Original Research Article

Roles of NK cells in sepsis

Abstract

Purpose: Sepsis is a dangerous sickness that happens when the body's insusceptible framework assaults its own tissues and organs in response to disease. The invulnerable framework is smothered past this beginning phase. Fever, expanded pulse, expanded breathing rate, and confusion are on the whole normal signs and manifestations. Indications of a specific ailment, like a hack with pneumonia or difficult pee with a kidney contamination, may likewise be available.

Methods: The gene expression data were retrieved from Gene Expression Omnibus (GEO) (GSE60424). Fold change and p value analysis, hierarchical clustering, and pathway analysis were performed.

Results: In this study, we identified altered genes involved in sepsis in NK cells. Ten genes corresponding 11 probe sets were differentially expressed following the sepsis. We identified a network between these genes and pathways they belong to. Pathway analysis showed that these genes are mostly associated with autoimmune response.

Conclusion: DLL1, SERPINA1, IFI44L, XCL1, CD3G, IGHM, PAX8-AS1, PACSIN1, PDE4B and SCML1 genes were found

to be associated with sepsis. Almost all of these genes are effective in the autoimmune response, especially during the sepsis. Therefore, it is hypothesized that downregulation or upregulation of these genes may affect immune response. And it is predicted that NK cells may be an important factor for autoimmune disease.

Keywords: Sepsis, NK cells, Autoimmune response.

Introduction

Sepsis is a dangerous sickness that happens when the body's insusceptible framework assaults its own tissues and organs in response to disease. The invulnerable framework is smothered past this beginning phase. Fever, expanded pulse, expanded breathing rate, and confusion are on the whole normal signs and manifestations. Indications of a specific ailment, like a hack with pneumonia or difficult pee with a kidney contamination, may likewise be available (1,2,3). Diseases are a predominant medical problem that influences individuals, everything being equal. As a rule, the reaction they get is fitting, and they require little consideration. Notwithstanding, in different cases, the resistant framework's response to the disease is lacking, bringing about organ breakdown; this is known as sepsis.

Sepsis can be brought about by microorganisms, growths, or infections, and there is presently no fix; treatment centers around controlling the contamination by source control and meds, as well as organ work support (4). Sepsis has been described as a microbial contamination that causes temperature (or hypothermia), tachycardia, tachypnoea, and blood leukocyte adjustments for over twenty years. Sepsis is currently broadly viewed as a dysregulated foundational incendiary and immunological reaction to microbial attack that outcomes in organ hurt and has passing paces of 15-25 percent (5). Sepsis is an incessant infirmity that has an unsuitably high casualty rate and, for the individuals who get by long haul horribleness. Expanded consciousness of the issue as a result

of consistent endeavors, as well as proof got from research directed over the most recent decade, has improved doctors' and laypeople's understanding of the issue, prompting better results. In 2017, the World Health Assembly and the World Health Organization (WHO) announced sepsis a worldwide wellbeing need and supported a goal to improve sepsis avoidance, recognition, and the board. A modified meaning of (Sepsis-3) was made in 2016. The expression "sepsis" today alludes to a contamination that causes organ glitch (6). Sepsis is an overwhelming reaction to disease that is related with critical bleakness and demise, requiring prompt treatment to further develop results. Getting through Sepsis is a worldwide drive pointed toward further developing sepsis results (7). During delayed sepsis, apoptosis and decreased immunological exercises of regular executioner (NK) cells add to patients' weakness to optional/nosocomial diseases and viral reactivation, bringing about unfortunate life quality and long-haul demise (8). Extreme sepsis and septic shock are as yet dangerous sicknesses that require the improvement of new therapies. For the advancement of novel immunointerventions, a superior information on the confounded modifications of the resistant framework in septic patients is required. CD3NKp46+CD56+ cells that can be cytotoxic or potentially discharge a lot of cytokines like IFN-are known as normal executioner (NK) cells. Crosstalks between NK cells and other insusceptible cells like dendritic cells, macrophages, and neutrophils are additionally normal. As referenced, NK cells might assume a significant part in the improvement of fundamental irritation during the beginning phases of septic shock (9).

Materials and methods

Microarray gene expression data

The gene expression data was obtained from the Gene Expression Omnibus (GEO)database. Transcription profile data of human Natural killer cells from Sepsis patients were obtained from GEO (GSE60424).

