

Plasma exosomes may mediate the development of lupus nephritis in patients with systemic lupus erythematosus

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Abstract

Background: Lupus nephritis (LN) is the most serious complication of systemic lupus erythematosus (SLE), and plasma exosomes may serve as a bridge. MicroRNAs (miRNAs) are abundant in exosomes, so this study aimed to explore the role of exosome-derived miRNA in the development of LN.

Methods: The publicly available data containing plasma exosomal miRNAs in SLE patients and healthy controls were researched, and differential expression and functional enrichment analysis of exosomal miRNA was conducted. Then, plasma exosomes from SLE patients were extracted, and the accuracy of differential expression and functional enrichment analysis was preliminarily verified. PKH26 dye was used to label exosomes to detect whether exosomes can enter HK2 cells. Evaluation of plasma exosomes impact on cell viability was done by utilizing CCK-8 assay. Flow cytometry was used to measure cell apoptosis.

Results: Plasma exosomes were successfully extracted and identified. Through differential expression analysis of the public data and subsequent qPCR validation, we observed that miR-20b-5p is overexpressed in plasma exosomes of SLE patients, whereas miR-181a-2-3p is downregulated. Then functional enrichment analysis revealed that these differential miRNAs primarily regulate processes such as apoptosis, autophagy, and inflammation. Then, flow cytometry analysis conducted after co-incubation of plasma exosomes and peripheral blood mononuclear cells confirmed that exosomes can indeed regulate apoptosis. And plasma exosomes can successfully enter HK2 cells without affecting cell activity. In addition, plasma exosomes promote HK2 cell apoptosis and autophagy. Overexpression of miR-181a-2-3p could inhibit HK2 cells apoptosis and upregulate the expression of bcl2, and beclin1. At the same time, a trend towards increased apoptosis rates was observed in HK2 overexpressed miR-20b-5p, although the difference did not reach statistical significance. And miR-20b-5p can enhance the expression of caspase3 and beclin1 while suppressing the expression of bcl2 and LC3β.

Conclusion: Our research indicates that the abundant presence of miR-20b-5p and the depletion of miR-181a-2-3p in plasma exosomes of SLE patients may mediate the promotion of apoptosis and autophagy in HK2 cells, thereby causing kidney damage and the development of LN.

Keywords

Lupus nephritis, systemic lupus erythematosus, exosome, apoptosis, autophagy

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Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease with complex pathogenesis, which affects multiple systems and organs.¹ Current studies have confirmed that the onset of SLE is closely related to genetics, environment, immunity, etc.,² but the specific mechanism still needs to be explored. SLE occurs predominantly in women, with a peak onset during the reproductive years, seriously impacting the quality of life of patients.³ It's reported that both the prevalence and incidence of SLE are increasing in Asia,⁴ and the

symptoms tend to be more severe in Asian populations than white patients,⁵ which has significantly increased our burden.

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As research progresses, the role of immune disorders in SLE has been gradually revealed. The use of immunosuppressants and biologics have significantly reduced the mortality of SLE.⁶ However, variations in the physical health of patients mean that not all SLE patients experience symptom improvement, and the high costs further limit the widespread use of these treatments. Therefore, it is still necessary for us to find new way to improve SLE prognosis.

Exosomes are extracellular vesicles secreted by most eukaryotic cells with a size of about 40-150 nm.⁷ They can act as a “bridge” of intercellular communication by carrying bioactive molecules, including proteins, RNA, DNA, and metabolites.⁸ The specific functions of exosomes depend on the molecules they carry, allowing them to play decisive roles in various diseases.^{9,10} Over the past two decades, exosome-related research in autoimmune diseases has gained worldwide attention. It has been reported that the number of serum exosomes in peripheral blood of SLE patients increases, and it is correlated with the severity of SLE.¹¹ Exosomes from bone marrow mesenchymal stem cells can promote the anti-inflammatory polarization of macrophages, thereby alleviating SLE.¹² Lupus nephritis (LN) is the most serious complication of SLE, and exosomes may serve as a bridge between the two conditions. Recent studies have demonstrated that miRNAs are abundant in exosomes and may play an important role in SLE and LN.¹³ Unfortunately, the role of exosomes obtained from SLE patients in LN and its specific mechanism have not been fully elucidated.

To address this issue, plasma exosomes from SLE patients were extracted to investigate their effects on renal tubular epithelial cells in vitro and to further explore their mechanisms in LN.

Methods

Human samples

A total of 21 individuals, including 11 healthy people and 10 SLE patients, were enrolled in this study (Supplemental Table 1). The inclusion criteria for SLE patients were those who met the American College of Rheumatology criteria for SLE, excluding other autoimmune diseases. Healthy controls were age- and sex-matched volunteers. Blood samples (10 mL) were obtained from each participant for the extraction of plasma exosomes. This study was approved by the Human Ethics Committee of The Second Affiliated Hospital of Kunming Medical University (PJ-2024-231), and written informed consent was obtained from all participants.

