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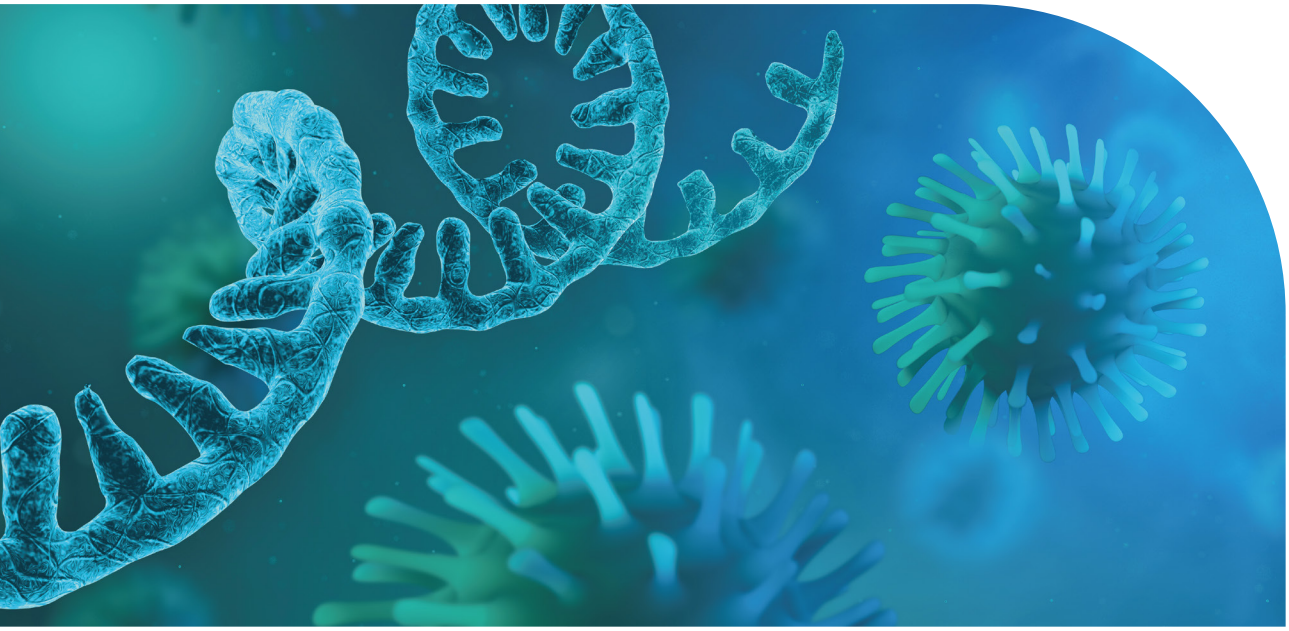
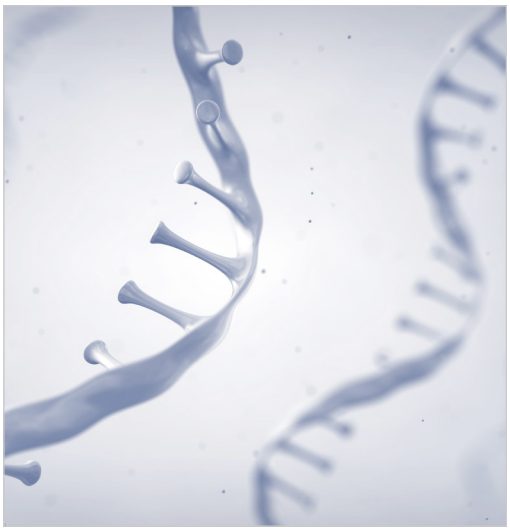


The Application of RNA Sequencing in Systemic Lupus Erythematosus

ADVANCING GENOMICS
IMPROVING LIFE

Case Study

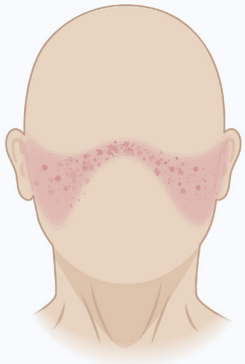
Summary



RNA sequencing (RNA-seq) has emerged as a powerful tool for gene expression analysis, allowing for the comprehensive study of the transcriptome. It has become an essential technique in various research fields, including disease research. Here, we will explore how RNA-seq was applied to investigate the mechanism of synaptic stripping by microglia in a mouse model of lupus. This study, from Nanjing Drum Tower Hospital collaborated with Nanjing University of Chinese Medicine titled, "Neuronal NR4A1 deficiency drives coordinated synaptic stripping by microglia in a mouse model of lupus"[1] , used RNA-seq and other research methods to clarify that synaptic loss is the early pathology of lupus brain injury, and microglia, complement signals and neurons participate in this process.