Processing and normalization of data

The raw data from GEO were normalized with the DESeq2 package in the R software. Normalized transcription profile data consists of 11,895 different genes/ 12,744 probe sets. The data contains 4 groups of control and 2 groups of patients with sepsis, whole genome expression data.

Fold change and p value analysis

Among the groups, significant genes with a fold change greater than |3| were identified. In order to group the identified genes more specifically P value was calculated and genes under 0.05 were selected.

Analyses were done using GraphPad Prism 9.0.0 (Graphpad Prism 9 Software, San Diego, CA, USA). Genes with a P value less than 0.05 and fold change greater than |3| were selected.

Hierarchical clustering

The Euclidean Gene Cluster 3.0 tool was used to hierarchically cluster genes discovered using linear regression analysis using mean standardized gene expression levels. After cluster analysis, the data was normalized, and the standardized data was examined in Treeview. Using Euclidian distance as a similarity metric and full linkage as a clustering approach, hierarchical clustering was done on both genes and arrays.

Pathway enrichment analysis

The "Database for Annotation, Visualization, and Integrated Discovery" (DAVID) software was utilized to investigate the biological relationship underlying these genes. The

pathways linked to our genes have been discovered.

GSEA was performed according to the GSEA guideline protocol (http://software.broadinstitute.org/gsea/docGSEAUserGuideFrame.html). The analysis was carried out using GSE60424 data. There are 12,744 probe sets in this data collection (11,895 different genes). To further understand the trend, a comparison was made between the four control groups and the two sepsis patient groups. The major goal of this study is to figure out which genes are considerably enriched in various GSEA gene sets, as well as to figure out which gene sets are enriched in which groups.

The enhancement score (ES), the standardized advancement score (NES), the notional P esteem (NOM P esteem), the bogus revelation rate q esteem (FDR q esteem), and the familywise mistake rate P esteem are totally determined utilizing GSEA (FWER). The ES esteem addresses a quality's most noteworthy deviation in a gathering of qualities; as such, this score supports the ID of the most upregulated qualities. The distinction or connection between quality sets and quality articulation is addressed by the NES esteem. The more prominent the NES esteem, the more prospects there are.

As a result, gene sets with a higher NES value have more relevance. The NOM P value, in addition to the ES and NES values, assesses the significance of the ES computation. As a result, the NOM P value was closely tied to the ES and NES values. The importance of ES is demonstrated by the increase in NOM P value. On the other side, the FWER P value reflects the likelihood of NES false positives, and a lower FWER P value is directly and strongly connected to the accuracy of NES computation. In addition, the FDR q value is the most important parameter in this study. This number must be less than 0.25, and the enrichment of gene sets becomes more significant as this value decreases.

Volcanoplot

Volcano plot was shown with the EnhancedVolcano package in the R software. volcano plot shows the log2 of the fold change on the x-axis and minus log10 of the p-value. Genes with P value lesser than 0.05 and fold change greater than |1,5| are shown.

Changes in gene expression in a sepsis patient's NK cell line Human NK cells were evaluated using whole genome expression data to see if there were any differences in gene expression between four control groups and two sepsis patient groups. According to the findings, 10 genes belonging to 11 probe sets revealed statistically significant expression changes with a fold change larger than|3| and a P value less than 0.05. We focused our additional research on the genes that influenced expression diversity between groups. Two genes were positively connected and elevated in human NK cells from four control groups and two sepsis patient groups, whereas eight genes were negatively linked and downregulated (Supplementary Table 1).

Gene changes were discovered using hierarchical cluster analysis in two groups: four healthy people and two sepsis patients. Eight genes were found to be negatively associated, strongly expressed in the control groups, and reduced in the sepsis groups. In contrast, two genes were positively associated, with low expression in the control groups and higher expression in the sepsis groups. The image depicted 10 genes, with the remainder provided as supplemental information (Fig. 1) Sepsis causes gene changes.

Fold change analysis and p value were done on 10 genes/11 probe set expression data from 4 control groups and 2 sepsis groups to see if this expression change was caused by sepsis in

patients. Thus, 11 probe sets with a fold change more than |3| and a P value less than 0.05 were determined for 10 statistically significant genes (DLL1, SERPINA1, IFI44L, XCL1, CD3G, IGHM, PAX8-AS1, PACSIN1, PDE4B, and SCML1) (Table 1).

Eight of the ten genes were shown to be downregulated and negatively linked. Figure 2 compares the expression of these 10 genes in four groups and two sepsis groups (Fig. 2).

