

Identification of biomarkers and pathways of mitochondria in sepsis patients

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Abstract

Sepsis is a life-threatening condition associated with significant morbidity and mortality, but limited treatment. Mitochondria are recently recognized to be related to the pathophysiology of sepsis, and mitochondria could serve as a potential drug target. In our study, we aim to identify biological functions and pathways of mitochondria during the processes of sepsis by using a bioinformatics method to elucidate their potential pathogenesis. The gene expression profiles of the GSE167914 dataset were originally created by using the Nanostring nCounter Elements™ TagSet preselected for mitochondrial biogenesis and function panel. The biological pathways were analyzed by the Kyoto Encyclopedia of Genes and Genomes pathway (KEGG), Gene Ontology (GO), and Reactome enrichment. KEGG and GO results showed the Neurodegeneration pathways such as Huntington and Parkinson pathways were mostly affected in the development of sepsis. Moreover, we identified several mitochondrial genes including TOMM40, TOMM20, TIMM22, TIMM10, TIMM17A, TIMM9, TIMM44 were involved in the regulation of protein translocation into mitochondria. Further, we predicted several regulators that had the ability to affect the mitochondria during sepsis by L1000fwd analysis. Thus, this study provides further insights into the mechanism of mitochondrial function during sepsis.

Introduction

Sepsis is caused by the dysregulated host response to infections from tissue damage or organ dysfunction¹. Sepsis is a serious disease with a mortality of 15-20%, which is characterized not only by the upregulation of inflammation but also by the strong immune suppression². Understanding the pathophysiology of sepsis may aid in the development of novel therapies³. The effects of impaired cellular functions such as mitochondrial dysfunction and cell death mechanism in sepsis-associated organ dysfunction are beginning to be unveiled⁴.

The significance of mitochondrial dysfunction in sepsis is previously recognized⁵. As the mitochondrion is a critical organelle for multiple cellular processes such as adenosine triphosphate (ATP) production, intracellular calcium homeostasis, and the production of ROS and some hormones^{6, 7}. Moreover, mitochondria are also associated with

triggering the intrinsic pathway of apoptosis, which is caused by the outer membrane permeabilization⁸. The mitochondrial functions are changed during sepsis, which includes reduced oxidative phosphorylation, increased ROS production, and altered mitochondrial biogenesis⁹. One hypothesis to explain the dysregulated mitochondrial function is that the decreased oxidative phosphorylation might contribute to the reduced production of potentially harmful ROS¹⁰. Mitophagy and mitochondrial biogenesis might be involved in sepsis as a mechanism to reduce the harmful effects of mitochondrial dysfunction⁵. Therefore, during the progression of sepsis, there are various mitochondrial alterations at different times. However, what kind of alteration affects the mitochondrial functions is unclear. Thus, studying the regulation of mitochondria and the related pathways may be a convincing strategy to study the mechanism of sepsis. In this study, we investigated the effect of mitochondrial alterations in sepsis patients. We identified several DEGs, candidate inhibitors, and the relevant biological process in sepsis patients by utilizing comprehensive bioinformatics analyses. The functional enrichment analysis and protein-protein interaction analysis were used for discovering significant gene nodes. These key genes and signaling pathways may be essential to therapeutic interventions of sepsis.

Methods

Data resources

The GSE167914 dataset was obtained from the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). The data was produced by Nanostring nCounter Elements™ TagSet preselected for mitochondrial biogenesis and function panel, Faculty of Medicine, Universitas Tarumanagara, Jakarta Barat, DKI Jakarta, Indonesia. RNA-Seq analysis was performed using peripheral blood of infection and sepsis patients as well as healthy controls.

Data acquisition and preprocessing

The GSE167914 dataset that contains gene expression related to mitochondrial function from the peripheral blood of infection and sepsis patients as well as healthy

controls was conducted by R script^{11, 12}. We used a classical t test to identify DEGs with $P < .01$ and fold change ≥ 1.5 as being statistically significant.

Gene functional analysis

Gene Ontology (GO) is a community-based bioinformatics resource that contains the model Biological Process, Molecular Function, and Cellular Component. Kyoto Encyclopedia of Genes and Genomes (KEGG) database is a useful tool that integrates functional information, biological pathways, and sequence similarity¹³. GO and KEGG pathway analyses were performed by utilizing the Database for Annotation, Visualization, and Integrated Discovery (DAVID) (<http://david.ncifcrf.gov/>) and Reactome (<https://reactome.org/>). $P < 0.05$ and gene counts > 10 were considered statistically significant.