Exosome isolation

The blood extracted was centrifuged (2,000g for 20 min) within 4 h to obtain plasma by removing cells and cell debris. ExoEasy Maxi Kit (QIAGEN) was used for extraction of

plasma exosomes according to kit handbook provided by the manufacturer. To put it simply, the obtained plasma was centrifuged at 12,000g for 15 min to remove cellular debris. The resulting plasma was mixed with an equal volume of XBP buffer, and the mixture was placed in a centrifuge column and spun at 500 g for 1 min to remove the liquid from the collection tube. Then, 10 mL of XWP buffer was added to the column, and it was centrifuged at 5,000 g for 5 min to wash the exosomes. Finally, 400 µl of XE buffer was added to the membrane in the centrifuge column to elute the exosomes, and the eluate was collected. The exosome content was quantified using a bicinchoninic acid (BCA) assay (Beyotime, Shanghai, China), aliquoted, and stored in the refrigerator for future use.

PBMC isolation

The collected blood sample was processed within 4 h. After mixing each blood sample (~3 mL) with an equal volume of PBS, it was gently layered on top of the lymphocyte separation medium (TBD, Shanghai, China). The samples were then centrifuged at 650 g for 20 min. The middle white membrane layer was collected, and 5 mL of PBS was added. This mixture was then centrifuged at 300 g for 5 min to collect the peripheral blood mononuclear cells (PBMCs) for subsequent experiments.

Cell culture

HK2 cells and their specialized culture medium were purchased from Procell Life Science & Technology Co., Ltd. Typically, HK2 cells are incubated in a specialized culture medium. When co-incubated with exosomes, the HK2 cells need to be cultured in modified Eagle's medium (MEM) (high glucose, Gibco, USA) mixed with 10% exosome-depleted fetal bovine serum (SBI, USA) and 1% antibiotics (Gibco, USA). HK2 cell were divided into three groups: control group (5% CO₂, 37°C), healthy plasma exosome co-incubated (HC_Exo) group, and SLE plasma exosome co-incubated (SLE_Exo) group (plasma exosomes were added to the medium with 10% exosome-depleted fetal bovine serum). These cells were placed in a cell culture incubator and cultured for 48 h.

Cell viability assay

The CCK8 assay kits used in our experiment were purchased from Beyotime Company, Shanghai, China. Logarithmic growth cells were digested with trypsin and inoculated into a 96-well plate at 8000 cells per well. After incubating these cells in a cell culture incubator for 24 h to adhere to the wall, the specialized medium was removed. And according to the groupings, the medium containing 10% exosome-depleted fetal bovine serum enriched with either HC_Exo or SLE_Exo

were added, the culture was continued for an additional 24 h. Then, 10 μ L of CCK8 solution was added to all wells. After incubation for 2 h. The absorbance (OD value) at 450 nm was measured, and the activity of the cells was calculated.

Exosome tracing

PKH26 dye (Duolaimi Biotechnology Co., Ltd, Wuhan, China) was used to label exosomes according to the instructions provided. 100 μ L of PKH26 working solution diluted 10 times with Diluent C was added into 100 μ g of BCA-quantified exosomes medium, mixed well for 10 min. To remove excess PKH26, 10 mL of PBS was added to the exosome solution containing PKH26. Then, the mixture was placed in a 50 mL ultrafiltration tube (100KD) and centrifuged at 2000g for 5 min. Repeat this process three times. The PKH26-labeled exosome solution was added to HK2 cells and incubated for 24 h. Afterwards, the cell coverslips were washed and fixed. A mounting medium containing DAPI was dropped onto a glass slide, the cell coverslip was placed on top. Inverted microscopes are used for observation.

Cell apoptosis

Annexin V-FITC/PI Apoptosis kit (Multi sciences, Hangzhou, China) was used to detect apoptosis of HK2 cells. After digesting the cells with Accutase, PBS was used to wash cell. Then, cells were resuspended in 500 μ L of 1 $\times$ Binding Buffer, and 5 μ L of Annexin V-FITC and 10 μ L of PI are added to each tube and mixed, the mixture was incubated for 5 min in the dark at room temperature. Eventually, flow cytometry was used to detect apoptosis.

Identification of DE miRNAs and Functional enrichment analysis

The datasets and literature on plasma exosomal miRNAs was retrieved,¹⁴ the differential expressed miRNAs (DE miRNAs) were filtered with a threshold p -value $< .05$ and log2-fold change (FC) > 1 or < -1 .¹⁵ And volcano plots of DE miRNAs were generated by R software. Target prediction was performed using the miRWalk website (https://mirwalk.umm.uni-heidelberg.de/search_mirnas/),¹⁶ and functional enrichment analysis was conducted based on the Metascape website¹⁷.

Mimics transfection

We purchased miR-20b-5p and miR-181a-2-3p from Genepharma (Shanghai, China). PolyFast Transfection Reagent was obtained from MedChemExpress (New Jersey, USA). When the cells in the 6-well plates reach 80% confluence, the specialized medium was removed and replaced with MEM medium. 3 μ L of transfection reagent and 1 μ g of mimics were added to 50 μ L of MEM medium, respectively, and incubate

them separately for 5 min. Then, the MEM medium containing the transfection reagent and the MEM medium containing the mimics were mixed, and continued to incubate for 15 min. Add the mixture of mimics and transfection reagent to the cells and transfect for 6 h. Afterwards, replace with the specialized HK2 culture medium and continue to incubate for 24 h before collecting the cells for subsequent analyses.

