Effective Diagnostic Biomarkers, Immune Infiltration, miRNA and N6-methyladenosine regulators in Lupus Nephritis

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Research

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Abstract

Objective

Lupus nephritis (LN) is the most common complication of systemic lupus erythematosus (SLE), which is an autoimmune disease involving multiple organs. This study aimed to explore the potential molecular mechanism of LN by bioinformatic analysis.

Method

In total, 130 LN, 25 living healthy donors were included to explore the differentially expressed genes (DEGs). The protein-protein interaction (PPI) network of DEGs were developed using the STRING database. Additionally, five algorithms were used to find the hub genes and their diagnostic effectiveness was predicted using receiver operator characteristic curve (ROC) analysis. CIBERSORT was used to evaluate the infiltration of immune cells in LN. Furthermore, the mRNA-miRNA network was constructed. Finally, we explored the landscape of N6-methyladenosine (m^6A) regulators in LN.

Result

Two hub genes—*FOS* and *IGF1*—were found by the intersection of five different algorithms. *FOS* and *IGF1* could jointly diagnose LN with the most excellent specificity and sensitivity. LN patients had lower activated and resting dendritic cells (DCs) while higher M1 macrophages and activated NK cells than HC. *FOS* had a positive correlation with activated mast cells and a negative correlation with resting mast cells. *IGF1* had a positive correlation with activated DCs and a negative correlation with monocytes. MiR-155 is considered as the hub miRNA. And m^6A modification is related to the severity of renal injury and involved in the pathogenesis of LN. MiRNA may affect the occurrence and development of LN by targeting m^6A regulators.

Conclusion

Activated DCs, resting DCs, M1 macrophages, and activated NK cells may play a role in LN pathogenesis. *FOS*, *IGF1* and miR-155 may be new potential molecular markers for the pathogenesis, progression and new molecular targets for treatment of LN. MiRNA may affect the occurrence and development of LN by targeting m^6A regulators.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease involving multiple systems, and lupus nephritis (LN) is a common and severe complication of SLE that affects approximately 50% of patients

with SLE[1]. LN is remarkably related to increased morbidity and mortality. The standard treatment for LN is glucocorticoid and immunosuppressant therapy, which prolongs the course of the disease. However, most patients present with chronic remission-relapse, a few patients achieve long-term remission, whereas some patients cannot tolerate the toxic side effects of drugs. In addition, currently, no evidence supports the use of traditional drug therapy in the prevention of organ damage[2]. The formation of autoantibodies against nucleic acid and nucleic acid binding proteins, as well as circulating immune complexes, deposit on various parts of the glomeruli, leading to LN[3]. The glomerulus, consisting of glomerular endothelial cells, glomerular basement membrane, and podocytes, plays a crucial role[4] in the pathophysiology of LN. To date, however, immune regulation in the pathogenesis of LN[1] is still poorly understood, and no drug has been approved specifically for treating LN[5]. Despite the broad recognition of the 2003 ISN/RPS classification system and the unequivocal value of histopathological findings in the diagnosis of LN, the need to incorporate insights from molecular studies, such as genetics, epigenetics, proteomics, and urine biomarker studies, was well acknowledged by lupus experts, and this will ultimately lead to personalized therapeutic strategies[6, 7].

The basic mechanism of LN is complicated; It is caused by the interactions among genetic, environmental, and immune factors. Immune factors play an important role in the whole process, especially in the early stages. Therefore, exploring the diagnostic biomarkers and revealing the immune mechanism of LN are the keys to the early prevention and treatment of LN. SLE is considered to be mainly caused by immune imbalance. The dysfunction of $CD8^+$ and NK cells in inhibiting $CD4^+$ cells in patients with SLE leads to abnormal activity of B cells, resulting in the generation of autoantibodies, which leads to the occurrence of LN[8]. In addition, the IFN pathway is considered as a key pathway in the pathogenesis of LN. Macrophages, plasmacytoid dendritic cells (pDCs), and other immune cells can activate the IFN pathway by secreting a large amount of IFN, thus stimulating the proliferation and activation of B cells[9]. As a result, the complement system is activated by autoantibodies in conjunction with autoantigens, and complement deposition in the renal system is a crucial factor in the pathogenesis of LN[5]. Therefore, this method would systematically explore immune cell infiltration in LN renal tissues. MicroRNA (miRNA) and N6 methyladenosine (m^6A) RNA modification have recently been considered to be closely related to the pathogenesis and development and may become a new molecular mechanism for the treatment of LN[10, 11]. A new research found that miR-19a might suppress the expression of YTHDF2 which can identify mRNA m^6A sites to regulate mRNA degradation and increase the m^6A levels of mRNAs in SLE[12].

In this study, we used bioinformatics approaches to screen out hub genes from four datasets between LN and healthy controls (HC). At the same time, miRNA and m^6A regulators centered on hub gene were searched. The objective of this study was to identify novel and effective diagnostic biomarkers for LN and to explore immune cells and molecular mechanism related to the pathogenesis of LN.

2. Materials And Methods

Search strategy

Using public data from the GEO database[13], we used the keywords “glomerulus” AND “lupus nephritis” OR “SLE” OR "systemic lupus erythematosus" OR "lupus erythematosus" OR "lupus"—for data searching. By January 27, 2022, a sum of 16,279 results were available in the GEO database. A total of 16,124 items not related to the purpose of this study were excluded by restricting the study type (expression profiling by array), the entry type (series), and tissue sources (Homo sapiens). After further selection with title, abstract, and samples, four series were included, and gene expression profiles (GSE32591, GSE127797, GSE37463, and GSE99339) were obtained, which had 155 sample data, including sample data of 25 healthy controls (HC) and 130 patients with LN.

Integration of microarray data and Principal Component Analysis (PCA)

These four raw datasets were chosen for integrative analysis after manipulation with the “*affy*” packet of the R language including background-adjusted and normalized raw data values. The microarray data were categorized into LN and normal groups based on clinical data. Afterwards, intra-group data repeatability of each group was verified using Pearson’s correlation test, and that of the dataset was tested by sample clustering analysis. Statistical analysis was performed using the R programming language, and the results were presented using the R package “ggplot2”.

Data processing and identification of DEGs

To obtain differential genes between the LN and HC groups, DEG analysis was performed with the “limma” package. Specifically, the expression profile dataset obtained, excluding genes with an expression value of 0 greater than 50%, was data transformed using the voom function and further multiple linear regression through the lmfit function. Genes with adjusted $P < 0.05$ and log-fold change (logFC) > 1 were considered as DEGs. Volcano plots were used to display all of the upregulated and downregulated DEGs using the “ggplot2” package in R. Thereafter, using the heatmap package in R, hierarchical clustering was performed. The IDs associated with the probe names were converted into gene symbols using the “clusterProfiler” packet in the R language.

GO and KEGG pathway enrichment analyses of DEGs

To analyze the functional and pathway enrichment of the proteins encoded by DEGs, packets in the R software, such as “clusterProfiler” and “pathview”, were used to perform Gene Ontology (GO)[14] and Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis[15]. A p-value of 0.05 and an FDR of < 0.05 were regarded as statistically significant.

PPI network integration

DEGs were submitted to the Interaction Gene Search tool (STRING) (version 11)[16] to investigate their protein interactions. Thereafter, the Cytoscape software (V 3.8.2)[17] was used to integrate and visualize

the PPI network. Each network node represented a different protein, and the association between these nodes represented the interaction among biological molecules, which can be used for identifying interactions and associated pathways among these proteins encoded by DEGs in LN. The PPI network co-DEGs were obtained by setting the confidence level to 0.4. The central node closely related to other corresponding proteins may be core proteins or key proteins, which play important physiological functions.