Gene Name	Fold change	P value
DLL1	-4.005240681	1.02E-07
SERPINA1	-3.903689361	0.023160899
IFI44L	-3.763217156	0.000325333
XCL1	-3.704187412	0.001232046
CD3G	3.586105442	1.50E-05
IGHM	3.42755781	0.004404048
PAX8-AS1	-3.389036491	0.000217374
PACSIN1	-3.345921535	0.000542793
PDE4B	-3.170424395	4.43E-05
SCML1	-3.116287166	0.005822007

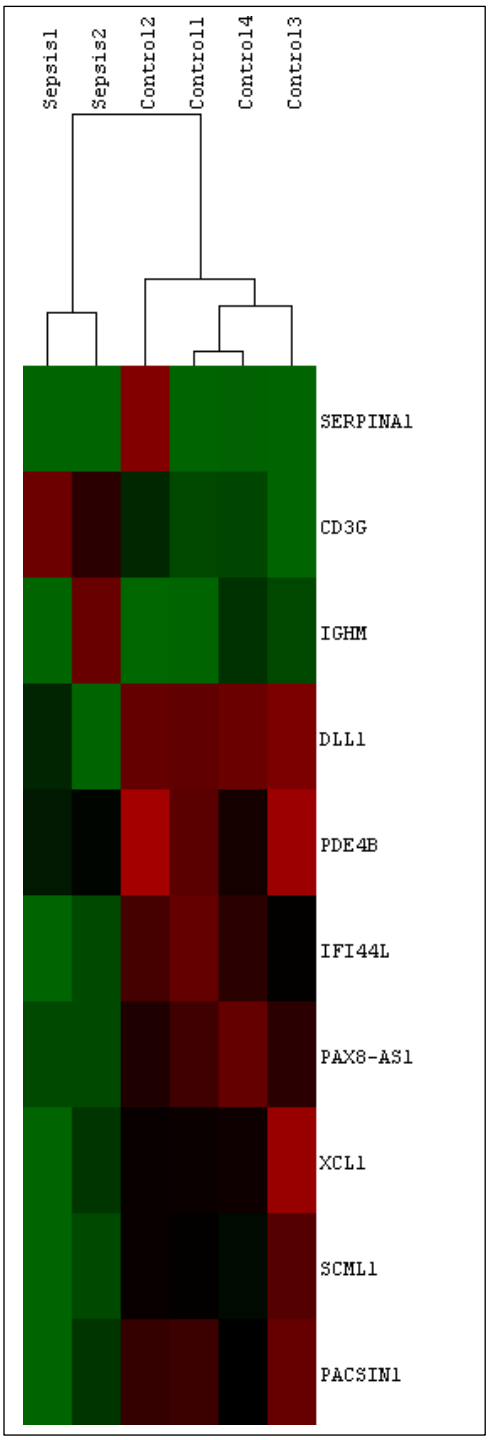
Table 1 The list of 10 genes (11 probe sets) which have the most alterations in expression. These genes have fold change greater than |3| and p value less than 0.05 between 4 control groups and 2 groups from patients with sepsis. These significant values indicate that the change occurred due to sepsis.

Functional enrichment of genes and pathway connections

DAVID software was used to do a pathway analysis of biological processes in order to discover the link between these 10 genes and cellular activities and pathways, as well as to better grasp their new significance. During sepsis, the four pathways are related with 10 genes: T cell signaling pathway, chemokine signaling pathway, and notch signaling cAMP

signaling pathway. The relevance of the cell cycle in stomach cancer cells, for example, demonstrates the link between these pathways and the development of cancer and autoimmune illness (Table 2).

Figure 1: Hierarchical clustering of 10 statistically significant variables in the six groups. For control and sepsis, the analysis revealed sensitive low expressions (green), intermediate expressions (black), and high expressions (red) of 10 genes. The classification of designated groupings is obvious. The top ten genes were included in the graph.



Strengthening Table 2 shows the significantly advanced quality sets, as well as their ES, NES, NOM P esteem, FWER P worth, and FDR q esteem. The negative controlled reaction to cytokine boosts and DNA blend engaged with DNA fix quality set were demonstrated to be firmly connected with the qualities contained in the information, as indicated by GSEA. Table 2 and other featured exploration observed that specific quality sets associated with pathway investigation.

