Noninfectious uveitis (NIU), an intraocular inflammation caused by immune-mediated reactions to eye antigens, is associated with systemic rheumatism and several autoimmune diseases. However, the mechanisms underlying the pathogenesis of uveitis are poorly understood. Therefore, we aimed to identify differentially expressed genes (DEGs) in individuals with NIU and to explore its etiologies using bioinformatics tools.

GSE69396 and GSE18781 datasets from the gene expression omnibus (GEO) database were merged and analyzed. Functional enrichment analysis was performed, and protein-protein interaction (PPI) networks were constructed. A total of 89 DEGs were identified. Gene ontology (GO) enrichment analysis identified 21 enriched gene sets. Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis identified four core enriched pathways: antigen processing and expression signaling, natural killer (NK) cell-mediated cytotoxicity signaling, glutathione metabolic signal transduction, and arachidonic acid metabolism pathways. PPI network analysis revealed an active component-target network with 40 nodes and 132 edges, as well as several hub genes, including CD27, LTF, NCR3, SLC4A1, CD69, KLRB1, KIR2DL3, KIR3DL1, and GZMK.

The eight potential hub genes may be associated with the risk of developing NIU. NK cell-mediated cytotoxicity signaling might be the key molecular mechanism in the occurrence and development of NIU. Our study provided new insights on NIU, its genetics, molecular pathogenesis and new therapeutic targets.

**Abbreviations:** AS = ankylosing spondylitis, BD = Behcet disease, DEGs = differentially expressed genes, GEO = gene expression omnibus, GO = gene ontology, KEGG = Kyoto encyclopedia of genes and genomes, KLRB1 = killer cell lectin-like receptor B1, NIU = noninfectious uveitis, PPI = protein-protein interaction, VKH = Vogt-Koyanagi-Harada.

**Keywords:** differentially expressed genes, GEO database, noninfectious uveitis, Protein-protein interaction

1. Introduction

Uveitis is a vision-threatening eye disease[1] that occurs mostly in people over the age of 36 years.[2] Approximately 10% of blindness in Western countries is caused by uveitis, and 25% of legal blindness in developing countries is associated with uveitis.[3–5] Uveitis cases can be classified into infectious or noninfectious categories based on the etiology.[6] Non-infectious uveitis (NIU) is an intraocular inflammation caused by immune-mediated reactions to eye antigens. Approximately 41% to 55% of the cases are non-infectious uveitis and are associated with systemic rheumatism and several autoimmune diseases[6,7] such as ankylosing spondylitis (AS), Behcet disease (BD), Vogt–Koyanagi–Harada (VKH), and multiple sclerosis.[8,9] Different diseases result in the expression of different proteins.[10]

Epidemiologically, uveitis exhibits geographical variations, with NIU being the leading cause of uveitis in industrialized countries affecting 121 in 100,000 people.[11,12] Higher incidences have been observed in some regional groups of the Chinese population,[13,14] but national epidemiological statistics are lacking. An epidemiological survey of uveitis in 8952 people over 40 years of age in Hengli Town, Dongguan City, Guangdong Province, China, suggested that the prevalence of uveitis was 904 per 100,000 individuals, and NIU accounting for 81.5% of the cases.[15] The clinical manifestations of NIU include red eyes, pain, and blurred vision. Vision loss often occurs if the lesions involve the choroid, retina, and macula. These manifestations are characterized by rapid onset, long course, easy recurrence, and many complications, and the blindness rate is higher than in infectious uveitis.[16]
The pathogenesis of uveitis is not yet fully understood, and disruption to the stability of the ocular immune microenvironment may be acritical mechanism associated with NIU. Previous studies have revealed that interleukins (IL-17, IL-6, and IL-8) and cytokines (interferon-gamma [IFN-γ] and chemokines) play vital roles in the pathogenesis of intraocular inflammation. Some immune-related pathways such as the interleukin (IL)-23/IL-17 pathway, reactive oxygen species signaling pathway, arachidonic acid signaling pathway, and natural killer (NK) cell-mediated cytotoxicity pathway play crucial roles in the pathogenesis of NIU. Clear diagnosis and appropriate treatment are essential for preventing irreversible intraocular structural damage and blindness.

