Diagnostic significance of circulating miR-485-5p in patients with lupus nephritis and its predictive value evaluation for the clinical outcomes

Qinghua Wu[a], Yanan Qin[b], Mei Shi[a], Liping Yan[c,*]

[a]Department of Nephrology, Dongying People's Hospital, Dongying, Shandong, China; [b]Department of Obstetrics, Dongying People's Hospital, Dongying, Shandong, China; [c]Department of Outpatient, Dongying People's Hospital, Dongying, Shandong, China

Abstract

Background: Lupus nephritis (LN) is one of the main risk factors contributing to morbidity and mortality of systemic lupus erythematosus (SLE). This study aimed to investigate the potential role of miR-485-5p in human LN.

Methods: Quantitative real-time polymerase chain reaction was used for the measurement of miR-485-5p levels. The levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) in serum were determined by enzyme-linked immunosorbent assay. The diagnostic role of miR-485-5p in LN was evaluated by the receiver operating characteristic (ROC) curve. The impact of miR-485-5p on end-stage renal disease (ESRD) was compared by Kaplan-Meier analysis and Cox regression analysis. The target gene was determined by a dual-luciferase reporter assay system.

Results: MiR-485-5p was highly expressed in SLE and LN patients compared with the healthy controls, and LN patients had the highest level of miR-485-5p. The expression level of miR-485-5p in active LN patients was significantly increased compared with that in nonactive cases. MiR-485-5p expression showed a positive correlation with the levels of estimated glomerular filtration rate, serum creatinine, proteinuria, SLE disease activity index score, and inflammatory cytokines. The ROC analysis results indicated that serum miR-485-5p was a promising biomarker for the early diagnosis of LN, and it can distinguish active LN patients from nonactive ones. Phosphatase and tensin homolog was a direct target of miR-485-5p, and negatively associated with serum miR-485-5p levels. More ESRD events were observed in cases with high miR-485-5p expression, miR-485-5p was an independent factor for the risk of ESRD in LN patients.

Conclusion: Serum miR-485-5p might be a novel promising diagnostic marker for LN and has potential predictive value for ESRD risk in LN patients.

Keywords: Biomarker; End-stage renal disease; Lupus nephritis; miR-485-5p; Phosphatase and tensin homolog

1. INTRODUCTION

Systemic lupus erythematosus (SLE), a heterogeneous systemic autoimmune disease, is characterized by the production of autoantibodies and inflammation.[1–3] Lupus nephritis (LN), one of the most serious complications of SLE, is an inflammation of the kidney and associated with the poor prognosis of SLE.[4,5] LN is prevalent in non-Caucasian females, especially those of child-bearing age.[6,7] Approximately 30% to 50% of SLE patients will develop LN over the course of the disease.[8,9] SLE patients with LN are more likely to develop end-stage renal disease (ESRD) with high mortality and poor prognosis.[10] The most common symptom of LN is progressive glomerulonephritis with a variety of pathological disorders, including glomerular damage, leukopenia, proteinuria, and hematuria.[11,12] The early diagnosis and aggressive treatment of LN exacerbations are of crucial importance to realize remission and prevent the development of irreversible lesions.[8] Therefore, there is an urgent need to find new noninvasive biomarkers and identify novel therapeutic targets for the treatment of LN.

MicroRNAs (miRNAs) are a kind of noncoding single-stranded RNA molecules with a length of 22 to 25 nucleotides.[13] MiRNAs participate in the regulation of multiple biological processes at the transcriptional level, including cell proliferation and apoptosis, differentiation, metabolism, and immune-inflammatory response.[1,14] MiRNAs are important genetic regulators, and play pathophysiologic roles in the development of many diseases. Abnormal miRNA expression may lead to the disturbance of important cellular processes. A number of miRNAs have been identified to be abnormally expressed in LN patients, containing the blood serum, urine, and renal tissue.[15] As Navarro-Quiroz et al[16] reported, there are significant differences in the expression levels of circulating miRNAs among patients with class IV LN, patients with lupus without nephritis, and healthy individuals, in which the serum expression of miR-485-5p was reported to be significantly increased. Considering the aberrant expression levels of miR-485-5p in LN, its specific role in LN attracts our attention.