Figure 4 likewise showed that the reaction to cytokine boost set plot was contrarily managed. This chart portrayed qualities that were enhanced in four benchmark groups, a peculiarity known as emphatically connected qualities. On the other hand, the same genes were downregulated and negatively correlated in the 2 sepsis groups. The same results were obtained for the DNA synthesis involved in DNA repair genes set (Fig. 5).

Table 2 Pathways related to the genes are linked. It is seen that important pathways in cancer progression and autoimmune disease are related to our genes. Most of these genes are linked to T cell signaling pathway, chemokine signaling pathway and notch signaling cAMP signaling pathway from the database for annotation, visualization, and integrated discovery (DAVID)

Category	pathways	related disease
KEGG_PATHWAY	T cell receptor signaling	arthritis and rheumatoid
KEGG_PATHWAY	chemokines signaling	leukemia
KEGG_PATHWAY	Notch signaling	
KEGG_PATHWAY	c amp signaling	

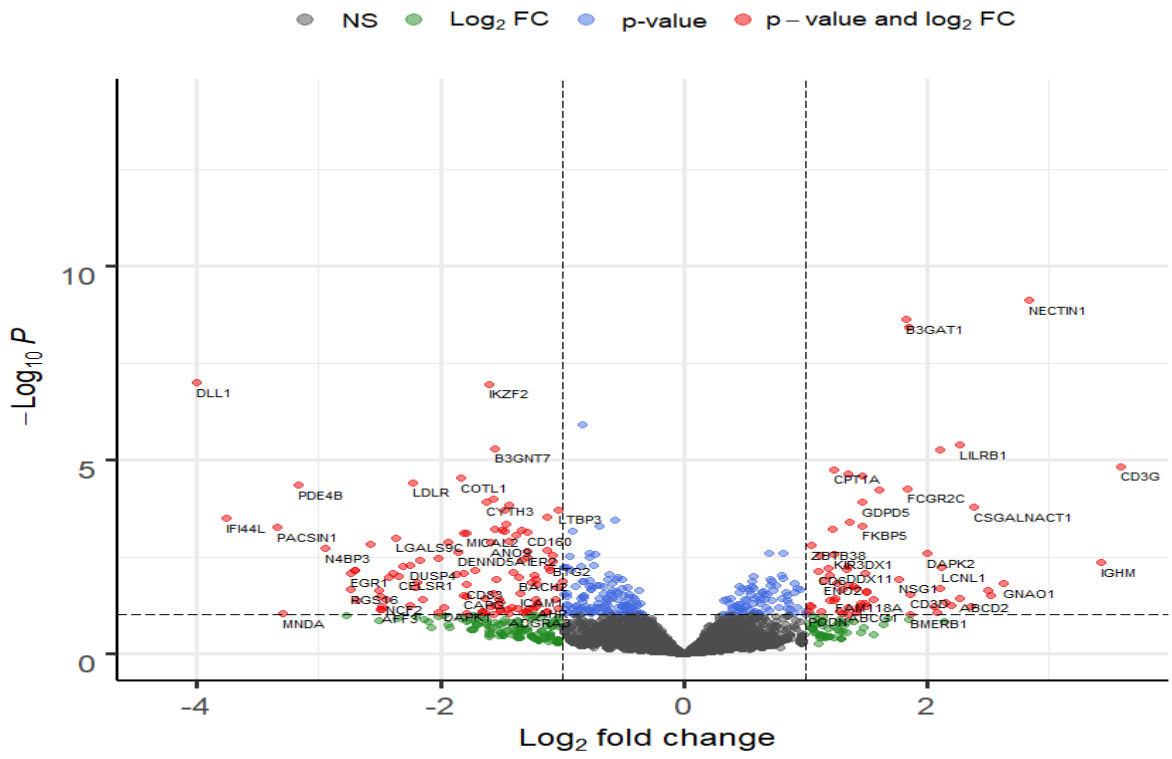


Fig 2 volcano plot shows the log2 of the fold change on the x-axis and minus log10 of the p-value. Genes with P value lesser than 0.05 and fold change greater than |1,5| are shown.

Result and Discussion

Sepsis is a frequent ailment that has an unacceptably high fatality rate and, for those who survive, long-term morbidity. Increased awareness of the issue as a consequence of continuous efforts, as well as evidence derived from research conducted in the last ten years, has enhanced physicians' and laypeople's comprehension of the problem, leading to better outcomes. In 2017, the World Health Assembly and the World Health Organization (WHO) declared sepsis a global health priority and approved a resolution to enhance sepsis prevention, detection, and management.