Although there are many clinical causes of NIU, the treatment is simple and mainly involves glucocorticoids and/or immune suppressants. In modern medicine, local and systemic corticosteroid therapy is generally used as first-line treatment. Immune modulators such as alkylating agents, antimetabolites, and T-cell inhibitor scan be used to treat patients with poor responses to corticosteroid therapy. Therefore, individualized immunomodulatory treatment programs are required to make the treatment more accurate and effective and to reduce the toxicity and side effects of the drugs.

Recently, biological agents targeting specific molecules have been used to treat NIU. These have attracted attention as alternative therapies for refractory uveitis due to their safety and efficacy. All molecules involved in the immune response, including cytokines, cell adhesion molecules, chemokines, and costimulatory molecules, may be potential targets for biotherapy. Notably, a substantial proportion of the mechanism of protein expression associated with NIU pathogenesis is still lacking and one possible explanation is that unknown genes may partially contribute to the immune deficiency. Therefore, many related genes remain to be identified, these will help us better understand the pathogenesis of NIU and facilitate the discovery of new diagnostic markers or therapeutic targets.

Gene chip technology is a powerful tool for exploring regulatory patterns and molecular mechanisms of targets associated with NIU genesis and development. However, some limitations are associated with current studies based on chip technology. These limitations include the use of small sample sizes.
sizes and experimental variability in different settings, even when studying the same disease. Integrated bioinformatic techniques are currently employed in biological research. These techniques may be used to identify protein expression and potential mechanisms underlying NIU and provide a strong basis for the precise diagnosis of NIU and the exploration of new drug targets.

In this study, transcriptomic data of patients with NIU and healthy individuals were downloaded from the gene expression omnibus (GEO) database, and multiple microchips were combined. The datasets were used to conduct a preliminary analysis of the potential mechanisms of action underlying the occurrence and development of NIU and provide a basis for experimental research on the pathogenesis of NIU and the identification of its novel therapeutic targets.

2. Materials and Methods

2.1. Data source

The GEO database (https://www.ncbi.nlm.nih.gov/) was accessed via the national center for biotechnology information website. “Uveitis” was taken as the keyword and used to search for related chip data. Original chip data files labeled GSE66936 and GSE18781 and chip gene annotation files labeled GPL570-55999 were retrieved. The GSE66936 dataset contains 21 samples, including 16 from patients with uveitis (experimental group) and 5 from healthy individuals (control group). The GSE18781 dataset contains 55 samples, including 34 from patients with uveitis (experimental group) and 21 from healthy individuals (control group). The 2 sample groups were all patients with NIU. The GSE66936 chip data was generated at NEI Institute in Bethesda, USA, while the GSE18781 chip data was generated at Casey Eye Institute in Portland, USA.

2.2.Data collection and analysis of differentially expressed genes (DEGs)

Practical extraction and reporting language script was used to merge data with the same gene names in the GSE66936 and GSE18781 chips, and the Sva package in R software (https://www.r-project.org/, Version 4.1.0) was used to correct for merged data in batches. The limma package was used to correct the filtered database on |LogFC| ≥ 1.5 and \( P < .05 \), and NIU-related differentially expressed genes were obtained. The heat map package was used to generate volcano plots and heat maps of all the DEGs.

2.3.Functional enrichment analysis of DEGs

ClusterProfiler, DOSE, and Enrichplot packages were used to conduct gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses of the DEGs. \( P < .05 \) was used to select enriched gene sets and pathways.

2.4. Protein-protein interaction (PPI) network construction and analysis

A PPI network was constructed using the STRING database (http://www.string-db.org). An interaction score >0.4 was used as the threshold. The PPI network was imported into R for visualization, and the connectivity between nodes was calculated to predict the core genes.