Quantitative real-time PCR (qPCR)

Total RNA was extracted using TRIzol (Vazyme Biotech Co., Ltd, Nanjing, China). A reverse transcription kit (TransGen Biotech, Beijing, China) was used to synthesize complementary DNA. Quantitative PCR was performed with Roche LightCycler 480 Real Time PCR System using using PerfectStart Green qPCR SuperMix (TransGen Biotech, China).^{18–20} GAPDH and U6 were used as internal reference genes of mRNA and miRNA, respectively. The qPCR amplification steps included: an initial activation of DNA polymerase at 94°C for 30s; denaturation at 94°C for 5s, annealing at 55°C for 15s, and extension at 72°C for 10s, for a total of 45 cycles. The results were analyzed using the $2^{-\Delta\Delta C_t}$ method. The primer sequences for each gene were as follows: caspase3, F: GGC GGT TGT AGA AGA GTT TCG, R: TCACGGCCTGG GATTTC AAG; bcl2, F: CCCGCGACTCCTGATTCATT, R: AGTCTACTTCCTCTGTGATGTTGT; beclin1, F: GGGCT CCCGAGGGATGG, R: CTGGGTCTCTCCTGGTTTCG; LC3 β , F: CGAACAAGAGTAGAAGATGTCCGA, R: GCCTGATTAGCATTGAGCTGTAAGC; GAPDH, F: TGA CCTGCCGTCTAGAAAAACCT, R: GCTGTTGAAGTCAGAGGAGACCA; U6, F: CTCGCTTCGGCAGCACA, R: AACGCTTCACGAATTTGCGT; miR-181a-2-3p, F: AC-CACTGACCGTTGACTGTACC, R: universal, miR-20b-5p, F: CAAAGTGCTCATAGTGCAGGTAG, R: universal.

Western blot

After washing the cells with PBS, total protein was extracted by adding 1 mL of lysis buffer mixed with protease inhibitors (Beyotime, Shanghai, China). The protein content was measured using the BCA method. The proteins were then separated by electrophoresis on a 10% SDS-polyacrylamide gel (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane (Millipore, Billerica, USA). The membrane was incubated with primary antibodies (anti-BCL2, R23309; anti-CASPASE3, R22842; anti-BECLIN1, 222,090; anti-LC3 β , R10025; anti-CD64, R23327; anti-TSG101, 381,538; Zen-bio, Sichuan, China) and IgG-HRP secondary antibodies (511203 and 550110, Zen-bio, Sichuan, China). Following this, the membrane was exposed to enhanced chemiluminescence (ECL) Plus reagent (Beyotime, Shanghai, China) for imaging. The densitometry analysis was performed by using Image J software.

Statistical analysis

SPSS 17.0 software was used for statistical analyses. When the data met the conditions of normal distribution and homogeneity of variances between two groups, the *t* test was used. If the data did not follow a normal distribution, the Wilcoxon-signed rank test was used for grouped data. For cases where the data met the conditions of normal distribution and homogeneity of variances and involved multiple groups, one-way analysis of variance (ANOVA) was employed. $p < .05$ indicates that the difference was statistically significant.

Result

Extraction and identification of serum exosomes from SLE patients

Under transmission electron microscope, the plasma exosomes of SLE and healthy control group showed a concave sac-like structure (Figure 1(a)). Nanoparticle tracking analysis (NTA) results showed that exosomes ranged in size from 70 to 160 nm (Figure 1(b)). Total protein of exosomes was extracted, and western blot was performed to detect the expression of protein markers in exosomes. Then, we detected the expression of CD63 and TSG101 in exosomes samples (Figure 1(c)). These characteristics are consistent with the previously reported exosome characteristics, suggesting that we have successfully extracted exosomes.

Differential expression and functional enrichment analysis of exosomal miRNA

In order to further explore the function of plasma exosomes, datasets (accession number: HRA000985) containing plasma exosomal miRNAs in SLE patients and healthy controls were retrieved in the Genome Sequence Archive.¹⁴ And differential analysis found 407 upregulated and 596 downregulated miRNAs. After removing miRNAs with fold changes of positive or negative infinity, 185 differentially expressed miRNAs (DEmiRNAs) were screened (Figure 2(a)). To verify the accuracy of the sequencing results, plasma exosomes from SLE patients and healthy people in our hospital were extracted, qPCR validation found that the expression of miR-20b-5p in the plasma exosomes of SLE patients (SLE_Exo) was significantly decreased, while the expression of miR-181a-2-3p was significantly increased. Furthermore, miR-186-5p and miR-144-5p also showed an increasing trend in SLE_Exo, although without statistical significance. These results are consistent with the sequencing findings (Figure 2(b)). Then functional enrichment analysis revealed that these differential miRNAs primarily regulate processes such as apoptosis, autophagy, and inflammation (Figure 2(c)). To investigate whether SLE plasma exosomes can regulate cell apoptosis, we co-incubated SLE patient plasma exosomes and healthy individual plasma exosomes with PBMCs from the peripheral

blood of SLE patients for 48 h, respectively. Flow cytometry results indicated that compared to the PBS group, the apoptosis rate in SLE_Exo group was significantly increased, and the apoptosis was also increased in the SLE_Exo group compared to the healthy plasma exosome (HC_Exo) group, although the difference was not statistically significant (Figure 2(d)).