Module analysis

Molecular Complex Detection (MCODE) of Cytoscape software was used to study the connected regions in protein-protein interaction (PPI) networks. The significant modules and clusters were selected from the constructed PPI network using MCODE and String (<https://string-db.org/>). The pathway enrichment analyses were performed by using Reactome, and $P < 0.05$ was used as the cutoff criterion.

Reactome analysis

The Reactom pathway (<https://reactome.org/>) was used to obtain the visualization, interpretation, and analysis of potential pathways. $P < .05$ was considered statistically significant.

Results

Identification of DEGs of mitochondria from the blood of sepsis patients

The peripheral blood of sepsis patients and healthy controls were harvested to analyze the DEGs (differentially expressed genes) of mitochondria. Patients fulfilling infection or sepsis criteria were recruited from the emergency department (Faculty of Medicine, Universitas Tarumanagara, Velma Herwanto, Indonesia). A total of 56 genes were

identified to be differentially expressed in sepsis patients with the threshold of $P < 0.05$. The top 10 up- and down-regulated genes are listed in table 1.

KEGG analysis of DEGs of mitochondria from the blood of sepsis patients

To identify the biological functions and potential mechanisms of DEGs of mitochondria from sepsis patients and healthy controls, we performed KEGG pathway enrichment analysis and created a visual graph (Supplemental Table S1). KEGG pathway (<http://www.genome.jp/kegg/>) is a gene collection for exploring the molecular interaction, reaction, and relation networks. Our study showed top ten enriched KEGG pathways including “Neurodegeneration”, “Platinum drug resistance”, “p53 signaling pathway”, “Huntington disease”, “Mitophagy”, “Apoptosis”, “Parkinson disease”, “Apoptosis-multiple species”, “Measles”, and “Colorectal cancer” (Figure 1).

GO analysis of DEGs from the blood of sepsis patients

Gene Ontology (GO) analysis is a commonly used tool for classifying genes, which includes cellular components (CC), molecular functions (MF), and biological processes (BP). Here, we identified the top ten cellular components including “mitochondrial inner membrane”, “organelle outer membrane”, “outer membrane”, “mitochondrial outer membrane”, “mitochondrial protein complex”, “intrinsic component of organelle membrane”, “intrinsic component of mitochondrial membrane”, “integral component of organelle membrane”, “integral component of mitochondrial membrane”, and “intrinsic component of mitochondrial outer membrane” (Figure 1). We then identified the top ten biological processes: “mitochondrial transport”, “protein localization to mitochondrion”, “establishment of protein localization to mitochondrion”, “protein targeting to mitochondrion”, “mitochondrial transmembrane transport”, “mitochondrial membrane organization”, “ATP transport”, “adenine nucleotide transport”, “purine ribonucleotide transport”, and “purine nucleotide transport” (Figure 1). We also identified the top ten molecular functions: “organic anion transmembrane transporter activity”, “anion transmembrane transporter activity”, “ATP transmembrane transporter activity”, “adenine nucleotide transmembrane transporter activity”, “purine ribonucleotide transmembrane transporter activity”, “purine nucleotide transmembrane transporter

activity”, “nucleotide transmembrane transporter activity”, “organophosphate ester transmembrane transporter activity”, “nucleobase-containing compound transmembrane transporter activity”, and “carbohydrate derivative transmembrane transporter activity” (Figure 2 and Supplemental Table S1).

PPI network and Module analysis

We constructed PPI networks to analyze the relationships of DGEs at the protein level. The criterion of combined score >0.7 was chosen and the PPI network was constructed by using 55 nodes and 106 interactions. Among these nodes, the top ten genes with the highest scores are shown in Table 2. The significant modules of DEGs of mitochondria from the blood of sepsis patients were selected to show the functional annotation (Figure 3).