Screening of Hub Genes

Plug-in CytoHubba[18] in the bioinformatics software, Cytoscape (3.8.2), was used to quantify the degree of connectivity between each protein node, and explore key nodes and fragile motifs in the PPI network by five topological algorithms. Each topological analysis method considered the top 10 nodes as significant genes in the network. Cross genes in the top 10 nodes in each topological algorithm were considered the most important hub genes in the network. Afterwards, Immune-related genes were obtained from the Immunology Database and Analysis Portal (ImmPort)[19].

The Association between Hub Genes and Clinical Features of LN

Nephroseq combines publicly available renal gene expression profiles—gathered with a sophisticated analysis engine and powerful web application designed for data mining and visualization of gene expression data. Correlation analysis and subgroup analysis between hub genes and clinical features were performed using the Nephroseq v5 online tool[20] to confirm the potential functions of hub genes involved in the pathogenesis of LN. Pearson's correlation coefficient of the association between the hub genes and glomerular filtration rate (GFR) was calculated to construct a scatter plot.

Analysis of the Predictive Value of Biomarkers

To evaluate the accuracy of hub genes, receiver operating characteristic (ROC) curve analysis was applied using the R package "pROC" in the normalized dataset. Using the Wilcoxon–Mann–Whitney test, the hub genes were identified based on the following criteria: AUC > 0.80 and relatively expressed levels of $P < 0.05$.

Evaluation and Correlation Analysis of Infiltration-Related Immune Cells

To estimate the number of 22 different types of immune cell types present in LN glomerular tissues and normal glomerular tissues, the normalized data were uploaded to the platform of CIBERSORT. The percentage of immune cells in the gene expression matrix and the relationship between the two immune cells were calculated by installing the "corrplot" package. Thereafter, using the "ggplot2" package, PCA was performed to determine the difference between LN and HC renal tissues. The "ggplot2" and "ggstatsplot" packages were used for analyzing the Spearman relationship between characteristic diagnostic markers and immune-infiltrating cells and illustrating the results.

Identified the microRNAs in LN and hub genes-miRNAs network construction

Comprehensive experimentally validated hub mRNA-miRNAs interaction data was collected from TarBase. Next, we further explore the miRNAs that regulate these risk genes in LN. The Human MicroRNA Disease Database (HMDD) is a database that curates experimentally supported evidence for human miRNA and disease associations. The intersection of hub genes-miRNAs in and miRNAs in LN were used to construct the mRNA-miRNA regulated network. Cytoscape software was used to visualize the network.

M ^{6} A RNA Methylation Regulator Detections

The m^{6}A RNA methylation regulator list we used was based on previous publications. Then, Using Wilcoxon rank sum test to determine expression differences of m^{6}A regulators between LN samples and HC. The R packet “ggplot2” was used to visualize the results. Spearman correlation analysis was used to determine the correlation between m^{6}A regulators and hub genes. Pearson’s correlation coefficient of the association between the hub genes and glomerular filtration rate (GFR) was calculated to construct a scatter plot.

3. Results

The details of our selection process are shown in Supplementary Fig. 1. LN was completely distinguished from HC by PCA analysis (Fig. 1a). An aggregate of 201 DEGs were acquired, of which 31 DEGs were upregulated and 170 was downregulated (Fig. 1b). As the adjusted p-value decreased, DEGs increased in ranking in this experiment. As shown in Fig. 1c, the R heatmap software was used to draw a heatmap of the 31 upregulated and 50 downregulated DEGs.

GO term and KEGG pathway enrichment analysis of DEGs

With an adjusted p-value of < 0.05, the GO term and KEGG pathway enrichment analyses of upregulated and downregulated genes were acquired, respectively. The results of the GO term in LN are shown in Fig. 2a–d. The visual analysis results of the KEGG enrichment of DEGs in LN are shown in Fig. 3a and b. GO analyses revealed that DEGs were enriched in biological processes (Fig. 2b),such as chemical response, cellular response to chemical stimulus, and response to external stimulus; molecular functions (Fig. 2c), such as transporter activity, transition metal ion binding, and anion transmembrane transporter activity; cellular components (Fig. 2d), such as extracellular region, vesicle, and extracellular region. These DEGs were enriched in KEGG pathways, including the peroxisome proliferator-activated receptor (PPAR) signaling pathway, phenylalanine metabolism, and renin-angiotensin system (Fig. 2e and f). The pathway map for targeted LN (Supplementary Fig. 2) was described using KEGG pathway enrichment. The significantly enriched terms and pathways may help us to further explore the role of DEGs in LN.

Analyzing DEGs in LN using a PPI network

To construct PPI networks, DEG expression products in LN were constructed using the STRING database. After deleting all isolated and partially disconnected nodes, as shown in Fig. 3a, an integrated network was built. The 30 most significant genes are shown in Fig. 3b.

Identification and Validation of Hub Genes

Finally, Venn diagram was adopted to illustrate the intersection of five algorithms (Fig. 3c, Supplementary Table 1). Based on the Immunology Database and Analysis Portal (ImmPort), two hub genes, FOS, and insulin-like growth factor 1(IGF1), were screened out. The number of nodes in the FOS network was 32, and the number of nodes in the IGF1 network was 32, both of which were > 30, indicating that the two genes were more strongly associated with other genes in the PPI network.

Association between the Hub Genes and Clinical Features of LN

Using Nephroseq v5, the expression of the hub genes (FOS and IGF1) showed the difference between LN and HC; both hub genes were downregulated in the LN renal tissues, compared with the HC renal tissues (Fig. 4a and b). In addition, the expression of FOS in LN renal tissue samples was negatively correlated with GFR (Fig. 4c and d), which is one of the important indices for measuring renal function.

Diagnostic Effectiveness of Biomarkers for LN

The normalized dataset was used to verify the diagnostic specificity and sensitivity of the biomarkers for LN by ROC analysis. As shown in Fig. 4e and f, the AUC values of FOS and IGF1 were 0.786 (95% CI, 0.676–0.878) and 0.885 (95% CI, 0.802–0.951), respectively. Therefore, FOS and IGF1 could diagnose LN.

Immune infiltration analyses

In studying immune cell infiltration, 155 tissues, including 25 HC and 130 LN tissues, were identified (Supplementary Fig. 3). PCA (Fig. 5a) showed that a significant difference ($P < 0.05$) in immune cell infiltration existed between LN with HC. The results of the immune cell infiltration analysis indicated that eosinophils were not ex-pressed in all tissues. As shown in Fig. 5b, 10 types of differentially infiltrating immune cells between the LN and HC groups were present. A clear correlation existed between increasing B cell memory and T cell gamma delta in LN, which suggests that humoral immunity plays an essential role in the mechanism of LN. Thereafter, the percentage of the 22 types of infiltrating immune cells was analyzed (Fig. 5c). Every differentially infiltrating immune cell was assessed using the Wilcoxon rank test. Activated and resting DCs were lower in LN than M1 macrophages and activated NK cells. This finding could reflect the heterogeneously inflammatory character of LN (Fig. 5d).

Correlation Analysis Between Key Biomarkers and Infiltration-Related Immune Cells

Based on the results of the correlation analysis, FOS showed a positive correlation with activated mast cells ($R = 0.27$, $P = 8e\text{-}04$) and showed a negative correlation with resting mast cells ($R = -0.23$, $P = 0.0037$). IGF1 had a positive correlation with activated DCs ($R = 0.33$, $P = 3.4e\text{-}05$) and a negative correlation with monocytes ($R = -0.22$, $P = 0.0053$) (Supplementary Fig. 4a–e).