Plasma exosome could promote cell apoptosis and autophagy

Increased renal tubular epithelial cell apoptosis and autophagy are important causes of LN.²¹ Therefore, whether SLE plasma exosomes can enter HK2 cells and cause LN by inducing their apoptosis and autophagy has aroused our interest. Plasma exosomes at concentrations of 10 µg/mL and 20 µg/mL were co-incubated with HK2 cells for 24 h, and it was found that there was no significant change in cell viability (Figure 3(a)). Considering that after 48 h of co-culture, the cell viability in the 20 µg/mL dose group was slightly lower compared to the 10 µg/mL dose group (Figure 3(a)), the lower dose (10 µg/mL) was used in subsequent experiments. Then, plasma exosomes labeled with PKH26 were co-incubated with HK2 cells for 24 h. Red fluorescence on the membrane of HK2 cells was observed, indicating that the exosomes could enter the HK2 cells (Figure 3(b)). Subsequent flow cytometry analysis with Annexin V-FITC/PI Apoptosis kit revealed an increase in apoptosis of HK2 cells treated with SLE plasma exosomes (Figure 3(c)). Then, the results of qRT-PCR suggested that compared with PBS group, LC3β mRNA level in HC_Exo group was significantly decreased, and LC3β mRNA level in SLE_Exo group was significantly increased compared with HC_Exo group (Figure 3(d)). These results suggest that SLE plasma exosomes could significantly promote apoptosis and autophagy of HK2 cells. Subsequent western blot results revealed that compared with HC_Exo group, the expression of CASPASE3 was increased in SLE_Exo group, and the expression of BCL2 was decreased (Figures 3(e) and (f)), which was consistent with the results of flow analyzer. At the same time, the expressions of LC3β in SLE_Exo group was significantly increased, while there was no difference in the expression level of BECLIN1 (Figures 3(e) and (f)).

miR-20b-5p and miR-181a-2-3p could regulate cell apoptosis and autophagy

To explore whether miR-20b-5p and miR-181a-2-3p including in SLE plasma exosomes regulate cell apoptosis, we transfected HK2 cells with miR-20b-5p mimics and miR-181a-2-3p mimics, qPCR result suggested that miR-20b-5p and miR-181a-2-3p were overexpressed (Figure 4(a), and (d)). Flow cytometry analysis revealed a trend towards increased apoptosis rates in HK2 cells transferred