Reactome Pathway analysis

To further understand the potential functions of DEGs, we also identified several signaling pathways by using Reactome Pathway Database. The top ten signaling pathways include “Mitochondrial protein import”, “Protein localization”, “Mitochondrial biogenesis”, “Cristae formation”, “The citric acid (TCA) cycle and respiratory electron transport”, “Formation of ATP by chemiosmotic coupling”, “Organelle biogenesis and maintenance”, “Intrinsic Pathway for Apoptosis”, “Respiratory electron transport, ATP synthesis by chemiosmotic coupling, and heat production by uncoupling proteins”, and “Activation of PUMA and translocation to mitochondria” (Supplemental Table S2). We then constructed the visual reaction map according to the signaling pathways (Figure 4).

Potential inhibitors for the treatment of sepsis

To further know the potential inhibitors for the treatment of sepsis, we introduced the L1000 fireworks display system that can predict bioactive molecules. The system indicated the potential pathways that may be inhibited. We selected the top ten molecules according to the DEGs and the inhibitor map: “BRD-K39757396”, “BRD-K39829853”, “veliparib”, “BRD-K32862555”, “halcinonide”, “DG-041”, “HC-toxin”, “UK-356618”, “XMD-1150”, and “GSK-461364” (Figure 5 and Supplemental Table S3).

Discussion

Sepsis is a kind of inflammation caused by a serious response of the immune system and finally led to multiorgan failure and death¹⁴. Sepsis is related to the activation of innate immunity through various pathological processes¹⁵. For example, the NF- κ B signaling pathway as the inflammation center in various diseases^{16, 17} is activated by the pathogen-associated molecular patterns (PAMPs) during the beginning of sepsis¹⁸. Mitochondria provide energy to cells and produce ATP through the basic process of respiration. Numerous cells respond to stresses such as cytokines by triggering mitochondria-dependent signals that initiate cellular protective responses¹⁹. Thus, based on the mitochondrial study on sepsis patients, our study may provide gene evidence for clinical trials of sepsis.

To understand the effects of mitochondria in sepsis, we analyzed the mitochondrial respiration and gene expression related to mitochondrial functions from the peripheral blood of sepsis patients and healthy controls. Ten proteins were selected according to the PPI network analysis, which may be important during the development of sepsis. TOMM40 is associated with apolipoprotein E (APOE), which further influences age-related memory performance during Alzheimer's disease²⁰. TOMM20 promotes proliferation, resistance to apoptosis, and chemicals²¹. In melanoma cells, enhanced ROS leads to the oxidation and oligomerization of the mitochondrial outer membrane protein Tom20²². TIM22 is the complex in the mitochondrial inner membrane²³. Mitochondrial acylglycerol kinase assembles with TIMM22 to support the import of a subset of multi-spanning membrane proteins²⁴. TIMM10 is identified as the blood-based biomarkers for pulmonary tuberculosis by modeling and mining molecular interaction networks²⁵. TIM17A is the stress-regulated subunit of the translocase of TIM23, which is downregulated by the eukaryotic initiation factor 2 α (eIF2 α)²⁶. TIM9 and TIM44 are responsible for the transport of proteins across the inner membrane²⁷.

KEGG and GO analyses indicated that neurodegeneration was the main pathological process during sepsis. The KEGG analysis showed "Neurodegeneration", "Huntington disease", and "Parkinson disease" were the most phenotypes during sepsis, which suggested that the brain or nerve damage was the most affected part during the infection. Similarly, Catherine N Widmann reported that sepsis could cause an increase

in the permeability of the blood-brain barrier, which may lead to a rapid decline in cognitive function or coma²⁸. Thus, sepsis may increase the brain's susceptibility to neurodegenerative disease. Moreover, we also found the cell death-related signaling processes “p53 signaling pathway”, “Mitophagy”, and “Apoptosis” were involved in sepsis. These signaling pathways were widely enhanced in the damaged cells or cancers²⁹⁻³⁴. Interestingly, these signaling pathways are regulated by the circadian genes such as Clock^{16, 35}. Circadian clocks play important roles in the physiological and pathophysiological processes³⁶⁻⁴⁰, such as controlling the immune checkpoint pathways in immune cells⁴¹. The GO analysis also showed the mitochondrial activity was enhanced during sepsis. Processes like “mitochondrial transport”, “protein localization to mitochondrion”, “establishment of protein localization to mitochondrion” were associated with the mitochondrial function, which suggested that sepsis could affect the translocation function of mitochondria.

