs to a family of protein tyrosine kinases (PTKs), play a critical role in initiating the numerous intracellular signaling pathways, which affect cell migration, adhesion, phagocytosis, cell cycle, and cell survival [19]. It have been identified to be essential for the recruitment and activation of monocytes, macrophages, neutrophils, and other immune cells, also play a critical role in the regulation of vascular permeability and inflammatory responses in tissue cells [20]. Toll-like receptors (TLRs) play an essential role in pathogen recognition and activation of innate immunity. TLR6 acts in a heterodimer form with TLR2, which mediates cell response to Gram-positive bacterial components. TLR2 regulates important neutrophil functions, including adhesion, generation of reactive oxygen species, release of chemokines, and activate major proinflammatory signaling pathways, such as NF-κB pathway [21]. IL1B is an important mediator of the inflammatory response, and is involved in a variety of cellular activities, including cell proliferation, differentiation, and apoptosis [22]. CD40 is a receptor on antigen-presenting cells of the immune system and is essential for mediating a broad variety of immune and inflammatory responses [23]. CCL2 is one of the key chemokines that regulate migration and infiltration of monocytes and macrophages [24].

Although the clinical manifestations of sepsis caused by gram-negative bacteria and gram-positive bacteria may appear similar, our study indicated that the host physiological responses to these pathogens may behave differently due to the inciting organism. The findings were concordant with the results of Feezor et al, the host inflammatory responses to gram-negative and gram-positive stimuli share some common response elements but also exhibit distinct patterns of cytokine appearance and leukocyte gene expression [12]. It was also confirmed by genome-wide gene expression analysis of a mouse sepsis model after infusion of either live Escherichia coli or Staphylococcus aureus [25]. The study of Li et al also determined that there was no significant difference in the expression profile between Gram-positive and gram-negative samples, however, several candidate genes may be biomarkers for distinguishing the different infections [16]. Unlike these reports, the current study mainly focuses on the differences in pathways or gene sets rather than single gene, because no single molecule can recapitulate the complex changes that occur in sepsis.

Gram-positive and Gram-negative bacteria activate different receptor pathways in the host, among which Toll-like receptors play a pivotal role [26]. TLR4 is regarded as the major lipopolysaccharide receptor for Gram-negative bacteria [27], whereas cellular responses to components of gram-positive bacteria are mainly mediated via TLR2 [28]. Individual TLRs differentially recruit specific adaptor molecules, such as MyD88, TRIF, TIRAP/MAL, or TRAM, leading to the activation of NF-κB and MAP kinases pathways [29]. The results were also confirmed in our study after KEGG analysis, the genes were mainly enriched in MAPK signaling pathway. It was also reported that combined signaling of TLR2 and CD137 augments antibacterial activities of neutrophils, while that of TLR4-CD137 diminishes them [30]. Gram-negative and gram-positive bacteria do not trigger monocyte activation through similar pathways. Lipopolysaccharide but not *Staphylococcus aureus* Cowan used CD14 internalization to induce cellular activation, resulting in p38 MAP kinase and ERK kinase activation pathways [31]. Besides that, host-response pathway correlated metabolites could be used to distinguish between bacterial- and host-induced metabolic changes [32].

According to the sepsis guidelines, empiric antimicrobial therapy was recommended before obtaining blood cultures [33]. However, the increasing antibiotic resistance requires novel approaches for early identification of the causative microorganism [34]. After analysis the plasma free circulating DNA from sepsis patients, Grumaz et al developed an alternative diagnostic platform for the identification of infectious microorganisms in roughly 30 hours by next-generation sequencing [35]. Recently, the focus for accurate and rapid diagnosis has moved from single disease-specific markers to bioprofiles or biosignatures comprising a well-defined set of reliable molecular indicators using platforms such as proteomics [36] transcriptomics [37] genomics [38] and metabolomics [39]. Thus, based our results, the differential gene sets between Gram-negative sepsis and Gram-positive sepsis could be further explored for diagnosis purpose.