Background

Systemic lupus erythematosus (SLE) is an autoimmune disease that is difficult to cure. 40-90% of SLE patients experience neuropsychiatric symptoms, known as neuropsychiatric lupus erythematosus (NPSLE)[2,3]. The early pathogenesis of NPSEL is still unclear, which is a bottleneck for clinical therapeutics to break through. Complement protein C1q is a promoter of classical complement signaling. Recent studies have found that C1q in the brain has a unique biological function, which can regulate the pruning of microglia to neural synapses, and is crucial to the precise development of neural circuits and synaptic loss during aging, Alzheimer's disease, schizophrenia and other pathological processes. However, little is known about C1q's effects in NPSLE. RNA-seq is a widely used tool to discover differentially expressed genes between organisms. Through the transcriptome data analysis, this study reveals the positive role of neurons in coordinating microglia mediated synaptic loss, and emphasizes that neurons Nr4a1 and C1q are key components suitable for NPSLE therapeutic intervention.



Lupus facial rasha，a typical sign of SLE.
Created by Biorender.

Materials

Hippocampus tissues from WT(MRL-mpj) mice in triplicate: WT1, WT2, WT3;
Hippocampus tissues from MRL-lpr mice with MSC (mesenchymal stem cell) administration in triplicate: MRL-lpr1, MRL-lpr2, MRL-lpr3;
Hippocampus tissues from MRL-lpr mice without MSC administration in triplicate: MRL-lpr+MSC1, MRL-lpr+MSC2, MRL-lpr+MSC3.

Methods (provided by Novogene)

1. mRNA Enrichment and Reverse Transcription

mRNA was purified from total RNA using poly-T oligo-attached magnetic beads. After fragmentation, the first strand cDNA was synthesized using random hexamer primers, followed by the second strand cDNA synthesis. Second strand cDNA synthesis was subsequently performed using DNA Polymerase I and RNase H.

2. Library Preparation

Remaining overhangs were converted into blunt ends via exonuclease/polymerase activities. After adenylation of 3' ends of DNA fragments, adaptor with hairpin loop structure were ligated to prepare for hybridization. In order to select cDNA fragments of preferentially 370~420 bp in length, the library fragments were purified with AMPure XP system. Then PCR was performed with Phusion High-Fidelity DNA polymerase, universal PCR primers and Index Primer. At last, PCR products were purified, and library quality was assessed on the Agilent Bioanalyzer system.

3. Sequencing Platform

The clustering of the index-coded samples was performed on a cBot Cluster Generation System using TruSeq PE Cluster Kit v3-cBot-HS according to the manufacturer's instructions. After cluster generation, the library preparations were sequenced on an Illumina HiSeq platform and 150bp paired-end reads were generated.

4. Data Processing and Analysis

Illumina Casava1.7 software used for basecalling.

The adaptor sequences and low-quality sequence reads were removed from the data sets. Raw sequences were transformed into clean reads after data processing. These clean reads were then mapped to the reference genome sequence. Only reads with a perfect match or one mismatch were further analyzed and annotated based on the reference genome. Tophat2 tools soft were used to map with reference genome. Quantification of gene expression levels were estimated by fragments per kilobase of transcript per million fragments mapped.

The following diagram shows the entire RNA-seq workflow (Figure 1).