In summary, we identified the potential pathways in sepsis patients by analyzing the mitochondrial gene functions. Neurodegeneration diseases such as Huntington's disease and Parkinson's disease are the mainly triggered diseases during sepsis. Future studies will focus on the administration of potential mitochondrial regulators on clinical trials. This study thus provides further insights into the treatment of sepsis, which may facilitate drug development.

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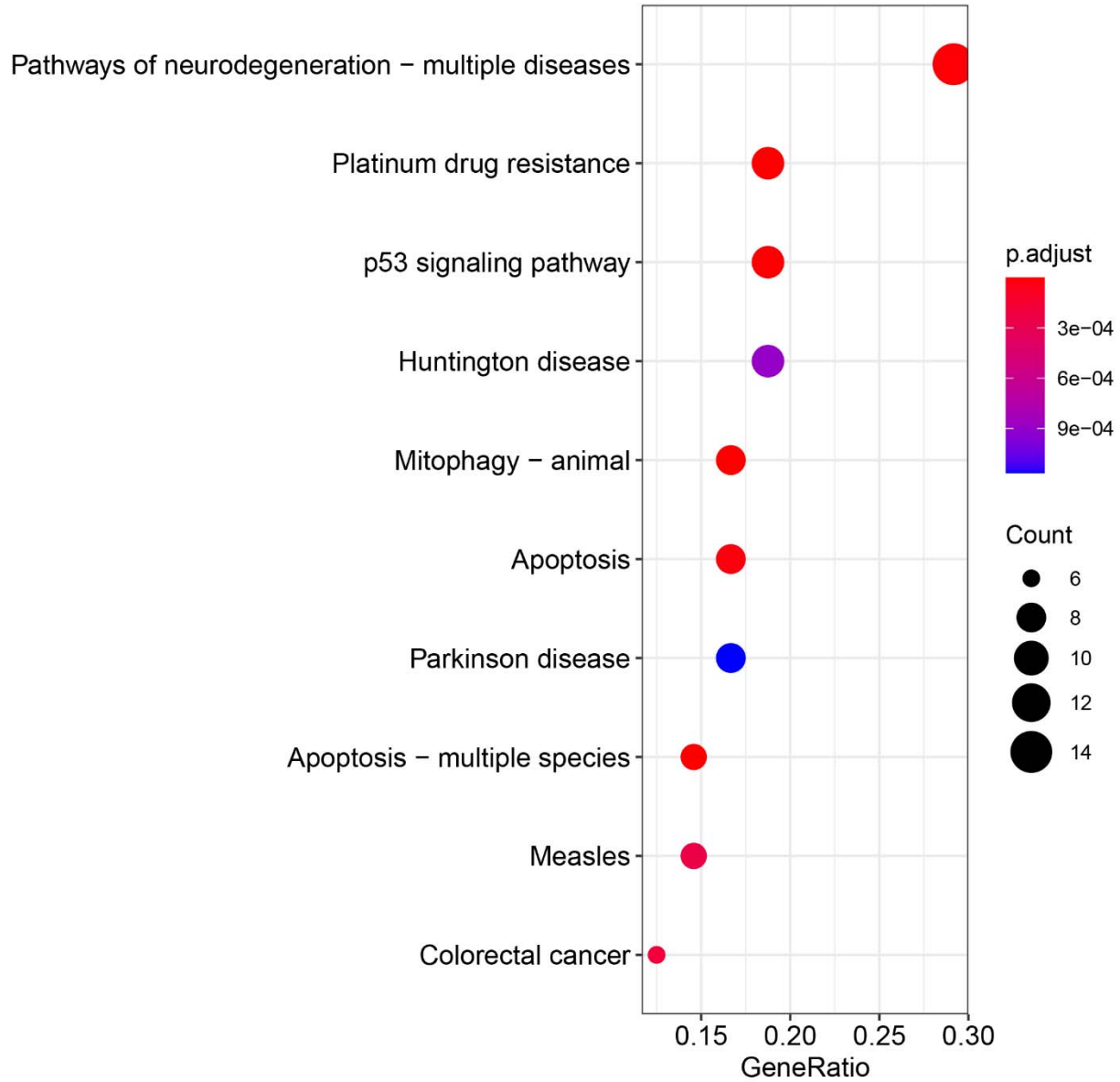


Figure 1. The KEGG pathways enriched by the DEGs. DEGs =differentially expressed genes, KEGG = Kyoto Encyclopedia of Genes and Genomes.