Identified and analysis of miRNAs in LN

According to the HMDD database, it was reported that 34 miRNAs were associated with LN. Based on Tarbase, we constructed the hub genes-miRNAs network and the LN-related miRNAs were shown in red on the network (Fig. 6). Among the miRNAs targeted for the hub genes, hsa-miR-155-5p, hsa-miR-1-3p, hsa-miR-182-5p and has-miR-16-5p were the top miRNAs that considered targeted for two hub genes. We crossed the miRNA screened from HMDD with these three RNAs and found the hub miRNA, has-mir-155-5p.

Landscape of m ^{6} A RNA methylation regulators in LN.

Wilcoxon rank-sum test (for unpaired comparisons) was used to compare the m^{6}A regulators' expression level between LN and normal cases. Unpaired comparisons showed that CBLL1, METTL5, WTAP, RBM15/15B, ZC3H13, ZCCHC4, FTO, YTHDF1/3, YTHDC1/2, and IGFBP1/2 were all downregulated in LN (Fig. 7A). Based on the correlation analysis between m^{6}A genes and hub genes, there was consistent variation in IGFBP3, YTHDF3, ZCCHC4, RBM15 and FTO in IGF1 and FOS (Figs. 7B-C). To further elucidate the roles of these m^{6}A markers in LN, correlation analysis between markers and GFR was carried out in the Nephroseq database (Figs. 8). RBM15, RBM15B, WTAP, YTHDF1 and ZC3H13 were all negatively correlated with GFR, indicating that these genes may aggravate kidney damage in patients with LN.

4. Discussion

SLE is a kind of chronic progressive systemic autoimmune genetic disease. Previous studies have suggested that autoantibodies, which are generated by abnormal activation of autoimmunity, combine with autoantigens and deposit in the skin, joints, small blood vessels, kidneys, and other systemic organs. These processes result in the special pathological changes that occur in SLE[21]. The complexity of etiology and difficulty of treatment have been the focus and challenge of medical research. Although traditional drugs can stabilize the disease and improve the prognosis of most patients with SLE, some drugs have poor efficacy or intolerance. In addition, the toxic side effects of these drugs, coupled with poor quality of life, can make SLE incurable[2]. LN, characterized by a complex pathogenesis, is a major risk factor for death in patients with SLE. The main initiating mechanisms are increased number of apoptotic cells, absence of phagocytic clearance mechanism of apoptotic products, and accumulation of neutrophil extracellular trapping net[22], leading to continued exposure of autoantigens to the immune system. IFN-mediated activation of the innate and adaptive immune systems is continuously activated, causing the chronic course of SLE[1].

According to the results of KEGG, PPAR may be a key pathway of LN. Recent studies have demonstrated that the PPAR pathway is linked to both innate and active immunity. Activation of the PPARγ pathway in mice significantly inhibited the ability of DCs to prime naive CD4$^+$ T cells[23] and selectively inhibited Th17 differentiation in mice CD4$^+$ T cells, which shares a strong relationship with inflammatory response and autoimmune diseases[24]. Besides, serum autoantibodies were significantly elevated in PPARγ-deficient

mice[25]. Related studies have shown both natural and synthetic drugs targeting PPARγ can promote the differentiation of monocytes into M2 phenotype and improve SLE prognosis[24]. The PPARγ agonist, rosiglitazone, can induce the differentiation of Tregs, inhibit effector T cells in lupus, reduce production of autoantibodies, and prevent renal lesions in SLE mouse. These features are beneficial to the early prevention of SLE in mice[26].

In the PPI network, the MCODE plug-in in Cytoscape was used to screen out the most significant modules, and CytoHubba plug-in was used to filter out the top 40 genes of the total score according to the degree of close correlation between proteins. Finally, two hub genes of *FOS* and *IGF1* were screened out. Both decreased significantly in LN, and in particular, *FOS* expression is inversely proportional to renal injury. The two genes have good diagnostic characteristics for the diagnosis of LN, and the combined diagnosis of the two genes are excellent.

As a general fast response nuclear transcription factor, FOS is upregulated in response to various inflammatory processes[27]; consists of FOS, FOSB, FOSL1, and FOSL2; and participates in the regulation of cell proliferation, differentiation, transformation, and apoptosis[28]. In LN, the expression of FOS in glomerular cells was significantly higher than that in mildly altered nephrotic syndrome and HC[29]. Compared with HC, FOS transgenic mice were more resistant to experimental autoimmune encephalomyelitis by inhibiting inflammatory cytokine production in DCs[30]. Moreover, the Fosl1-JunB complex plays a central role in the development of RA[31]. FOSL2 is a key factor in the control of autoimmunity and in the generation of Tregs[32]. Previous studies have indicated the clinical relevance of FOSL2 expression in podocytes and its role in inducing podocyte injury[33]. Hence, the FOS family may probably be involved in the pathogenesis of LN and may be a potential specific therapeutic target for LN.

IGF1 and its receptor (IGF1R) are both members of the insulin-like growth factor family. IGF1 is regarded as a critical regulator of energy metabolism and growth, promoting cell proliferation and prolonging cell lifespan[34]. Meanwhile, according to recent studies, IGF1 is implicated in immune and autoimmune diseases and plays an anti-inflammatory role[35]. Activation of the IGF1 pathway can stimulate the proliferation of thymus immune cells, increase $CD4^+$ T cells, but inhibit $CD8^+$ T cells[36]. Upregulated IGF1R participates in the development of Graves' disease and RA and their transfer to inflammatory and tissue remodeling sites[36]. Furthermore, IGF1 accelerates the occurrence of inflammation and aggravates injury by activating the PI3K/Akt signaling pathway[37], which is inseparable from the pathogenesis of LN[38]. Therefore, blocking IGF1 and its downstream signaling may be an effective therapeutic method for these diseases[36]. In a word, the IGF1 pathway is probably involved in the genesis and development of LN.

To further investigate the role of immune cell infiltration, we used CIBERSORT to conduct a comprehensive evaluation of SLE immune infiltration. In contrast to HC, LN had a significantly increased number of activated M1 macrophages and activated NK cells significantly. Whereas resting DCs and activated DCs significantly decreased. M1 macrophages may efficiently present antigens and secrete various cytokines, such as IL-1β, IL-10, and TNF-α, which play a crucial part in the pathogenesis of LN[1].

DCs are extremely strong antigen-presenting cells (APCs) capable of initiating primary T cell responses by presenting antigenic peptides associated with major histocompatibility complex (MHC) class I and II molecules on the cell surface[39]. According to previous research, IFN-α produced by pDCs plays a vital role in LN pathogenesis[1, 40]. Immune cells, including macrophages, overproduce IL-18, stimulate the differentiation of Th1 cell, and recruit pDCs from peripheral blood to the glomerulus, thus leading to the occurrence of glomerular inflammation, and LN subsequently[41]. In particular, T follicular helper cells activate germinal centre B cells to produce autoantibodies, and IL-17-producing T helper 17 cells drive kidney injury.