The data used in training set was obtained from neutrophils collected within 24 hours. We chose neutrophils rather than other leukocytes because neutrophils are crucial components of an early host's innate immune response [40]. Experimental conditions were similar for all patients to minimize the difference

between individual patients. Nonetheless, there are some limitations. The findings were based on a microarray dataset from a single institution with a small sample size. Although similar results were obtained in the validation set, a large sample from multiple centers is needed to further verify our results. One other hand, gene expression profiles are known to change rapidly in the early stages of sepsis [41]. Thus, the timing of microarray analysis should also be considered to consolidate our results. In addition, specimens of different sources may affect the genome expression characteristics. In the validation dataset GSE13015, whole blood contains a mixed population of leukocytes, the proportion of which varies depending on the stage of sepsis and between individuals. However, the 10 common gene sets in the two datasets also indicated the molecular difference between gram-negative and gram-positive sepsis.

Conclusions

In general, our results highlight the heterogeneity of sepsis between gram-negative bacteria and gram-positive bacteria at the molecular level. The screened differential gene set indicated that host response may differ dramatically depending on the inciting organism. The findings offer new insight to investigate the initiating mechanisms of sepsis and provide a potential method to identify the causative organism at the onset of sepsis.

Abbreviations

GSVA: Gene set variation analysis; GSEA: Gene set enrichment analysis; PPI: Protein-protein interaction; ES: enrichment score; GO: Gene Ontology; KEGG: Kyoto encyclopedia of Genes and Genomes; BP: Biological Process; CC: Cellular Component; MF: Molecular Function

Declarations

Ethical approval and consent to participate

The original trial whose dataset was analyzed was approved by the local ethics committees at each participating institution and by the responsible state data protection boards.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

JG and BH conceived and developed the study, obtained funding for the study. QW and JL wrote the manuscript, prepared the figures. JG, XG and ZX conducted the biostatistical analysis. YZ contributed to the data collection. All authors read and approved the final manuscript.

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Tables

Table 1 The differential gene sets between gram-negative and gram-positive sepsis based on GSVA and GSEA.

	GSE6535	GSE13015
1	MANNE_COVID19_NONICU_VS_HEALTHY_DONOR_PLATELETS_UP	GSE17721_12H_VS_24H_CPG_BMDC_
2	GSE19825_NAIVE_VS_IL2RALOW_DAY3_EFF_CD8_TCELL_UP	GSE41978_WT_VS_BIM_KO_KLRG1_L
3	GSE4142_PLASMA_CELL_VS_MEMORY_BCELL_DN	GSE28737_FOLLICULAR_VS_MARGIN
4	GSE21546_UNSTIM_VS_ANTI_CD3_STIM_SAP1A_KO_AND_ELK1_KO_DP_THYMOCYTES_UP	GSE13485_CTRL_VS_DAY3_YF17D_V
5	GSE45365_CD8A_DC_VS_CD11B_DC_IFNAR_KO_UP	GSE13485_PRE_VS_POST_YF17D_VA
6	GSE1432_CTRL_VS_IFNG_24H_MICROGLIA_DN	REACTOME_CASPASE_ACTIVATION_V
7	MIKKELSEN_MEF_LCP_WITH_H3K4ME3	RUAN_RESPONSE_TO_TNF_TROGLIT
8	GSE34006_WT_VS_A2AR_KO_TREG_DN	GALE_APL_WITH_FLT3_MUTATED_DN
9	GSE40273_EOS_KO_VS_WT_TREG_DN	RASHI_RESPONSE_TO_IONIZING_RAD
10	GSE21927_SPLENIC_C26GM_TUMOROUS_VS_BONE_MARROW_MONOCYTES_UP	NAKAMURA_CANCER_MICROENVIRO
11	REACTOME_RHO_GTPASES_ACTIVATE_WASPS_AND_WAVES	LIM_MAMMARY_LUMINAL_MATURE_
12	GSE41176_UNSTIM_VS_ANTI_IGM_STIM_TAK1_KO_BCELL_6H_UP	REACTOME_CASPASE_ACTIVATION_V
13	HUPER_BREAST_BASAL_VS_LUMINAL_UP	BOWIE_RESPONSE_TO_TAMOXIFEN
14	GSE17721_CTRL_VS_LPS_1H_BMDC_UP	SUMI_HNF4A_TARGETS
15	GSE21360_NAIVE_VS_QUATERNARY_MEMORY_CD8_TCELL_DN	WP_INTRACELLULAR_TRAFFICKING_
16	GSE37533_PPARG1_FOXP3_VS_FOXP3_TRANSDUCED_CD4_TCELL_PIOGLITAZONE_TREATED_UP	SCHAEFFER_PROSTATE_DEVELOPME
17	GRAESSMANN_RESPONSE_TO_MC_AND_SERUM_DEPRIVATION_UP	KUMAR_AUTOPHAGY_NETWORK
18	GSE37534_UNTREATED_VS_PIOGLITAZONE_TREATED_CD4_TCELL_PPARG1_AND_FOXP3_TRASDUCED_DN	QI_HYPOXIA
19	GSE21546_WT_VS_SAP1A_KO_DP_THYMOCYTES_UP	BILANGES_SERUM_RESPONSE_TRAN
20		BILANGES_SERUM_AND_RAPAMYCIN
21		WP_CYTOPLASMIC_RIBOSOMAL_PRO
22		CHNG_MULTIPLE_MYELOMA_HYPERI
23		REACTOME_EUKARYOTIC_TRANSLAT
24		KEGG_RIBOSOME
25		REACTOME_RESPONSE_OF_EIF2AK4_
26		REACTOME_ACTIVATION_OF_THE_MI
27		REACTOME_EUKARYOTIC_TRANSLAT
28		REACTOME_NONSENSE_MEDIATED_I
29		HOLLEMAN_ASPARAGINASE_RESIST_
30		REACTOME_SELENOAMINO_ACID_ME
31		GUENTHER_GROWTH_SPHERICAL_VS