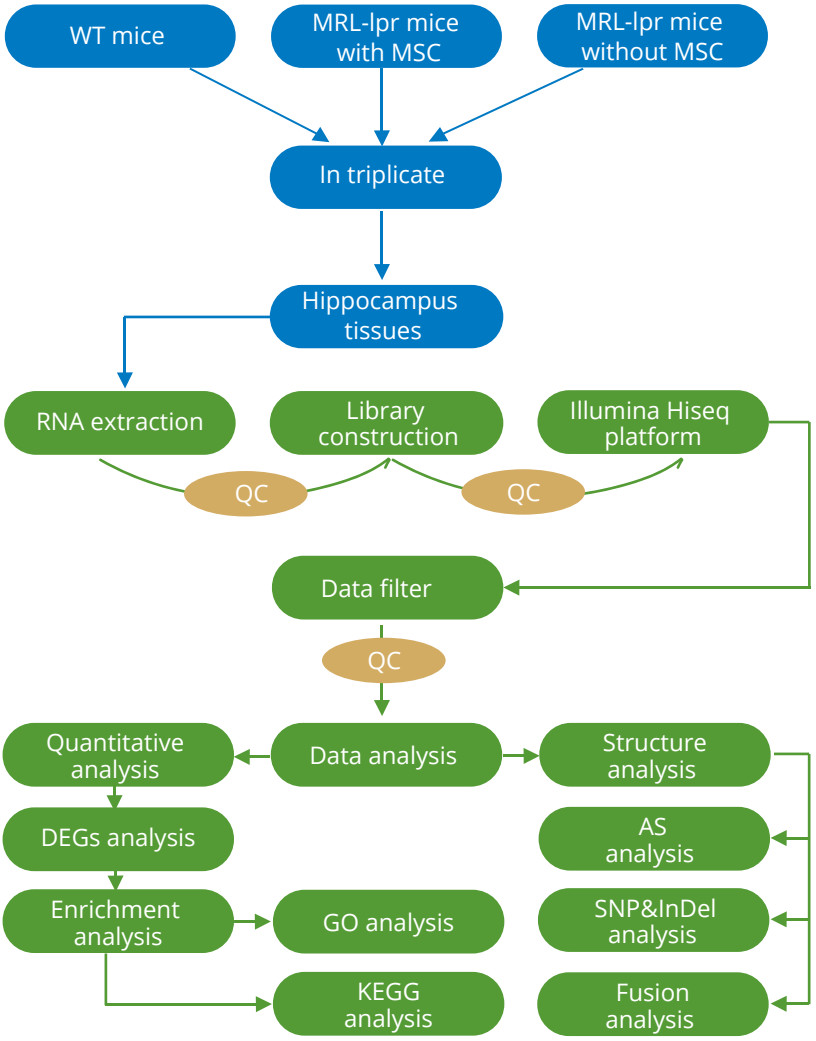


Figure 1 RNA-seq workflow

Results

1. Transcription profiling reveals molecular changes in the hippocampus of MRL/lpr mice

The authors performed transcriptome DGE analysis on the hippocampus of 6-week MRL/lpr and MRL/mpj mice. KEGG and GO analysis showed that MRL/lpr mice showed enrichment of immune activation pathways, including microglia mediated phagocytosis category and classical complement pathway.

2. Synaptic loss caused by phagocytosis of microglia is the cause of early behavioral defects in MRL/lpr mice

RNA-seq analysis detected expression changes in synaptic activity related genes. To further determine the cause of synaptic loss, the author treated MRL/lpr mice with the phagocytic inhibitor minocycline, and further observed the synaptic density in the hippocampus and analyzed the ultrastructural remodeling of glial phagocytic synapses. The observed evidence together shows that the MRL/lpr mice have synaptic loss due to the increased phagocytosis of synapses by microglia.

3. Complement C1q increases and accumulates at synapses in MRL/lpr mice

4. Transcriptome analysis combined with Western blot showed that the abundance of C1q protein in lupus hippocampus was much higher.

The author conducted immune co-staining co-IP, and other experiments, and the data showed that increased C1q accumulated around the synapses of the MRL/lpr brain and was associated with neurodegeneration.

Conclusion

In this study, researchers used RNA-seq in lupus-prone mouse model to study the crosstalk between microglia, complement C1q and its target neurons. Researchers found both microglia and C1q are necessary, and emphasized that the intrinsic Nr4a1 deficiency in neurons plays an active role in coordinating the synaptic engulfment of the microglia-C1q axis. These results offered a novel guide for therapeutic intervention in NPSLE.

Highlight

In this case, Novogene provided RNA-seq and analysis services to help researchers resolve four key points:

1. Specific gene expression profile of hippocampus in lupus-prone mice.
2. Identification of two pathways related to lupus by functional enrichment analysis, including immune-activated pathway and classical complement pathway.
3. Detection alterations in synaptic activity-related genes by RNA-seq analysis to provide a speculation that synaptic defects might be involved in behavioral defects in MRL/lpr mice.
4. Identification of lupus-specific genes (C1q and Nr4a1) by differential expressed genes analysis.

References

1. Han X, Xu T, Ding C, et al. Neuronal NR4A1 deficiency drives complement-coordinated synaptic stripping by microglia in a mouse model of lupus. *Signal Transduct Target Ther*. 2022 ;7(1):50.
2. McGlasson S, Wiseman S, Wardlaw J, et al. Neurological disease in lupus: toward a personalized medicine approach. *Front Immunol*. 2018;9:1146.
3. Dörner T, Furie R. Novel paradigms in systemic lupus erythematosus. *Lancet*. 2019; 393(10188):2344-2358.

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