Remarkably, in the miRNA-mRNA network, as a hub miRNA, miR-155 was decreased in LN patients in tandem with a lowered GFR[42]. Overexpression of miR-155 could enhance the ability of mature DCs to activate NK cell production of IFN-γ and abnormally activate T and B cells[10]. MiR155 regulates the depletion of $CD8^+$ T cells in long-term chronic infection, while FOSL2 could antagonize it[43]. MiR155 also promotes apoptosis of colonic smooth muscle cells by targeting IGF-1[44]. Importantly, owing to its central role in the development of the immune system, miR-155 is deeply involved in SLE[45]. These confirm our research and further guide us to explore the role of miRNA in the pathogenesis of LN with FOS and IGF1

M^6A modification is considered to be related to the pathogenesis of SLE[46]. Considering that, we further carried out m^6A analysis. The results show that compared with HC, RBM15B, WTAP, YTHDF1, ZC3H13, FTO and IGFBP2 are low expression in LN. It is worth noting that the expression of RBM15B, WTAP, YTHDF1 and ZC3H13 was negatively correlated with renal injury. Among them, YTHDC1 and FTO was closely and positively correlated with FOS while RBM15B and IGFBPs with IGF1. Interestingly, miR-155 regulates m^6A level by targeting FTO[47], while FTO retards FOS-dependent ovarian aging[48], which is suggested that miR155 may regulate the methylation level of FOS through FTO. Our miRNA prediction of FTO also confirmed this (Fig. 9).

Significantly, overexpression of IGF1 and IGFBP2 in glomeruli may play important roles in renal function and morphological changes in MRL/LPR mice[49]. Studies have shown that IGFBP2 have great potential as a SLE and LN biomarker[50]. Thus, m^6A modification participates in the pathogenesis of LN and may become a new biomarker and targeted therapeutic mechanism. MiR155 may regulate the methylation level of FOS through FTO in LN. However, further studies are needed to determine how miRNA and methylation regulators regulate FOS or IGF1 and participate in the pathogenesis of LN.

5. Conclusions

Activated DCs, resting DCs, M1 macrophages, and activated NK cells are likely to participate in the occurrence and progress of LN. FOS and IGF1 are excellent diagnostic markers of LN. Additionally, as a hub miRNA related to hub genes, miR155 participates in immune response and is related to LN. Meaningfully, miR155 may target m^6A regulators to participate in the occurrence of LN. However, how miR-155 and m^6A participate in the occurrence of LN through FOS or IGF1 remains to be further studied.

Abbreviations

LN
Lupus nephritis
SLE
systemic lupus erythematosus
HC
Healthy controls
DEGs
differentially expressed genes
PPI
protein-protein interaction
ROC
receiver operator characteristic curve
M^6A
N6-methyladenosine
DCs
dendritic cells
MiRNA
microRNA
GO
Gene Ontology
BP
Biological process
MF
Molecular function
CC
Cellular Component
KEGG
Kyoto Encyclopedia of Genes and Genomes
ImmPort
the Immunology Database and Analysis Portal
GFR
glomerular filtration rate
APCs
antigen-presenting cells
MHC
major histocompatibility complex
HMDD
The Human MicroRNA Disease Database

Declarations

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Author Contributions

S.W, Z.P designed and conducted the whole research. Z.P and W.L. applied for the dataset analysis of LM. XLiang, Z.P and XLin drafted the manuscript. XLiang, XLin and Z.L revised and finalized the manuscript. All authors contributed to the article and approved the submitted version.

Data Availability Statement

The datasets generated and analyzed during the current study are available in the Gene Expression Omnibus (GEO) datasets. The datasets supporting the conclusions of this article are included within the article and its additional files.

Declaration of conflicting Interests

The authors declare no conflict of interest and the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate

Not acceptable.

Consent for publication

Not acceptable.

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Figures

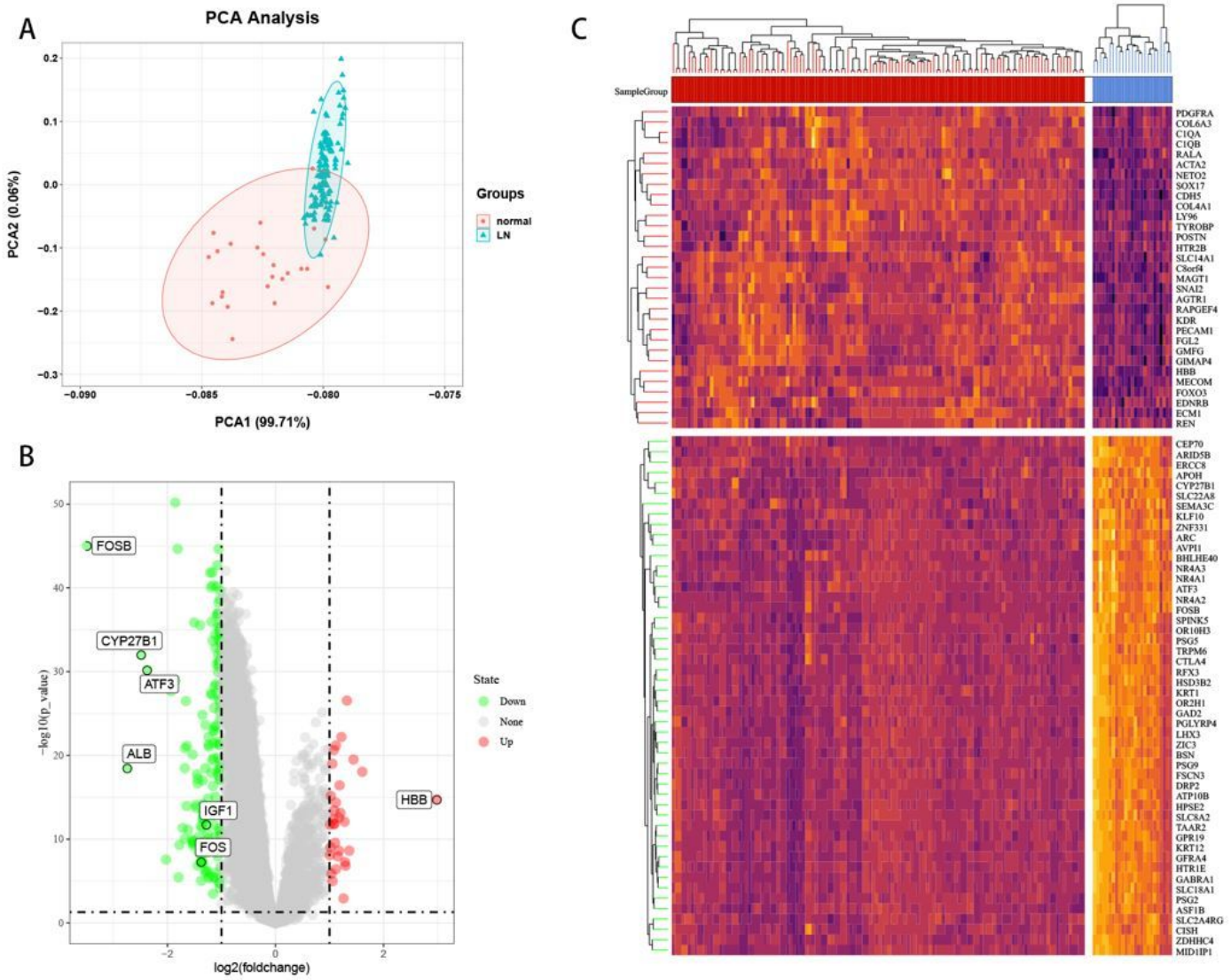

Figure 1

Identification of differentially expressed genes (DEGs). a. Principal component analysis (PCA) was performed on two groups. LN samples are indicated by green dots and ellipses, whereas HC samples are indicated by red dots and ellipses; b. Volcano plot of the DEGs between LN and HC tissues. Gray points represent the adjusted p-values >0.05. Green dots represent adjusted p-values <0.05 and downregulated genes. Red dots represent adjusted p-values <0.05and the upregulated genes; c. Heatmap of the top 201 DEGs according to the adjusted p-values and logFC. Higher gene expression is indicated by yellow, whereas lower gene expression is indicated by black.