Figures

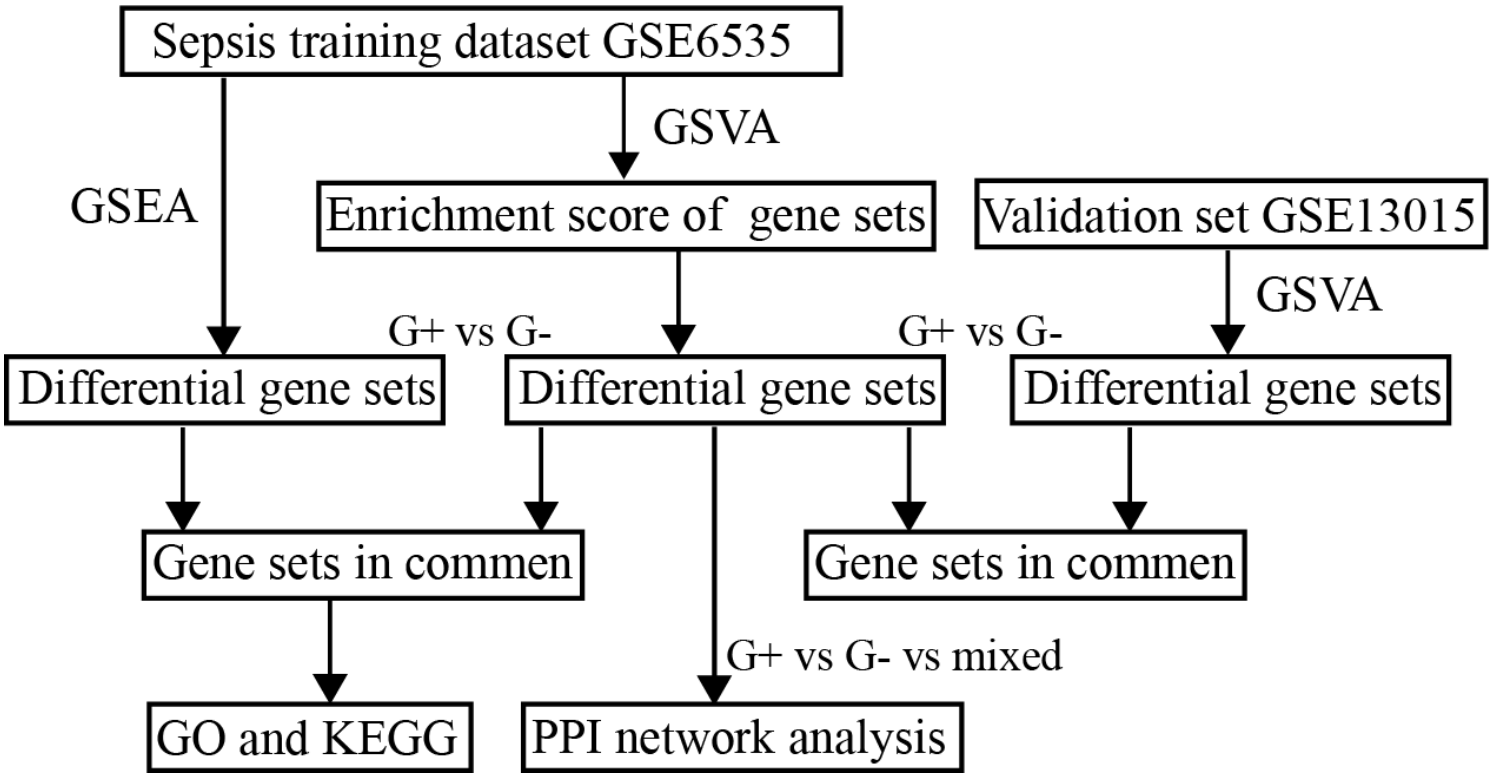

Figure 1

Analysis workflow of this study.

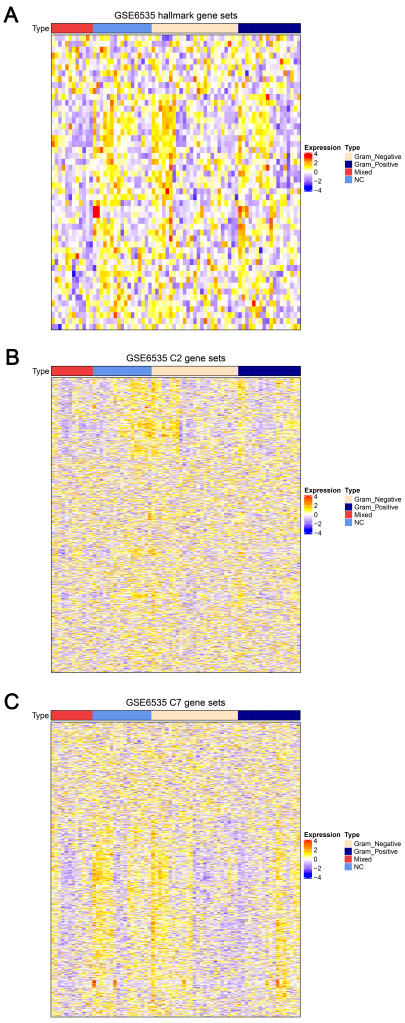

Figure 2

Heatmap of enrichment score of hallmark gene sets (A), C2 gene sets (B) and C7 gene sets (C) in patients with gram-positive sepsis, gram-negative sepsis, mixed sepsis and normal control.