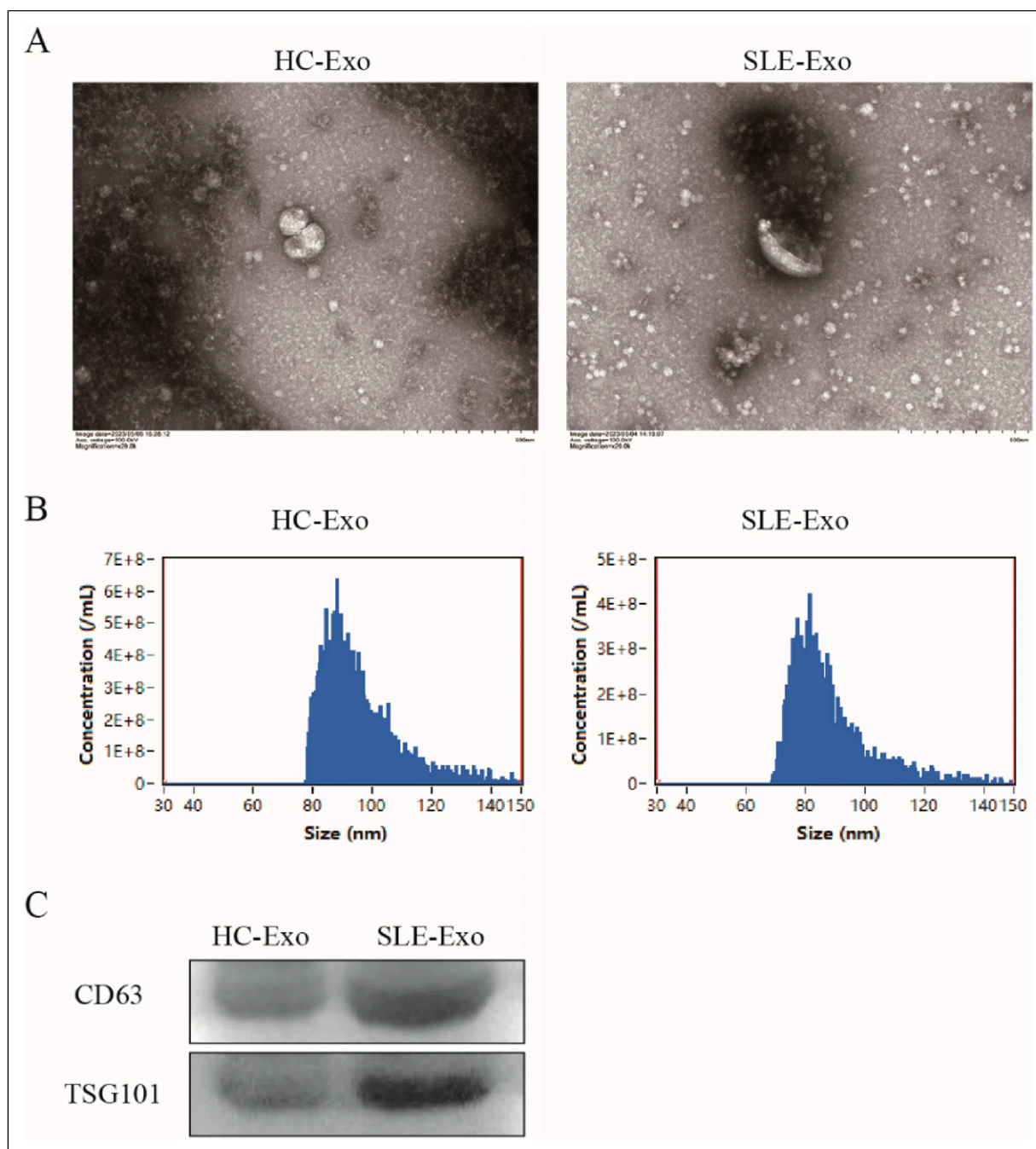


Figure 1. Plasma exosomes from SLE patients were extracted. (a) Transmission electron microscopy revealed the spherical characteristics and size of exosomes by ultracentrifugation pellet from plasma. (b) Nanoparticle tracking analysis (NTA) to quantify the nanosize and concentration of exosomes. (c) Western blotting showed that the pellet after ultracentrifugation was strongly reactive for antibodies against the exosomal membrane markers CD63 and TSG101.

miR-20b-5p mimics, although the difference did not reach statistical significance (Figure 4(b)). However, miR-20b-5p can enhance the expression of caspase 3 and beclin1 while suppressing the expression of bcl2 (Figure 4(c)). Moreover, we further explore the function of miR-181a-2-3p. Compared with negative control (NC), overexpression of miR-

181a-2-3p inhibited HK2 cells apoptosis as measured by flow cytometry with Annexin V-FITC/PI Apoptosis kit (Figure 4(e)), and qPCR results indicate that miR-181a-2-3p can significantly upregulate the expression of bcl2 and beclin1 while have no impact on the expression level of caspase3 and LC3 β (Figure 4(f)).