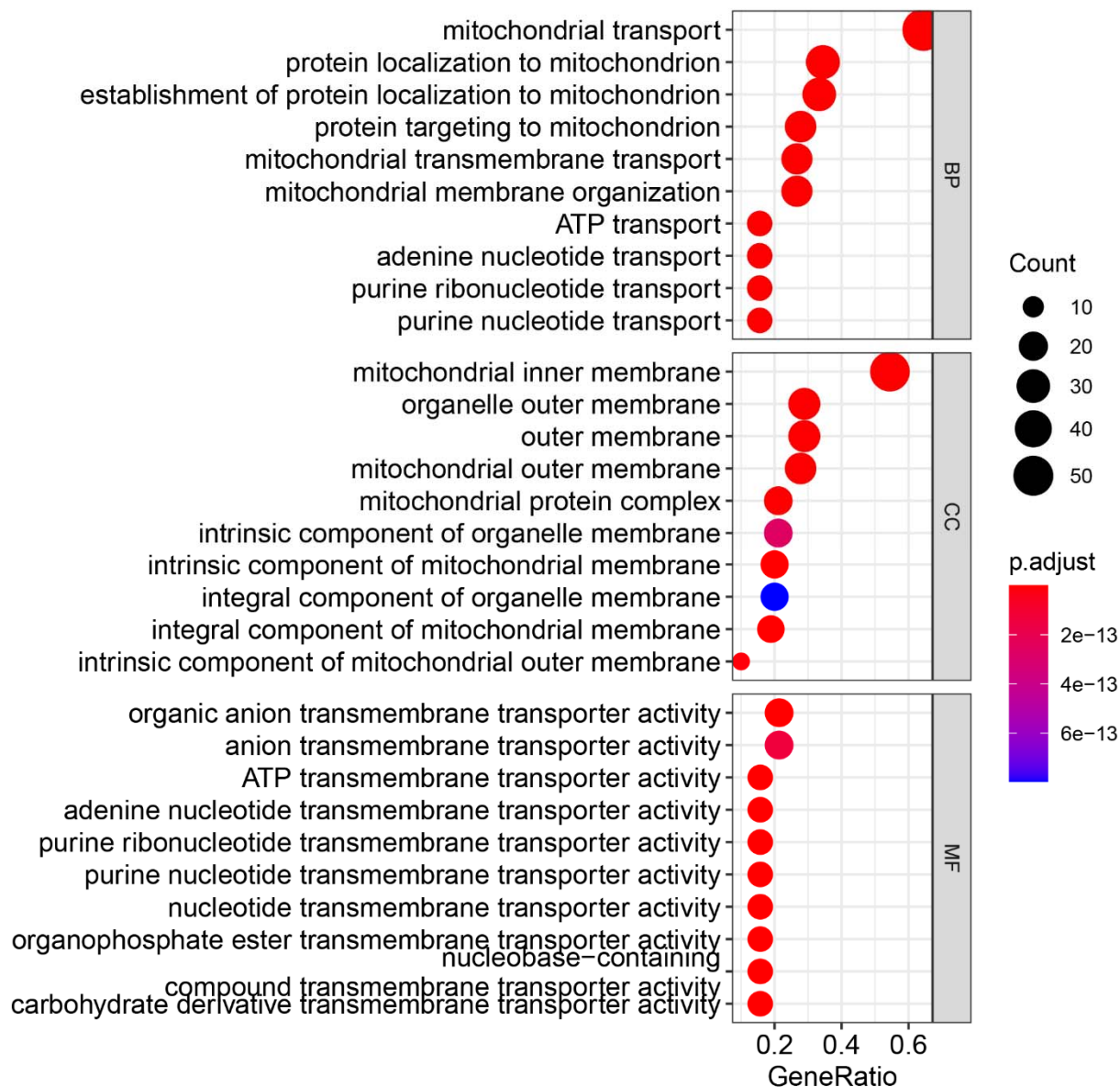


Figure 2. The biological process (BP), cellular component (CC), and molecular function (MF) terms enriched by the DEGs.