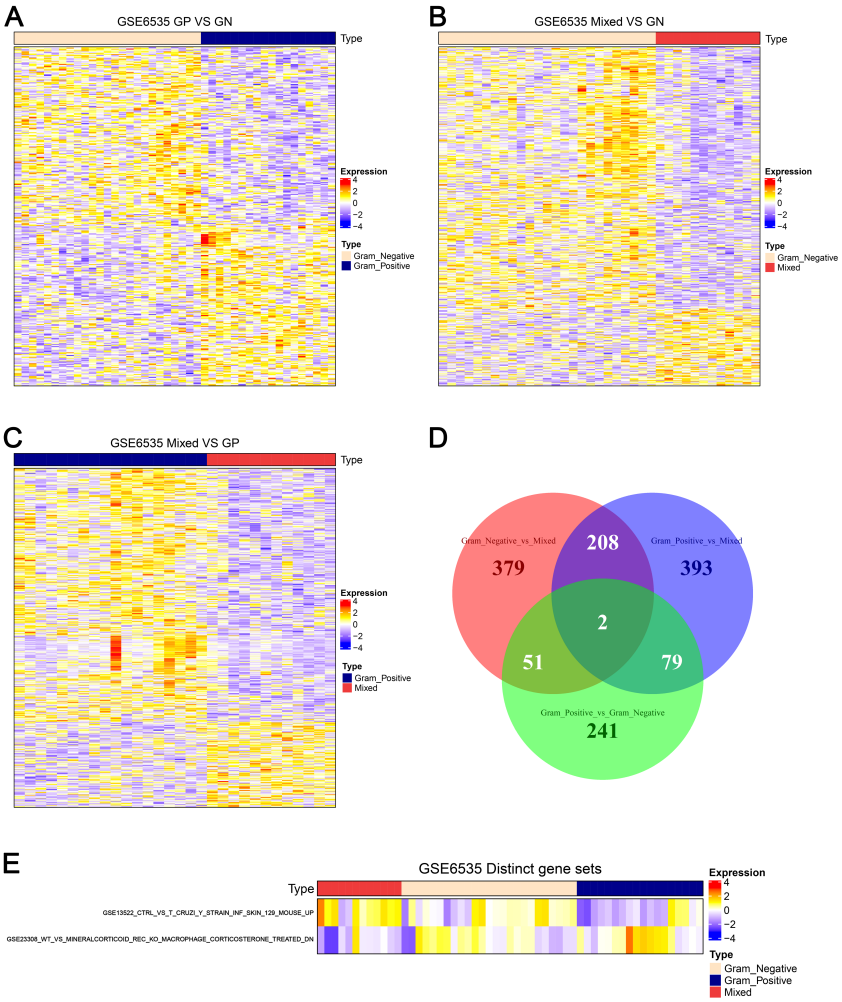

Figure 3

Heatmap of deferential gene sets between Gram-positive sepsis and Gram-negative sepsis (A), mixed sepsis versus Gram-negative sepsis (B), mixed sepsis versus Gram-positive sepsis (C). Venn diagram of differential