2.5. Ethics and dissemination

Ethical approval is not required for this differentially expressed gene analysis as we did not use data related to individual patient. The final report of this paper will be published in a peer-reviewed scientific journal or at conferences to provide evidence-based medical support on NIU, its genetics, molecular pathogenesis and new therapeutic targets for clinical workers, and dataset will be made freely available.

3. Results

3.1.Identification of DEGs

Analysis of GEO chip data identified genetic variations between healthy individuals and patients with uveitis (|LogFC| ≥ 1.5 and \( P < .05 \)) after batch correction for 89 different genes, including 31 upregulated genes (Table 1) and 58 downregulated genes (Table 2). R software was used to create a volcano plot (Fig. 1A) and heat map (Fig. 1B) of the DEGs.

![Figure 1. Visualization of differentially expressed genes (DEGs). (A) Volcano map, the vertical coordinate represents the function of gene enrichment, the horizontal coordinate represents the ratio of the number of differential genes to the total number of differential genes, and different colors represent the \( P \) value of the significance of enrichment. (B) Heat map, the vertical coordinate represents the function of gene enrichment, the horizontal coordinate represents the ratio of the number of differentially expressed genes to the total number of differentially expressed genes in the function of gene enrichment work set, and the different colors represent the \( P \) value of enrichment significance.](image-url)
3.2. GO and KEGG enrichment analysis of DEGs

GO enrichment analysis helped identify 21 enriched core gene sets \((P < .05)\) (Table 3 and Fig. 2A). KEGG pathway analysis of DEGs identified four enriched pathways \((P < .05)\): antigen processing and expression, NK cell-mediated cytotoxicity, glutathione metabolism, and arachidonic acid pathways (Fig. 2B). In these pathways, the ones most closely related to the treatment of NIU were: NK cell-mediated cytotoxicity (Fig. 3).

3.3. PPI network construction and analysis

The network of interactions between proteins encoded by the DEGs was analyzed using the STRING database. The PPI network comprised of 40 nodes and 132 edges (Fig. 4A). Genes with connectivity ≥12 were selected as core genes. The most significant core gene was CD27, with connectivity of 20, followed by CD69, KLRB1, KIR2DL3, KIR3DL1, GZMK, NCR3, and SLC4A1 (Fig. 4B).

4. Discussion

In this study, we analyzed NIU-related gene expression profiles in GSE66936 and GSE18781 microarray datasets containing 76 samples, including 50 samples from patients with uveitis (experimental group) and 26 samples from healthy individuals (control group). Data in the 2 chips were generated from peripheral
blood samples collected from patients with NIU. Bioinformatics analysis-based screening for NIU-related genes helped identify 89 DEGs, including 31 upregulated and 58 downregulated genes. GO enrichment and KEGG pathway analyses revealed that the DEGs were enriched in biological processes such as peptidase regulatory activity, immune receptor activity, and cysteine endopeptidase inhibitor activity. Enriched pathways were mainly involved in antigen processing and presentation, NK cell-mediated cytotoxicity, glutathione metabolism, and arachidonic acid signaling. Among these, peptidase regulatory activity, immune receptor activity, and cysteine endopeptidase inhibitor activity are associated with immunity and inflammation. The corresponding genes included CXCR6, KIR3DL1, CD160, CD27, SNCA, SLPI, NKK3-1, PI3, LTF, SERPINF1, and KIR2DS5. NK cell-mediated cytotoxicity leads to the expression of inhibitory signals on cell surfaces that are associated with uveitis. Immune balance can be achieved by regulating the secretion of cytokines and may contribute to the treatment of uveitis. CD27, a member of the tumor necrosis factor receptor superfamily, is a marker of NK cell maturation. CD69 expression was significantly enhanced in inflamed eye tissue of an experimental autoimmune uveitis model and was associated with uveitis pathogenesis.