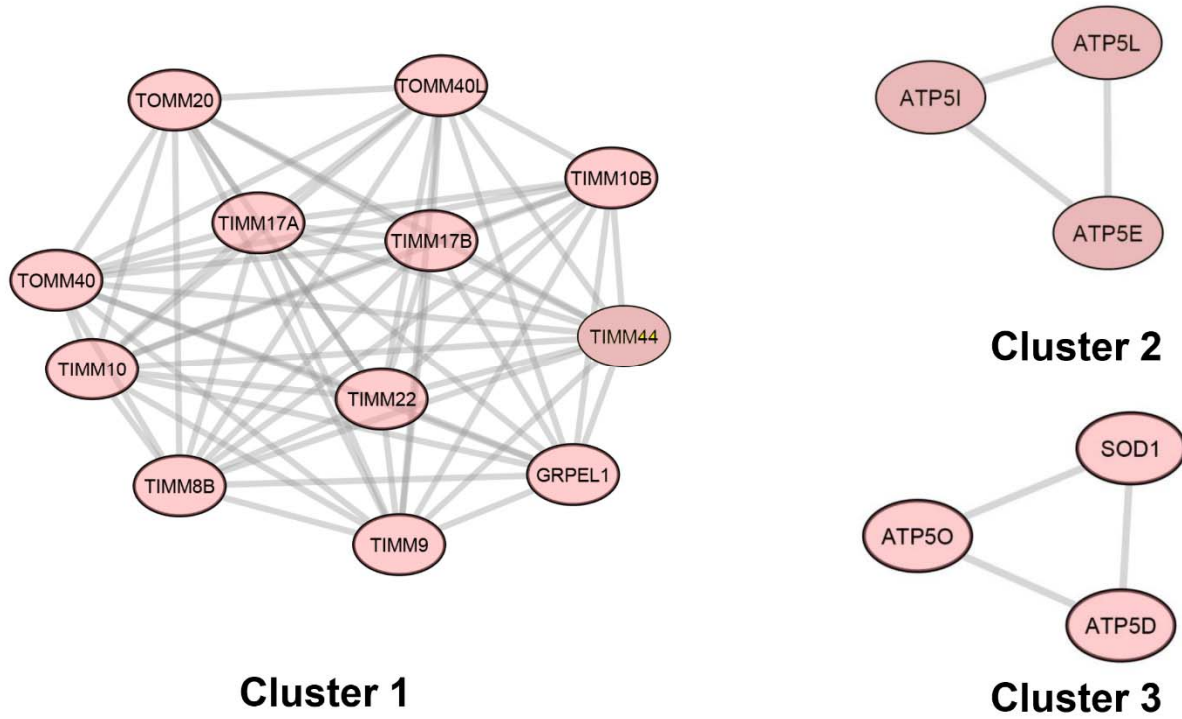


Figure 3. Top three modules (Cluster1-3) from the blood of sepsis patients

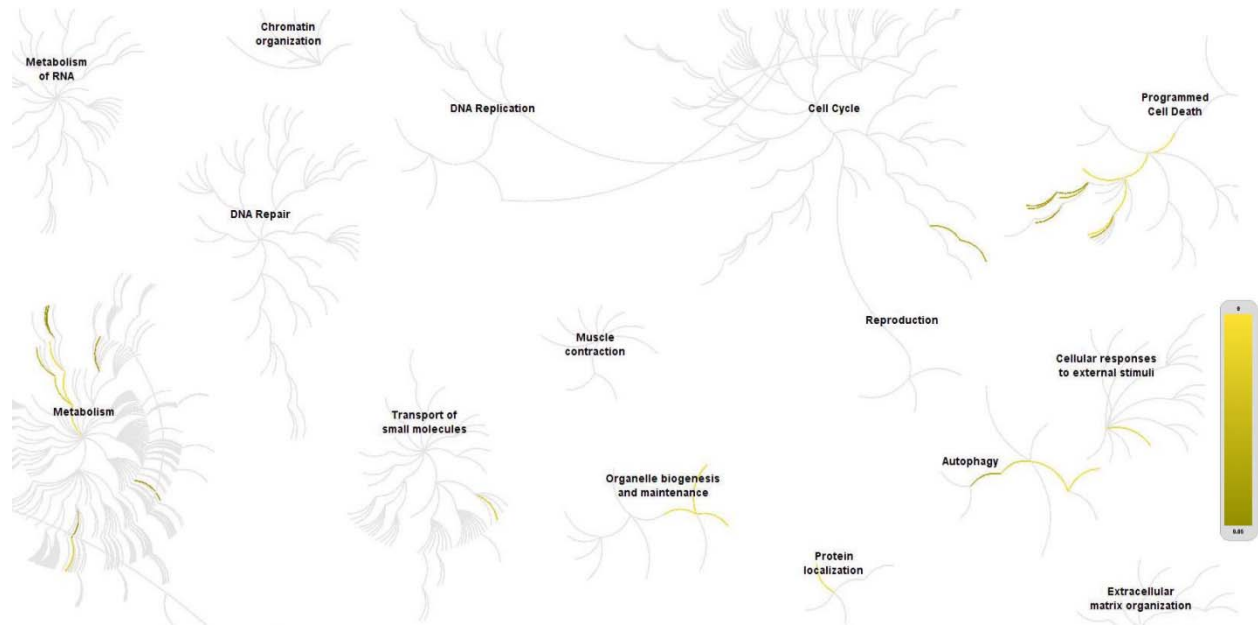


Figure 4. The Reactom pathway visualization map. Input genes are represented by the top significantly changed genes obtained from the GSE167914 dataset ($P < 0.01$). The yellow color represents the most relevant signaling pathways.

p-value predicted_MOA

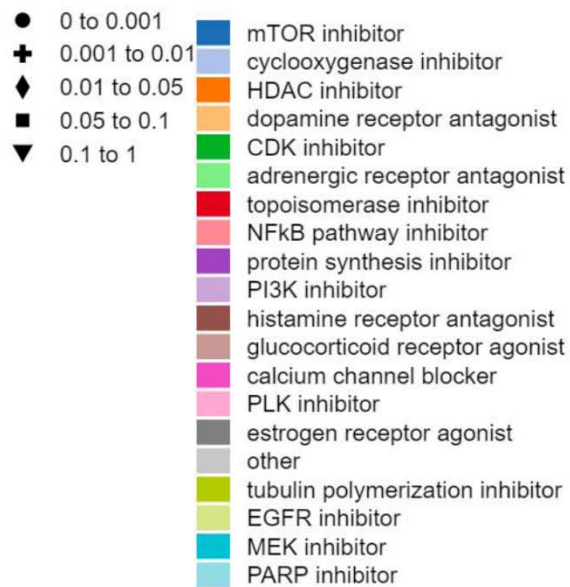


Figure 5. Inhibitors by L1000FDW visualization. Input genes are represented by the significantly changed genes obtained from the GSE167914 dataset. Dots are the Mode of Action (MOA) of the respective drug.

Table 1

Entrez gene	Gene Symble	Fold-change	Regulation
Top 10 down-regulated DEGs			
706	TSPO	-1.45828702346694	Down
26521	TIMM8B	-1.43210769844688	Down
51100	SH3GLB1	-1.28619452774405	Down
29957	SLC25A24	-1.01302822644703	Down
55288	RHOT1	-1.01271887616278	Down
788	SLC25A20	-0.901108073452252	Down
84134	TOMM40L	-0.857069247581503	Down
10245	TIMM17B	-0.829410945662233	Down