gene sets across various infection types (D) and the two distinct gene sets (E).

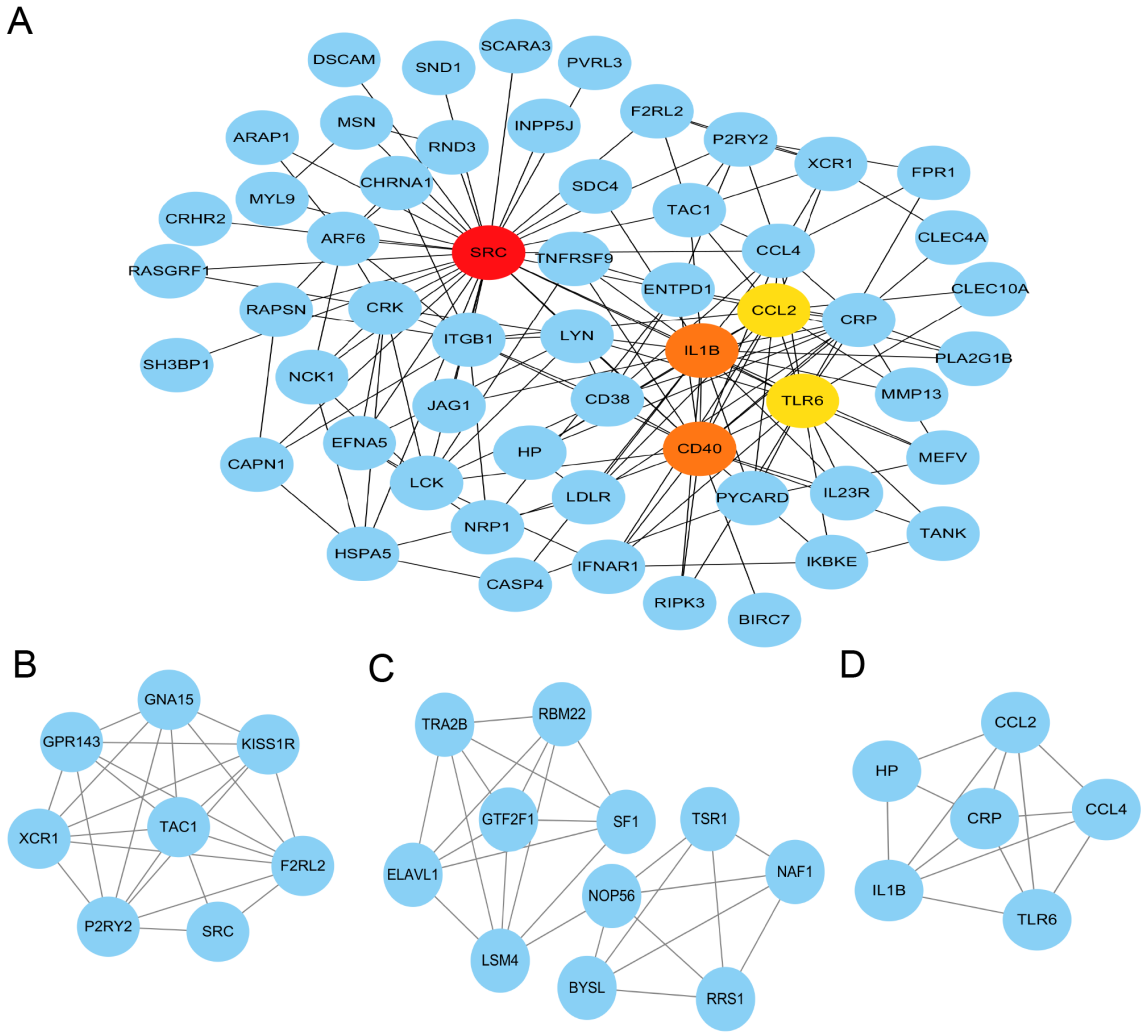

Figure 4

Protein-protein interaction network of the two distinct gene sets, the top 5 hub genes (A) and the top 3 clusters (B, C, D).