A revised definition of sepsis (Sepsis-3) was created in 2016. The term "sepsis" today refers to an infection that causes organ malfunction. (10)

In this study, it is aimed to identify sepsis related gene expression alterations, their associated pathways using all genome expression data of NK cells from sepsis patients.

(DLL1, SERPINA1, IFI44L, XCL1, CD3G, IGHM, PAX8-AS1, PACSIN1, PDE4B and SCML1

gene expression alterations were identified in NK cells from sepsis patients. These genes were identified as differentially expressed in NK cells from sepsis patients and groups ($P < 0.05$ and fold change greater than $|3|$) were

hierarchically forming a very distinct cluster, as expected. Gene set enrichment analysis supported the results that some crucial genes function effectively in the negative regulated of response to cytokine stimulus and DNA synthesis involved in DNA repair as a result of sepsis. Most of the genes showed a statistically significant difference in negative regulated of response to cytokine stimulus and DNA synthesis involved in DNA repair gene sets. Genes found statistically significant because of the comparison between the 4 control groups and 2 groups patients with sepsis were enriched in the specified biological gene sets. This enrichment supports that these genes have important effects on the immune system.

At the point when monocytes are animated by microorganisms, they produce Delta-like Protein 1 (DLL1), an exemplary Notch ligand. Given the job of monocytes in sepsis pathogenesis, it was anticipated that this interaction may likewise happen in the clinical setting, and DLL1 could be utilized as a biomarker for dangerous bacterial disease. DLL1 is another host-inferred biomarker for sepsis recognition that beats known biomarkers, undoubtedly because of its extraordinary strength in non-irresistible incendiary responses. (11,12)

The anti-inflammatory protein alpha-1 antitrypsin (AAT) has a well-known safety profile. AAT's therapeutic potential has been investigated in a variety of autoimmune illness types. AAT gene transfer reduced the development of type 1 diabetes (T1D) in the non-obese diabetic (NOD) mouse model in the first research, which used a recombinant adeno-associated viral (rAAV) vector. Treatment with the AAT protein prevented and reversed type 1 diabetes in subsequent investigations. Other autoimmune disease models, such as rheumatoid arthritis and systemic lupus erythematosus, have shown that AAT therapy is effective. (13)

Although AAT lack has been connected to the advancement of bronchial asthma and bronchiectasis, there is no convincing proof that it influences the recurrence or seriousness of these problems. Lung emphysema brought about by AAT inadequacy has a promising beginning of 35-45 years, with determined,