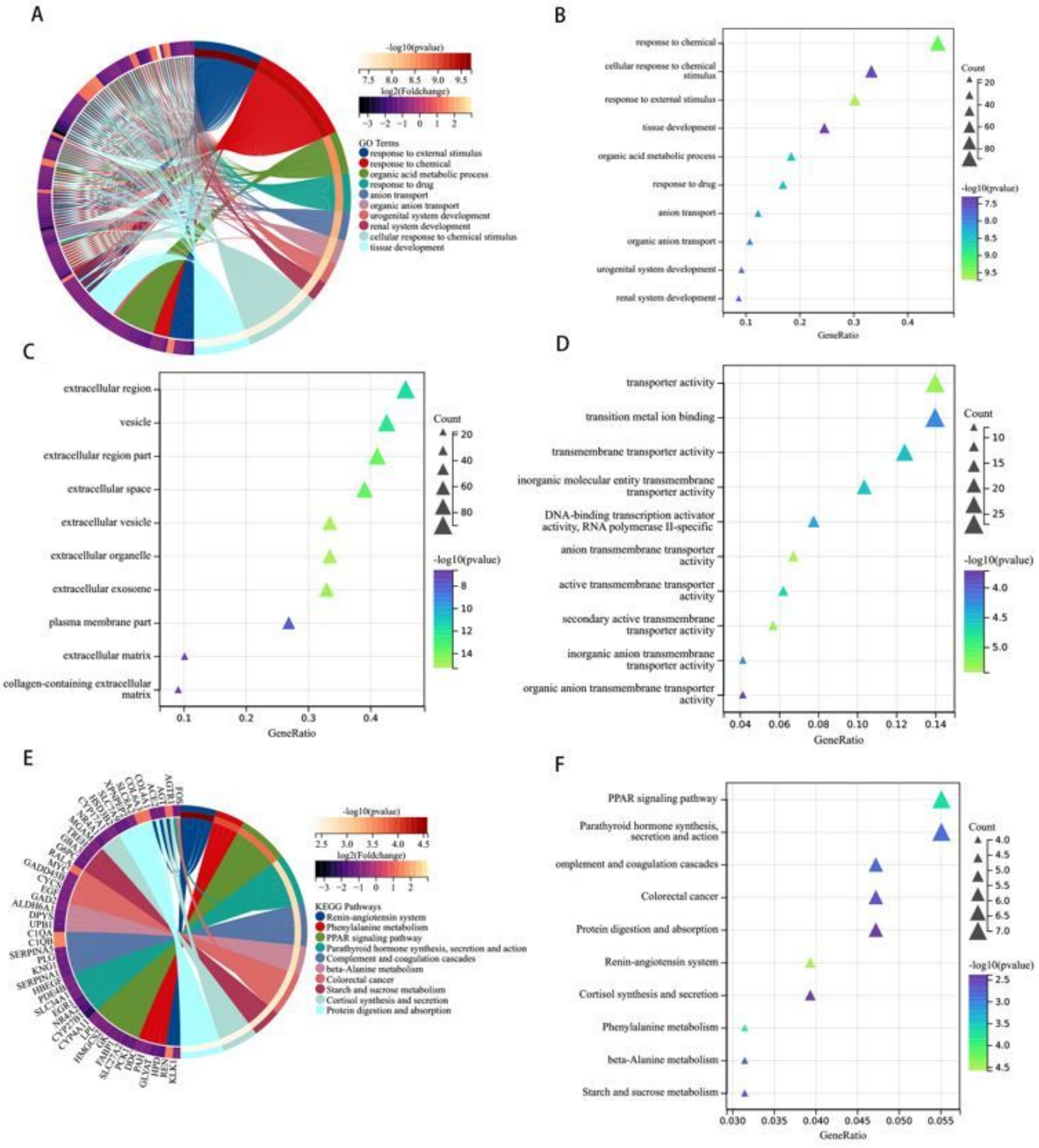

Figure 2

GO and KEGG pathway enrichment analysis of DEGs enrichment analysis of DEGs. a. GO term enrichment analysis of DEGs; b. The top ten enriched BP of DEGs; c. The top ten enriched MF of DEGs; d. The top ten enriched CC of DEGs. e, f. KEGG pathway terms in the enrichment analysis of DEG.

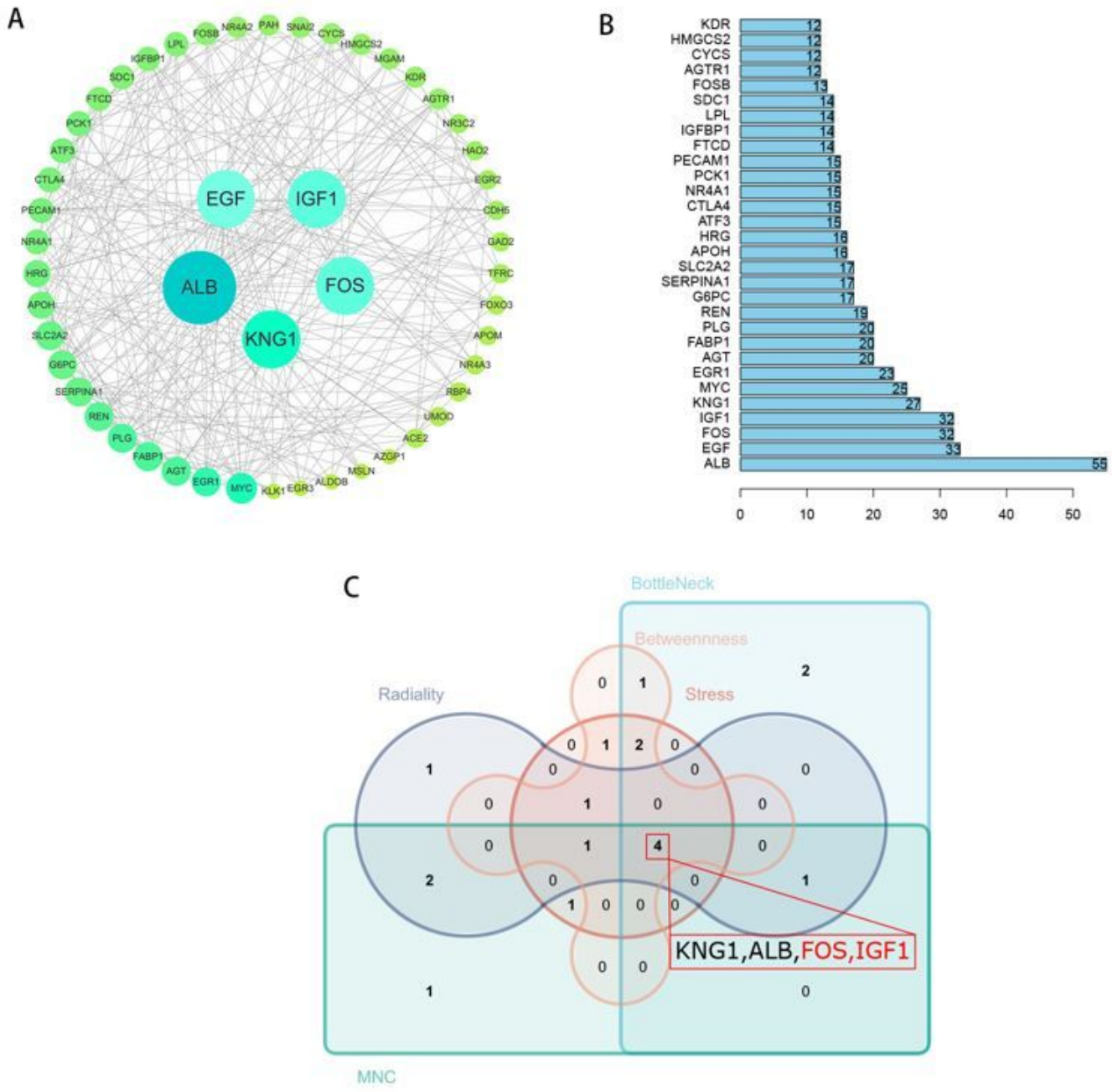

Figure 3

Protein-protein interaction (PPI) network of differentially expressed genes (DEGs) and hub genes identification. a. PPI network 201 DEGs filtered into the PPI network that contained 199 nodes and 602 edges); b. The predicted association rank (from low to high) of the top 30 genes in the PPI network; c. Venn diagram was adopted to illustrate the intersection of five algorithms, genes marked in red are associated with immune mechanisms.