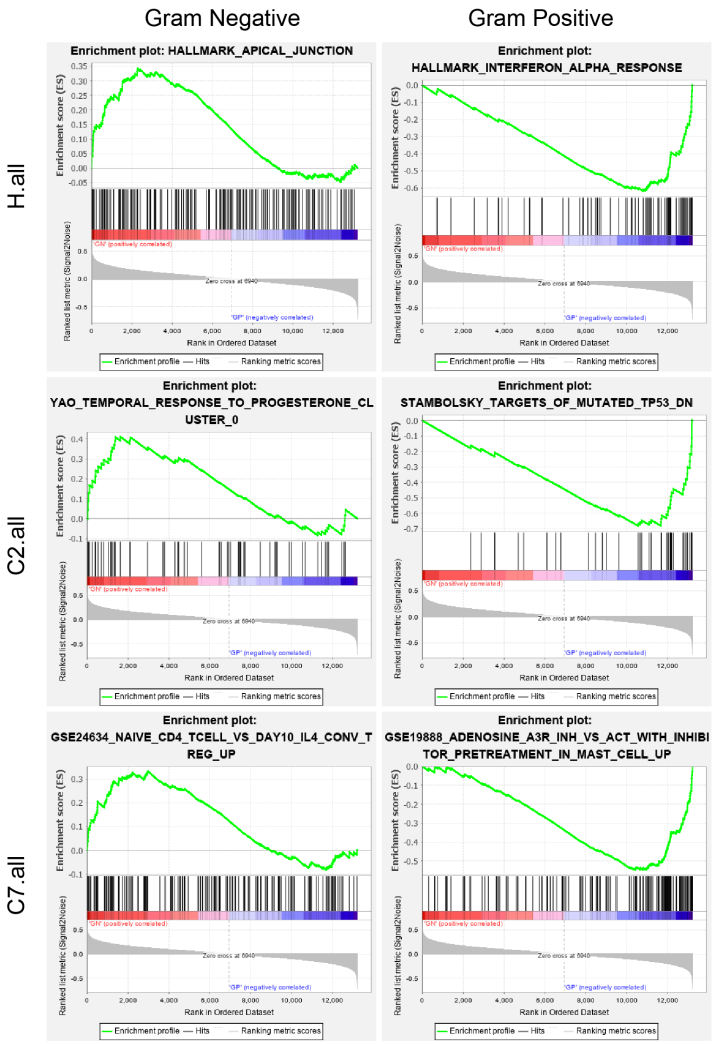

Figure 5

Representative images of gene set enrichment analysis for annotated gene sets.