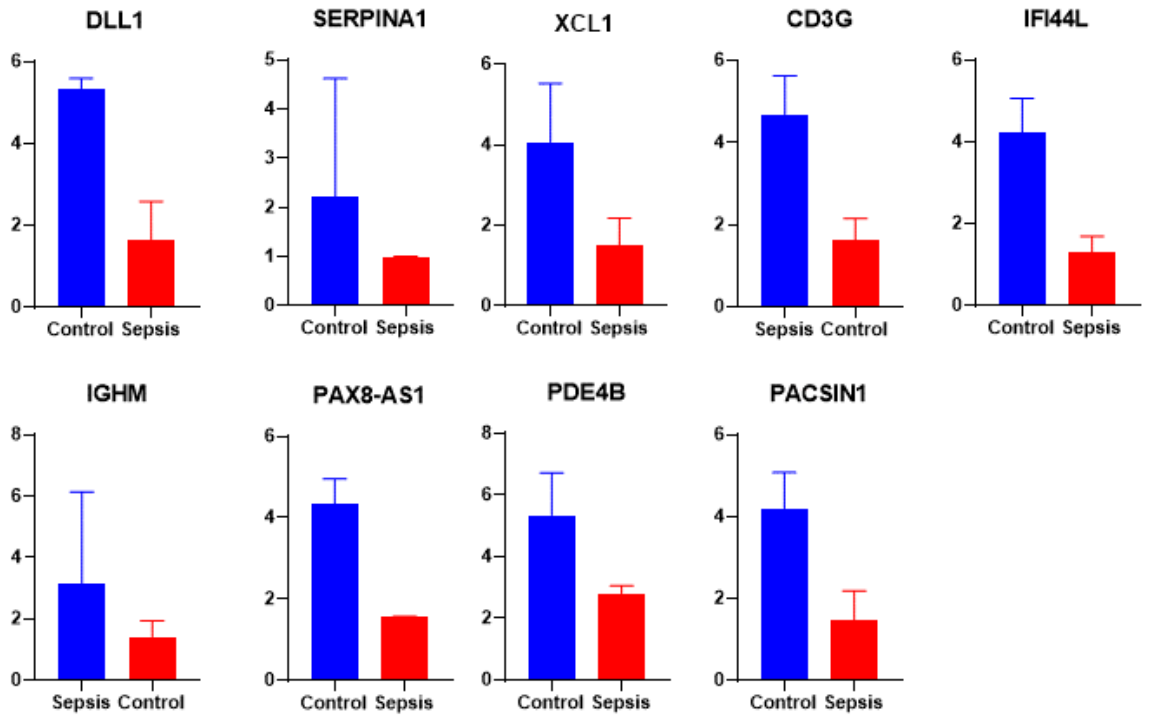


Fig 3 Ten genes that show significant differentially expressed in patients with sepsis. Statistically significant alterations were detected between 4 control groups and 2 sepsis groups.

expanding sleepiness and other vague respiratory manifestations. (14) The traditional worldview of protease/antiprotease awkwardness emerged from the disclosure that alpha-1 antitrypsin (AAT) was an effective inhibitor of neutrophil elastase, tying lung harm to the unopposed effect of proteases in people with the shortfall. Regardless of its significance as an antiprotease, alpha-1 antitrypsin has been displayed to have fundamental calming and safe administrative properties, which might assume a part in lung annihilation. (15,16) The connection among lymphotactin and XCR might direct unique lymphocyte subsets to incendiary regions. Lymphotactin can possibly control the provocative reaction. The NF-B flagging pathway might impact lymphotactin articulation, essentially to some degree.

Lymphoctactin is a powerful attractant of lymphocytes, strikingly T-cells and regular executioner (NK) cells, with a ruling articulation in actuated CD8+ T cells and enacted normal executioner cells, despite the fact that it is inactive for invigorating the relocation of neutrophils and monocytes. The presence of a lymphotactin-explicit receptor, XCR, which is a particular G protein-coupled receptor communicated in circling lymphocytes and NK cells, recommends the presence of a lymphotactin-explicit receptor, which is a particular G protein-coupled receptor communicated in flowing lymphocytes and NK cells. The lymphotactin-XCR communication has been displayed to play a part in the improvement of provocative problems like rheumatoid joint pain, foundational sclerosis,

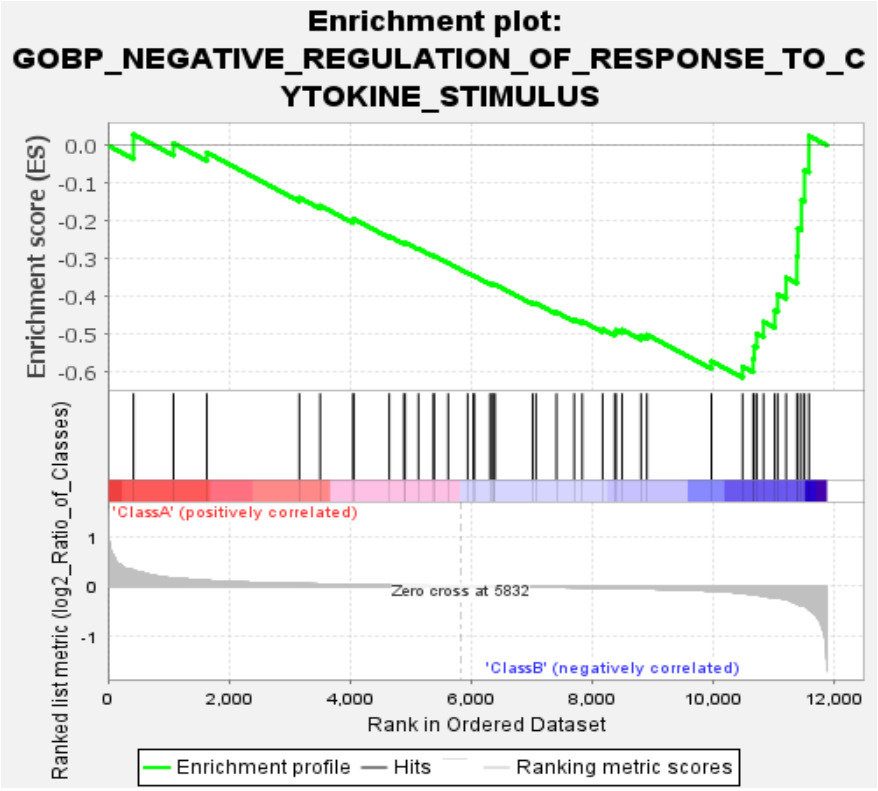


Fig. 4 The negative regulation of response to cytokine stimulus plot was represented. The black straight line refers the enriched genes in the groups. Red part contains the genes that positively correlated in 4 control groups and were upregulated in control groups. On contemporarily, the blue line includes downregulated or in other words negatively regulated genes that belong to sepsis.