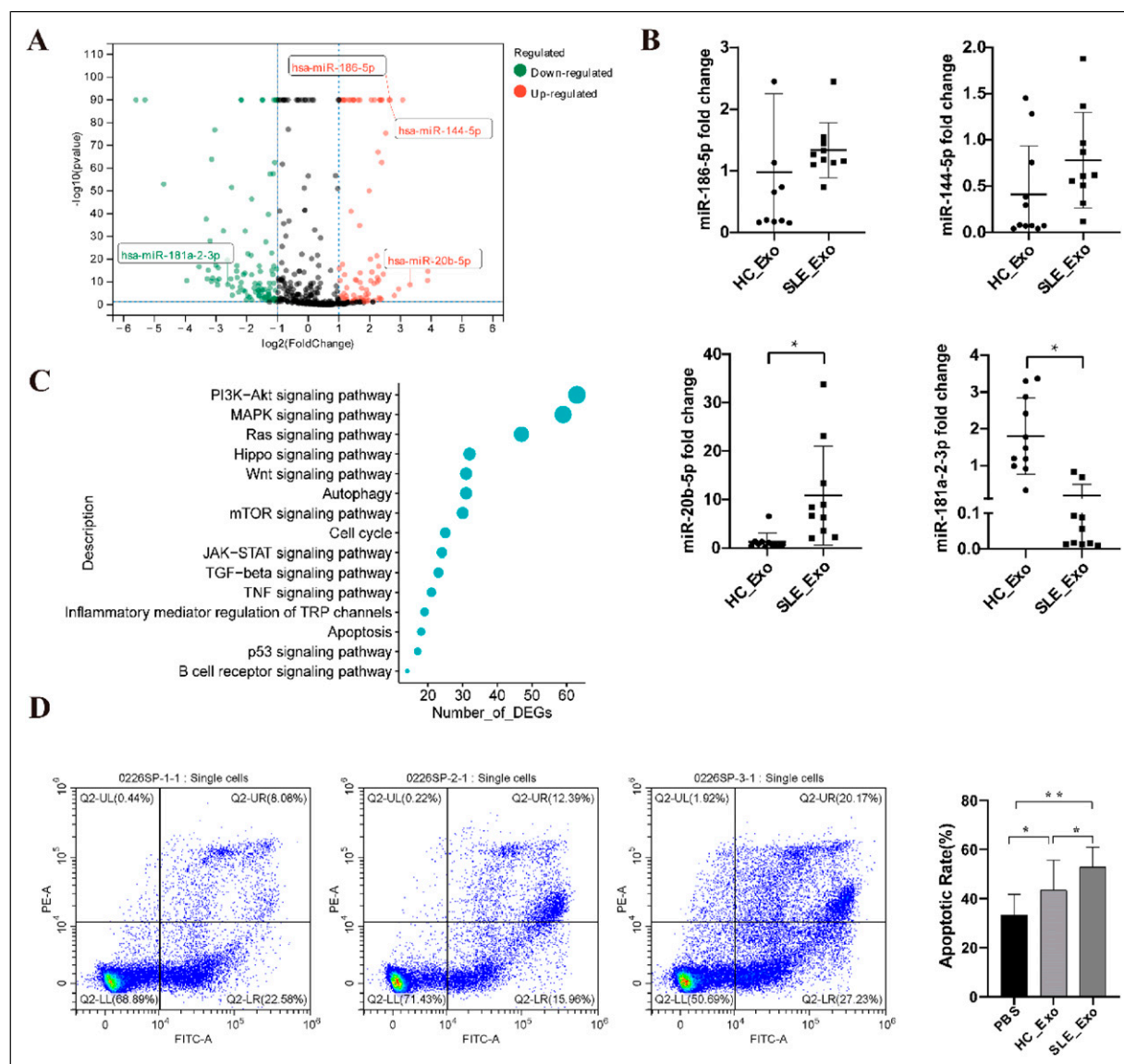


Figure 2. Differential expression of miRNAs in plasma exosomes. (a) The volcano plots depicted differential expression of microRNAs between SLE patients and healthy people in plasma. (b) qPCR results of differential expressed miRNAs (* $p < .05$). (c) Functional enrichment analysis of exosomal miRNA-targeted genes. (d) The apoptosis of PBMC after 24h of co-incubation with 10 $\mu\text{g/ml}$ exosomes was measured with the flow cytometry ($N = 3$, * $p < .05$, ** $p < .01$).

Discussion

Lupus nephritis (LN) is one of the gravest complications arising from systemic lupus erythematosus (SLE), critically endangering patients' lives.²² Proteinuria caused by the deposition of immune complexes and renal tubules damage are identified pathological features in LN.²³ Until now, the underlying mechanisms by which SLE leads to LN remain unclear. Exosomes, functioning as vehicles for the transport of diverse cargoes including RNA, proteins, enzymes, and lipids, enable inter-organ communication.²⁴ Therefore, they could be pivotal in connecting SLE with

LN.²⁵ Although the pathogenesis of LN is multifaceted (including environmental impacts, genetic predisposition, and epigenetic modifications), microRNAs (miRNAs) have proven to be noteworthy epigenetic components that significantly regulate gene expression.²⁶ At the same time, due to their small molecular weight, miRNAs are easily loaded into exosomes and are abundant within them.²⁷ Moreover, exosomes can enhance the stability of miRNAs, protect their integrity, and transport them to distant target cells or tissues, thereby promoting intercellular communication.²⁸ Therefore, exosomal miRNAs may play a crucial role in SLE complicated with LN.