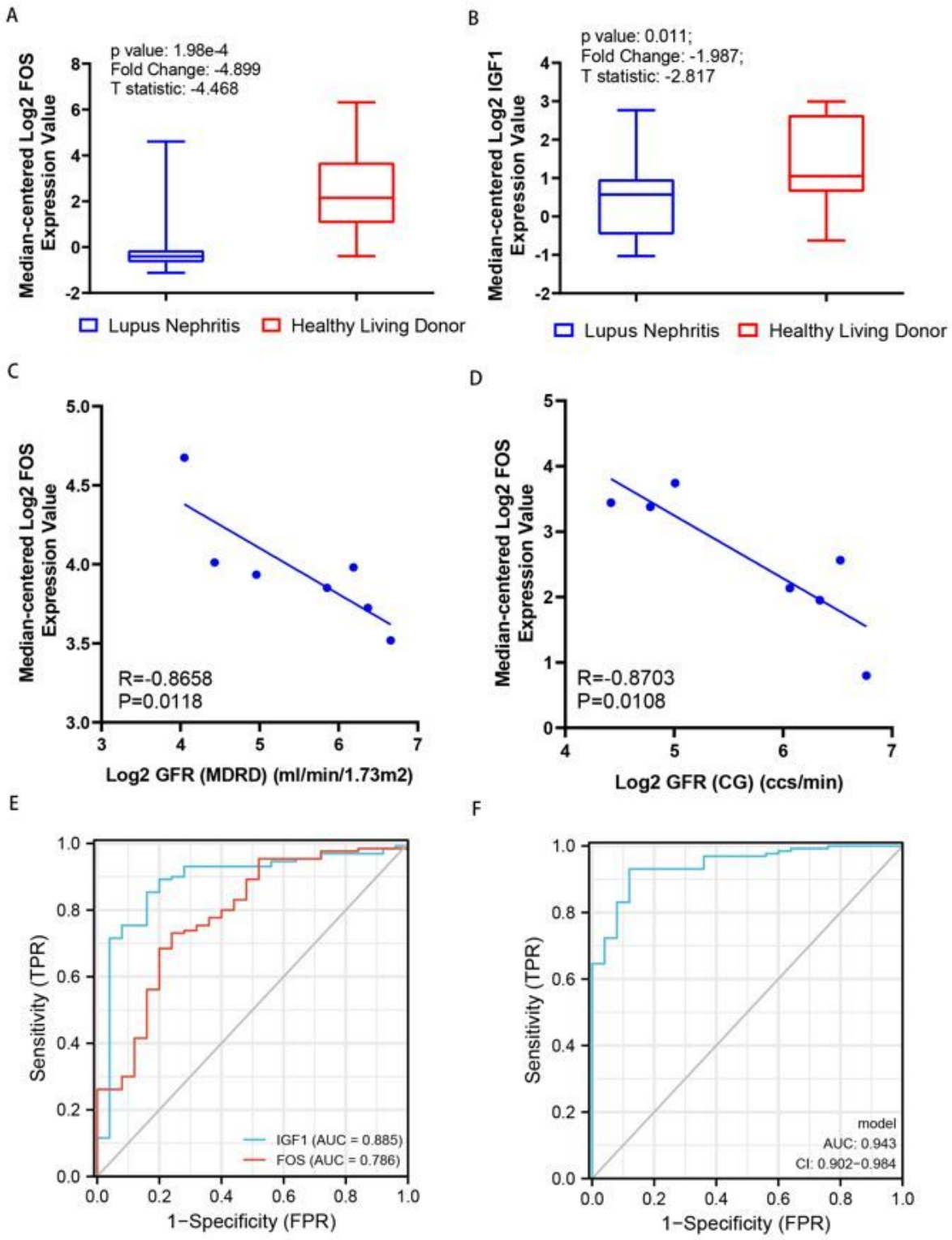

Figure 4

Expression of hub genes, correlation between expression with GFR and diagnostic effectiveness of the biomarkers for LN. a, b. The expression of FOS and IGF1 in LN and HC; c, d. The correlation analysis between FOS and GFR in LN; e, f. The diagnostic effectiveness of the combined hub genes for LN by ROC analysis.