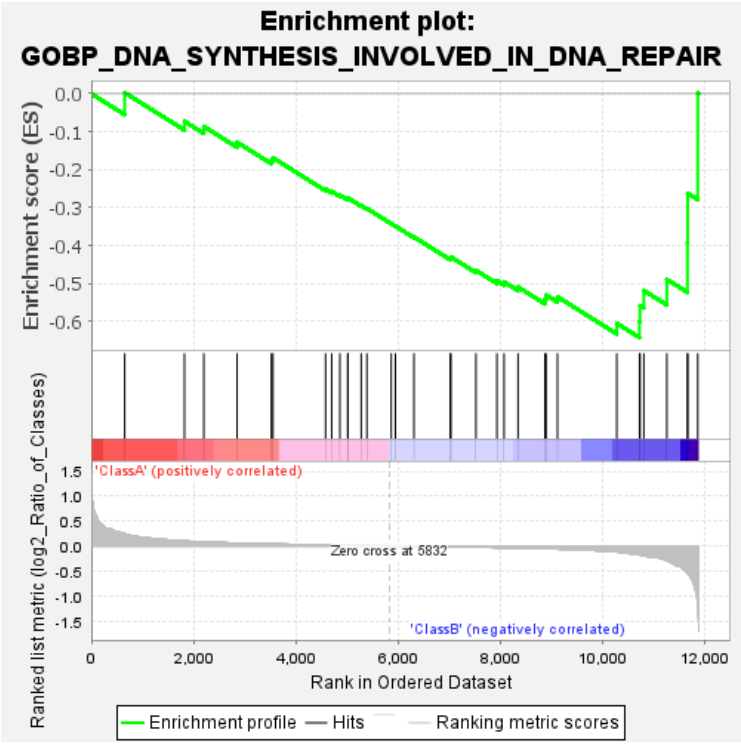


Fig. 5 The DNA synthesis involved in DNA repair plot was represented. The black straight line refers the enriched genes in the groups. Red part contains the genes that positively correlated in 4 control groups and were upregulated in control groups. On contemporarily, the blue line includes downregulated or in other words negatively regulated genes that belongs to sepsis.

fiery entrail sickness, glomerulonephritis, and HIV disease in past clinical and creature research. (17,18,19,20) XCL1 is a C-class chemokine created by resistant cells like CD8+ T cells, CD4+ T cells, T cells, NK cells, NKT cells, and medullary thymic epithelial cells. Accordingly, this is the first in vitro and in vivo evidence that the association somewhere in the range of XCL1 and α9 integrin plays a part in immune system problems, as far as anyone is concerned. XCL1 might play a part in the movement of incendiary joint inflammation. (21,22,23) Mutations in the CD3G gene (which codes for the CD3 chain) cause a range of immunodeficiencies and autoimmunity. (24,25)

The majority of the genes found in this study are significantly expressed in autoimmune illness and are linked to a strong immune response, according to the findings. As a result, cell suppression may result in diminished effects in sepsis patients. This study was the first to reveal a link between some of these genes and sepsis. Following network and pathway analysis, it was shown that these genes are linked to critical sepsis pathways. The T cell signaling pathway, the chemokine signaling pathway, and the notch signaling cAMP signaling pathway are all examples of these pathways. . The majority of these pathways are clearly linked to sepsis and other autoimmune diseases. As a result, we are able to favorably act in sepsis.

Conclusion

In this study, it has been shown that suppression of NK cells may have important effects on patients with sepsis. The results are important indicators that NK cells response to sepsis directly or indirectly have an effect on the immune system response, and this effect may favor the host in fighting with sepsis. Most of the genes we identified have functions that cause high immune response. More in vitro and in vivo studies are needed to demonstrate the role of natural killer cells in patients with sepsis.

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