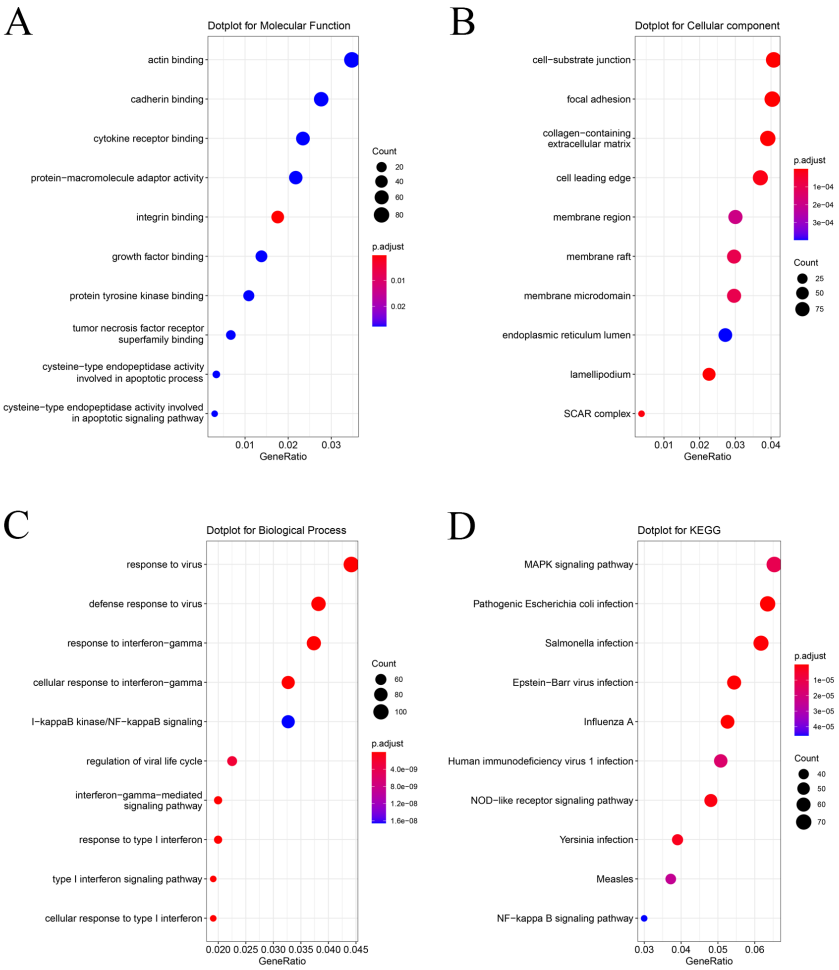

Figure 6

Gene Ontology (GO) and Kyoto encyclopedia of Genes and Genomes (KEGG) pathway of the genes involved in the intersection gene sets. Molecular function (A), Cellular component (B), Biological process (C) for GO analysis. The top 10 of KEGG pathway enrichment (D).

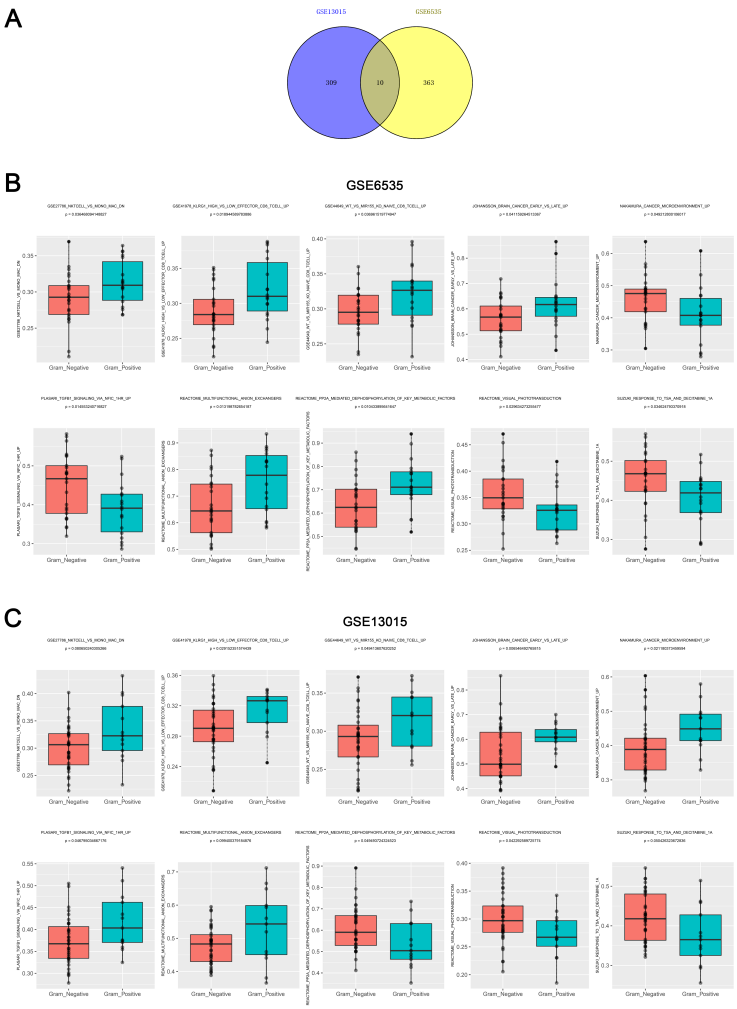

Figure 7

Venn diagram of the common gene sets between GSE6535 and GSE13015 based on gene set variation analysis (A) and the expression of in GSE6535 (B) and (C).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- tableS1.docx