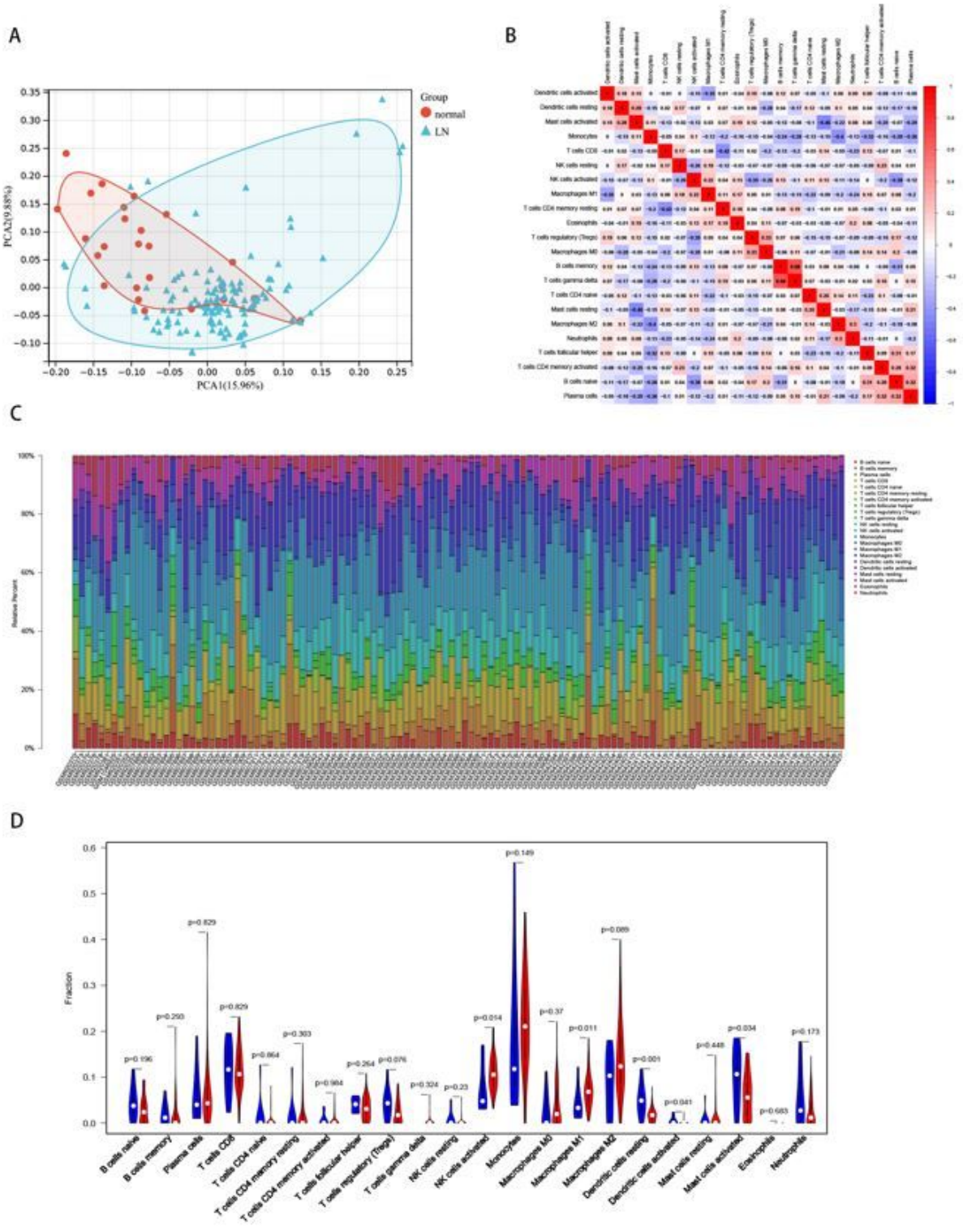

Figure 5

Immune cell infiltration in LN and HC tissues. a. Principal component analysis (PCA) was performed on two groups. Blue points and ellipse indicate LN sample, and red points and ellipse indicate HC samples; b. Correlation matrix of infiltration degree of immune cells in LN samples. Red indicates trends consistent with the positive correlation, and blue indicates trends consistent with the negative correlation between two immune cells. A higher statistical data size represents a more positive or negative correlation; c.

Landscape of immune cell infiltration; d. Violin diagram of immune cell proportions in two groups. The blue fusiform fractions on the left represent the HC group, and the red fusiform fractions on the right represent the LN group.

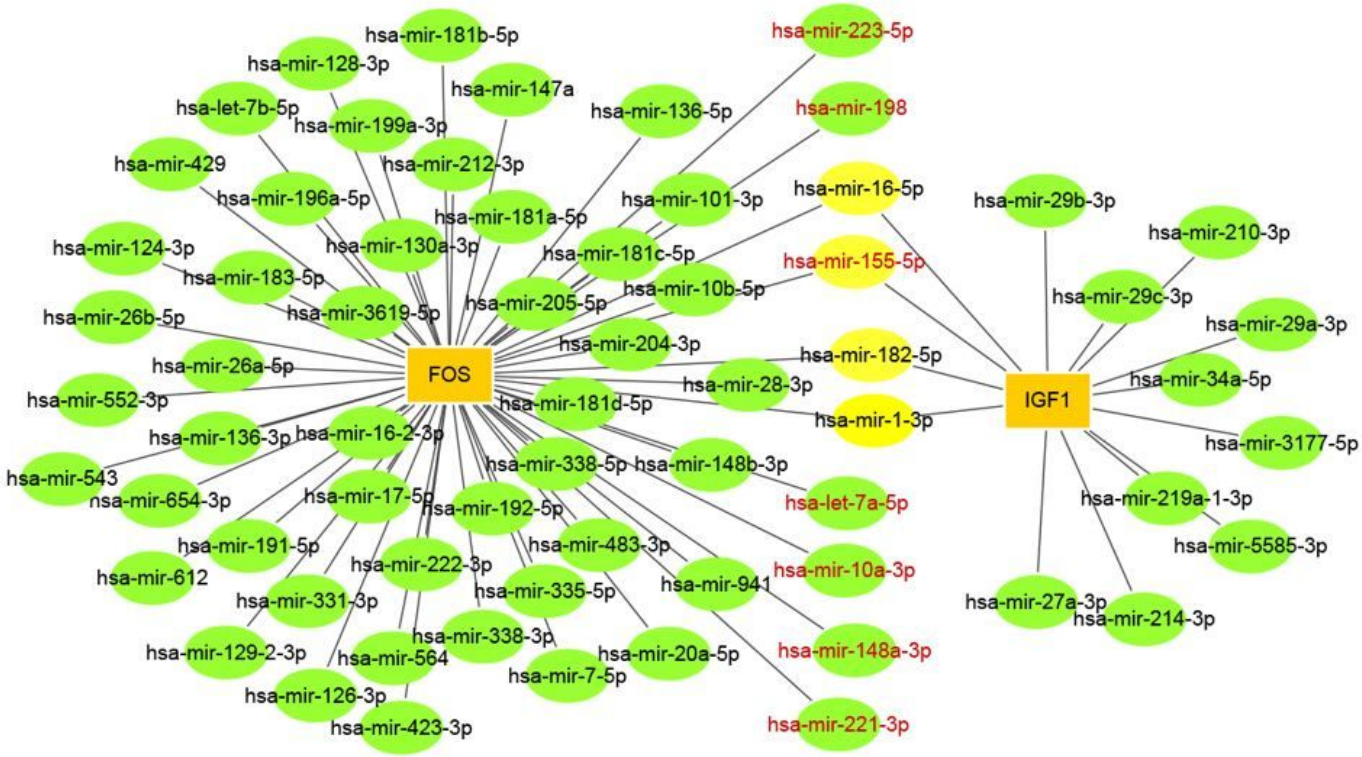

Figure 6

hub genes-miRNAs network. Red rectangles represent hub genes, and ovals represent miRNAs. green, yellow ovals represent miRNAs targeted by 1, 2 hub genes, respectively. MiRNAs in red are miRNAs in LN based on HMDD.