CD69 expression in NK gene clusters is a marker of CD4+ T cell activation. Local antigen stimulation triggers CD4+ T cell activation, resulting in the regulation of the immune response through CD69 on the cell surface. CD69 expression is upregulated during the inflammatory response in BD, AS, VKH, and other immune diseases. Killer cell lectin-like receptor B1 (KLRB1; CD161) is expressed in NK cells, CD4+ and CD8+ T cell subsets, and NKT cells and it is correlated with uveitis. Bioinformatics analysis revealed that KLRB1 expression is also significantly upregulated in AS. Therefore, we inferred that high KLRB1 levels during AS might increase the risk of NIU.

Paule et al. reported that the CD4/CD8 ratio in the cerebrospinal fluid can be used to infer the etiology of intermediate and/or posterior uveitis, providing novel insights into the role of KLRB1 in NIU pathogenesis. Killer cell immunoglobulin-like receptor 3DL1 (KIR3DL1) and KIR3DL3, members of the killer cell immunoglobulin-like receptor (KIR) family, are NK and T cell subset modulators that protect target cells by inhibiting NK cell activation. KIR3DL1 expression may be involved in the pathogenesis of BD, VKH, and AS. KIR3DL3 is considered a susceptibility gene that is associated with....

Figure 3. Natural killer cell metabolism mediated cytotoxicity. Core targets are marked in red.
with BD. KIR3DL3 may affect the function of NK cells through genetic differentiation, thus participating in the pathogenesis of BD. \[47\]

NCR3 encodes NKp30, \[48\] which can initiate an activation signal cascade in NK cells, leading to cytokine production or cytotoxicity.\[49\] NK cells are a subgroup of lymphocytes. Kucuksezer et al \[23\] confirmed that NK cells play a role in experimental autoimmune uveitis and may be a potential pathological factor. Considering that NCR3 is highly expressed in patients with NIU, inhibition of NCR3 activation may play a vital role in the treatment of NIU.\[50\]

SLC4A1 stimulates the functional activity of innate immune cells and is involved in immune responses, T cell activation, toll-like receptor-binding regulatory pathways, and granulocyte activation, all of which are closely associated with NIU.\[51\] Granzyme K (GZMK) is one of the pro-inflammatory members of the GZM family that can combine with IFN-\(\gamma\) to induce mouse fibroblasts to secrete inflammatory cytokines,\[52\] enhancing the inflammatory function of non-immune cells.\[53\] Its abnormal expression is associated with various auto immune diseases, including RA, AS,

and scleroderma,\[54\] that can lead to the development of NIU. Additional studies on GZMK may provide new perspectives on the diagnosis, treatment, and pathogenesis of NIU.

In the current study, we discussed 8 potential crucial genes that are involved in the occurrence and development of NIU. These 8 crucial genes were involved in the activation and regulation of NK cells. NK cell-mediated cytotoxicity was the key pathway enriched in this study, suggesting that these genes may serve as potential biomarkers and therapeutic targets for NIU. However, the limitations of this study should be considered. First, it is difficult to consider some important factors such as region, race, and age. Considering that the etiology of NIU is complex, undetermined factors such as region and history should be evaluated in further studies. In addition, potential key genes need to be further validated by RT-qPCR in clinical samples. Finally, the mechanisms by which these genes work are not fully understood. More evidence is needed to investigate their biological foundation.

5. Conclusions

Understanding the cellular and biochemical events involved in NIU is essential for treating the disease. In this study, gene expression data were analyzed to identify core genes involved in NIU and to determine the underlying mechanism associated with the disease. Bioinformatics analyses revealed that the NK cell-mediated cytotoxicity pathway plays a vital role in the pathogenesis of NIU, and genes associated with this pathway included CD27, CD69, KLRB1, KIR3DL1, KIR3DL3, and NCR3. The results of this study provide novel insights for studying the underlying mechanisms and activation factors associated with NK cells in NIU. In addition, analysis of NIU core genes suggested that NIU is associated with several autoimmune diseases and that different diseases induce the release of diverse proteins. Thus, using bioinformatic techniques to identify the expression of genes and the potential mechanisms underlying NIU may provide a strong basis for its precise diagnosis and exploration of novel drug targets.

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