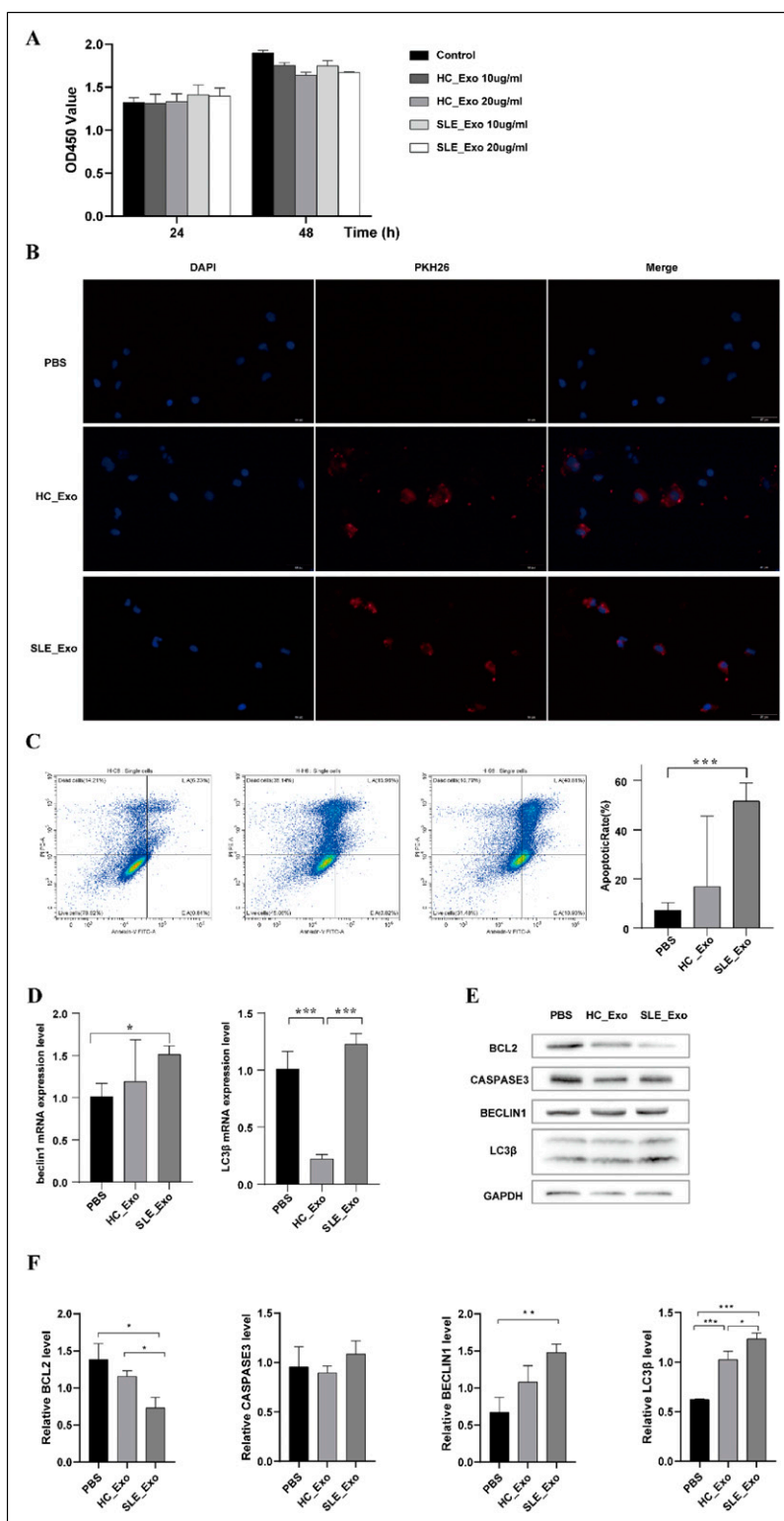


Figure 3. Effect of plasma exosomes on HK2 cells. (a) The cell viability of HK2 cell after 24h or 48h of co-incubation with 10 μ g/ml and 20 μ g/ml exosomes was measured by CCK8, respectively. (b) Exosomes were observed to enter the cells under an inverted microscope after 24h of co-incubation. (c) The apoptosis of HK2 cell after 24h of co-incubation with 10 μ g/ml exosomes was measured with the flow cytometry. (d) The qPCR results of beclin1 and LC3 β in HK2 cell after 48h of co-incubation with 10 μ g/ml exosomes. (e) and (f) The western blot results of CASPASE3, BCL2, BECLIN1 and LC3 β in HK2 cell after 48h of co-incubation with 10 μ g/ml exosomes. $N = 3$, * $p < .05$, ** $p < .01$, *** $p < .001$.