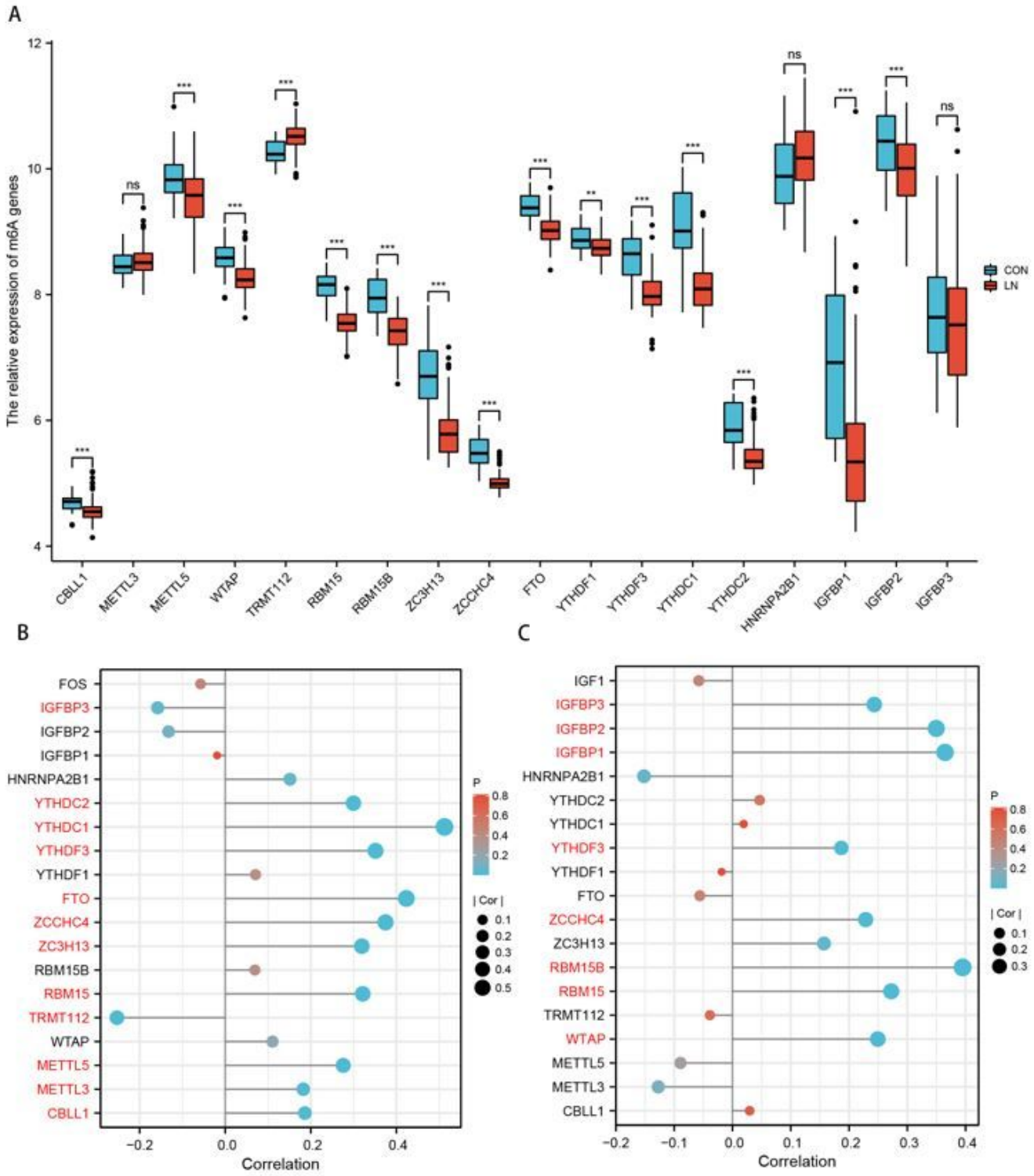

Figure 7

Landscape of m^{6}A RNA methylation regulators in LN. a. Box plot demonstrating the expression level of 18 m^{6}A regulators in glomeruli between healthy donor and LN; b, c. Correlation between m^{6}A regulator expression and hub genes expression in LN: Genes annotated in red indicate the results as significant (P<0.05);

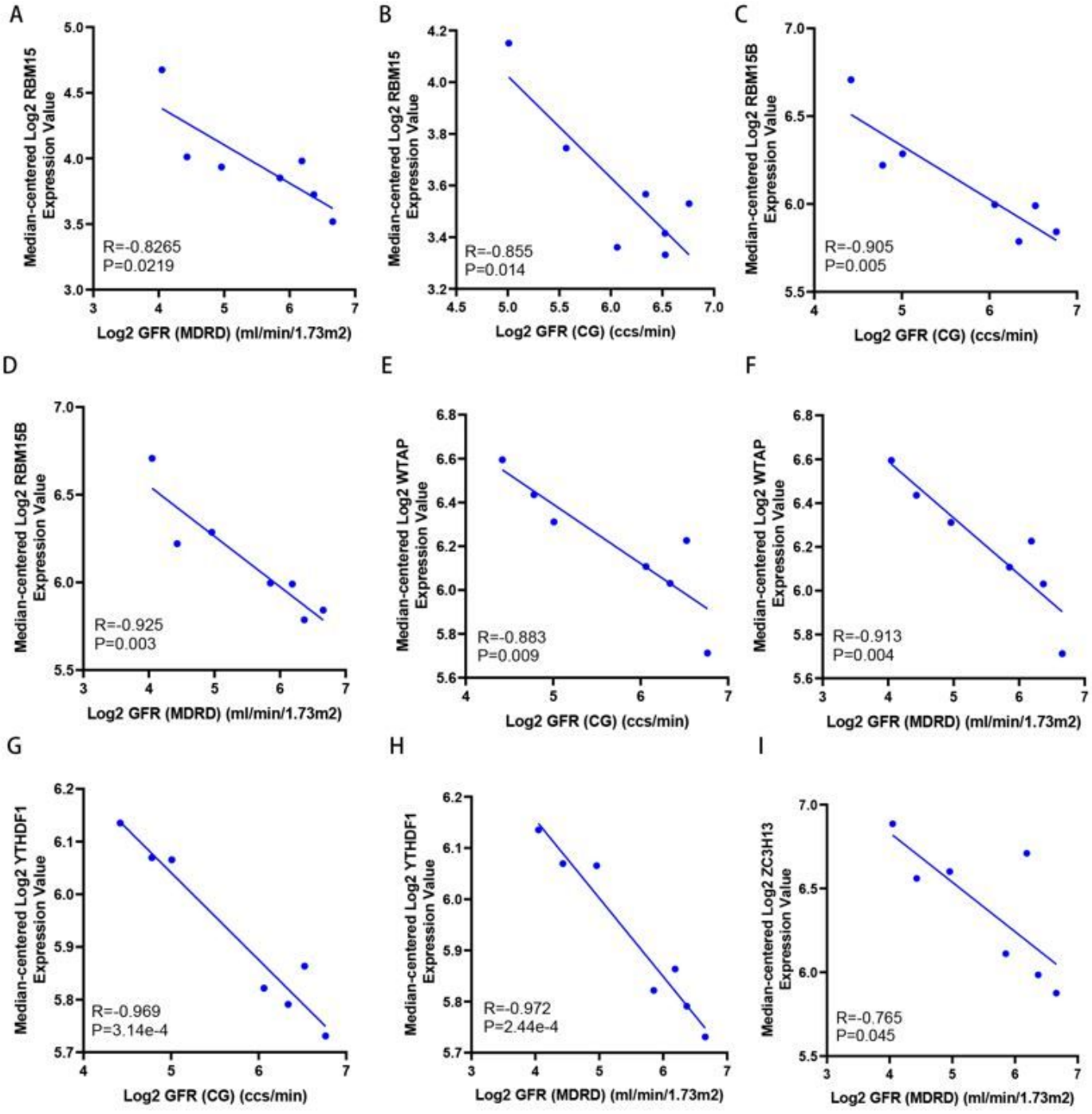

Figure 8

Relationship between m^{6}A methylation pattern markers and renal function (glomerular filtration rate).

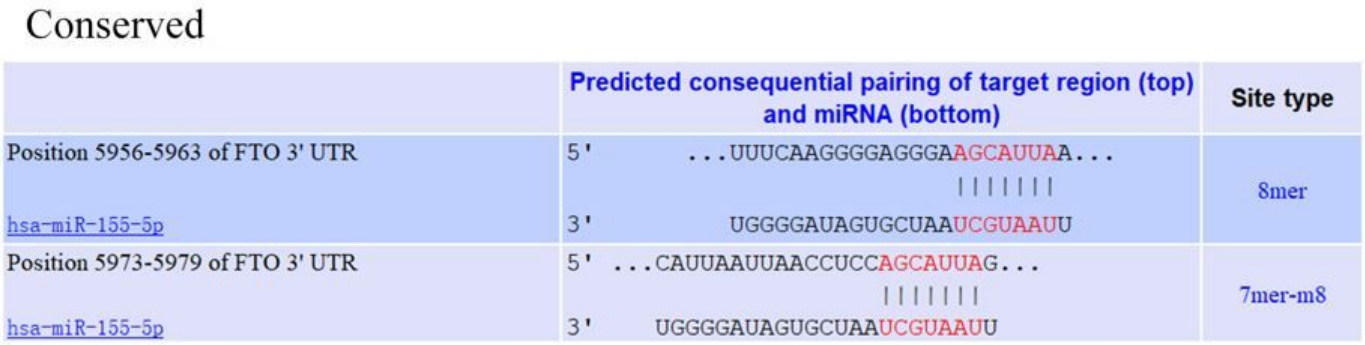

Figure 9

Predicted the potential miRNAs targeting the 3'-UTR of FTO by TargetScan, and miR-155-15p had binding sites for FTO

Supplementary Files

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- Supplementarymaterial.docx