3939	LDHA	-0.800959299777023	Down
67126	ATP5E	-0.784378992381854	Down
Top 10 up-regulated DEGs			
7157	TP53	1.12448582743376	up
23082	PPRC1	1.03266502881293	up
596	BCL2	0.994531085428525	up
66043	ATP5D	0.949586740919676	up
7351	UCP2	0.864981370741031	up
84172	POLR1B	0.728977834365151	up
3418	IDH2	0.644515388301858	up
10128	LRPPRC	0.642054261768502	up
23	ABCF1	0.632440787164533	up
10953	TOMM34	0.623887917139965	up

Table 2. Top ten genes demonstrated by connectivity degree in the PPI network

Gene Symbol	Gene title	Degree
TOMM40	translocase of outer mitochondrial membrane 40	16
TOMM20	translocase of outer mitochondrial membrane 20	12
TIMM22	translocase of inner mitochondrial membrane 22	12
TIMM10	translocase of inner mitochondrial membrane 10	12
TIMM17A	translocase of inner mitochondrial membrane 17A	11
TIMM17B	translocase of inner mitochondrial membrane 17B	11
CYCS	cytochrome c, somatic	10
TIMM9	translocase of inner mitochondrial membrane 9	9
TIMM44	translocase of inner mitochondrial membrane 44	9
TAZ	tafazzin	7