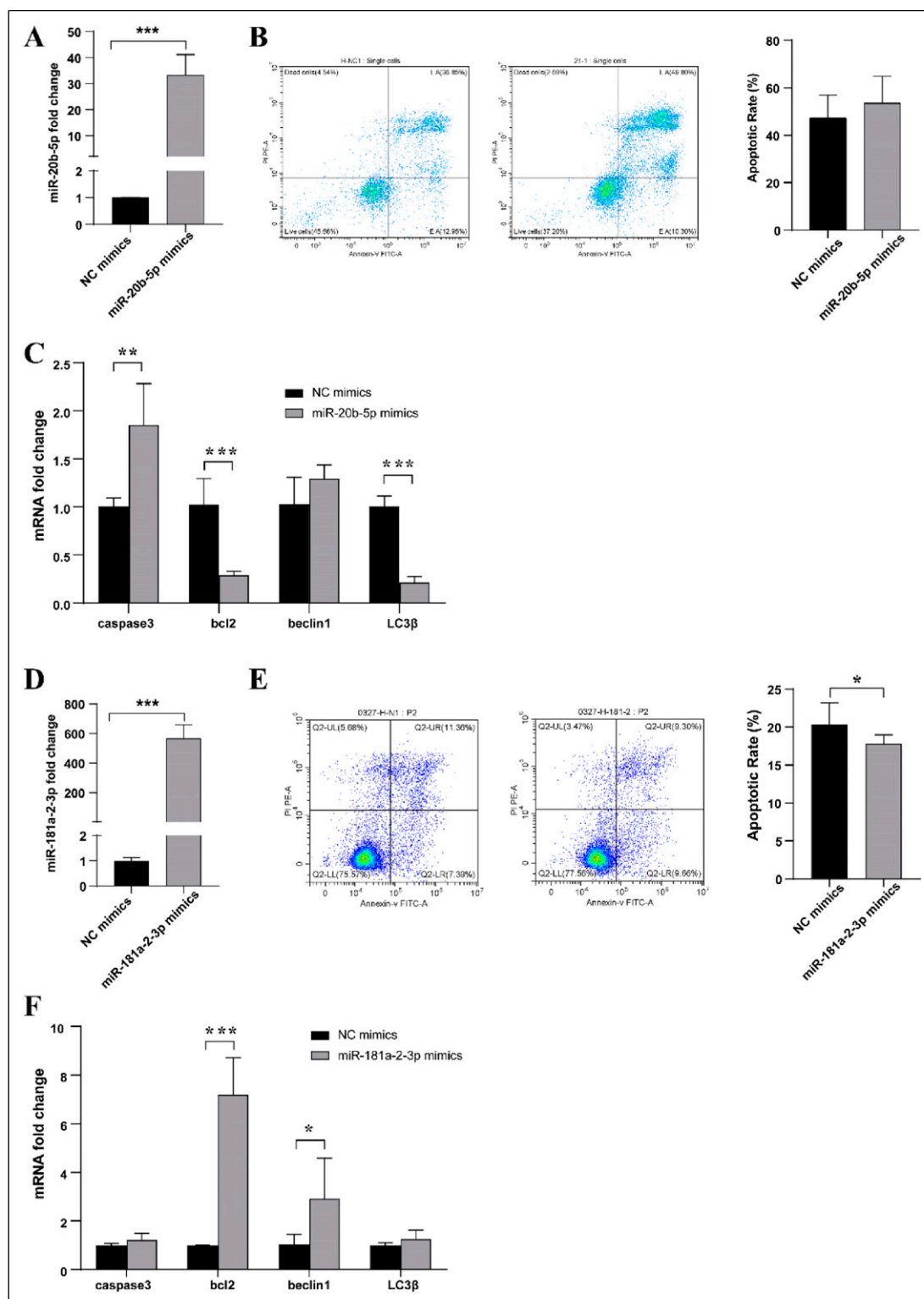


Figure 4. MiR-20b-5p and miR-181a-2-3p regulate cellular apoptosis and autophagy. (a) The expression of miR-20b-5p was detected with qPCR. (b) The apoptosis of HK2 cell transfected with miR-20b-5p was measured with the flow cytometry. (c) The qPCR results of caspase3, bcl2, beclin1 and LC3 β in HK2 cell transfected with miR-20b-5p. (d) The expression of miR-181a-2-3p was detected with qPCR. (e) The apoptosis of HK2 cell transfected with miR-181a-2-3p was measured with the flow cytometry. (f) The qPCR results of caspase3, bcl2, beclin1 and LC3 β in HK2 cell transfected with miR-181a-2-3p. $N = 3$, * $p < .05$, ** $p < .01$, *** $p < .001$.

In this study, plasma samples from patients with SLE and healthy controls were obtained, and exosomes were extracted for validation of sequencing results. The expression of miR-181a-2-3p was decreased and the expression of miR-20b-5p was increased in plasma exosomes of SLE patients, which supported the sequencing results. Subsequent functional enrichment analysis enriched in apoptosis, autophagy, the Wnt signaling pathway, and other processes. This result is similar to that of Ana *et al.*²⁹ who reported that plasma exosome could cause kidney injury by Wnt signaling pathway. Then, plasma exosomes from SLE patients were co-incubated with human renal tubular epithelial cells (HK2 cells), it was observed that exosomes could not only enter the HK2 cells but also promote their apoptosis and autophagy, indicating that plasma exosomes of SLE patients could induce LN by promoting cell apoptosis and autophagy. Similarly, Wu *et al.*³⁰ reported that exosomal miRNAs are involved in the development and progression of autoimmune diseases by regulating cellular apoptosis, inflammatory responses, and immune modulation. Furthermore, we observed that the depletion of miR-181a-2-3p in plasma exosomes of SLE patients is one of the reasons for the promotion of autophagy and apoptosis in HK2 cells by SLE plasma exosomes. Fan *et al.*³¹ found miR-181a-2-3p suppressed CSE-induced apoptosis, inflammation and cytotoxicity in 16HBE cells to relieve chronic obstructive pulmonary disease. In addition, miR-181a-2-3p alleviates the inflammatory response and cell apoptosis of renal tubular epithelial cells in septic -induced acute kidney injury *in vivo*.³² At the same time, the abundant presence of miR-20b-5p in plasma exosomes from SLE patients may be another significant factor underlying the ability of these exosomes to promote apoptosis and autophagy in HK2 cells. It was reported that miR-20b-5p regulated STAT3 expression to promote cell apoptosis in hepatic stellate cells *in vitro*,³³ which was consistent with our results. Another study indicated that miR-20b-5p could prevent the inflammation, apoptosis and enhance angiogenesis via enhancing autophagy.³⁴ This discrepancy may be attributed to the difference in cell types.

Interestingly, in our study, co-incubation of 10 µg/ml plasma exosomes with HK2 cells did not affect cell viability but significantly enhanced apoptosis and autophagy. Firstly, when cells are stimulated, intracellular stress response mechanisms are activated, potentially leading to compensatory proliferation that balances the increase in apoptosis and autophagy.³⁵ Secondly, the initiation and execution of apoptosis and autophagy are time-dependent processes.³⁶ Within the 48-h co-culture period, cells may have undergone multiple stress responses. Lastly, while the 10 µg/ml dose did not markedly affect cell viability, it may have been sufficient to trigger apoptosis and autophagy signaling pathways. Apoptosis and autophagy are cellular responses to different stress signals, which may be activated to varying degrees at different doses.³⁷ In our study, the 10 µg/ml dose did not significantly

affect cell viability, but it did slightly reduce viability, supporting this explanation.

In summary, our study identified differentially expressed miRNAs in SLE plasma exosomes that may contribute to the pathogenesis of SLE and LN by regulating cellular apoptosis and autophagy. Notably, we found that the enrichment of miR-20b-5p and the depletion of miR-181a-2-3p in the plasma exosomes of SLE patients may mediate the promotion of apoptosis and autophagy in HK2 cells, leading to kidney damage and the development of LN. However, these findings require validation through *in vivo* experiments.

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Author Contributions

All authors made a significant contribution to conception, study design, analysis, manuscript drafting, revising, and had the final responsibility for the decision to submit the manuscript for publication.

Declaration of conflicting interests

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Ethical Statement

Ethical approval

The current study was approved by the Human Ethics Committee of The Second Affiliated Hospital of Kunming Medical University (PJ-2024-231).

Informed consent

Written informed consent was obtained from the participants.

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Supplemental Material

Supplemental material for this article is available online.

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