Accordingly, we hypothesized that miR-485-5p could serve as a novel diagnostic marker in human LN. In the present study, we

*Address Correspondence. Dr. Liping Yan, Department of Outpatient, Dongying People's Hospital, 317, Dongcheng South 1st Road, Dongying, Shandong 257091, China. E-mail address: yanlipingdy@163.com (L. Yan).

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

Journal of Chinese Medical Association. (2021) 84: 491-497.

Received July 8, 2020; accepted December 29, 2020.

doi: 10.1097/JCMA.0000000000000522.

detected the expression profile of miR-485-5p in LN serum samples. MiR-485-5p was identified to be upregulated in LN patients, and had relative ability for the early diagnosis for LN. Furthermore, the predictive value of miR-485-5p for ESRD risk was investigated.

2. METHODS

2.1. Study population and sample collection

A total of 65 SLE patients without LN (mean age: 45.06 ± 10.81 years, range 17-69 years) and 116 LN patients (mean age: 43.86 ± 14.28 years, range 16-71 years) were recruited from June 2012 to January 2015 in Dongying People's Hospital. One hundred sixteen LN patients were divided into active LN patients (n = 80; mean age: 43.10 ± 14.73 years, range 19-71 years) and nonactive ones (n = 36; mean: 45.56 ± 13.25 years, range 16-68 years) according to their kidney function status. Another 50 healthy individuals (mean: 47.34 ± 14.16 years, range 23-70 years) were enrolled as the control group. SLE was diagnosed according to the American College of Rheumatology diagnostic criteria.[17] LN was diagnosed according to the criteria of the International Society of Nephrology/Renal Pathology Society.[18] None patients had received immunosuppressive treatment or immune modulators within 3 months. Patients with malignant tumors, acute or chronic infections, or other autoimmune diseases were ruled out. Patients with proteinuria <500 mg/d and nonactive urinary sediment with stable kidney function were divided into nonactive LN group.[15] Active LN patients were individuals with impaired kidney function, including proteinuria >500 mg/d or active urine sediment, or active glomerulonephritis in kidney biopsy. SLE disease activity index (SLEDAI-2000) scores were used to evaluate the SLE activities of patients. Six milliliters of peripheral blood was collected from each subject and stored in EDTA K3 tubes.

The protocols of this study were approved by the Ethics Committee of Dongying People's Hospital, and all subjects signed written informed consent.

2.2. Follow-up survey

All patients were followed up for 5 years. The primary endpoint was the occurrence of ESRD.

2.3. RNA extraction and quantitative real-time polymerase chain reaction

According to the manufacturer's instructions, total serum RNA was extracted using Trizol reagent (Invitrogen, Carlsbad, CA). After quantification, RNA was reverse transcribed into cDNA with RevertAid First Strand DNA Synthesis (RT) kit (Thermo Fisher Scientific, Waltham, MA, USA). Then, quantitative real-time polymerase chain reaction (qRT-PCR) was performed with the SYBR Premix Ex Taq system (TaKaRa, Dalian, China) using the Bio-Rad CFX96 Real-Time PCR System (Bio-Rad, Hercules, CA). The relative expression level of miR-485-5p and phosphatase and tensin homolog (PTEN) were determined using $2^{-\Delta\Delta Ct}$ method and normalized to U6 or GAPDH.

2.4. Enzyme-linked immunosorbent assay

Serum levels of interleukin-1β (IL-1β), IL-6, and tumor necrosis factor-α (TNF-α) were quantified with specific enzyme-linked immunosorbent kits (Beyotime Biotechnology, Shanghai, China) according to the instructions of the manufacturer.

2.5. Dual-luciferase assay

TargetScan7.2 (http://www.targetscan.org/) was used to screen the potential target and PTEN was identified to be the candidate target gene of the miR-485-5p (Fig. 4A).

Luciferase assays were performed using a Dual-luciferase assay kit (Promega Corporation, Fitchburg, WI, USA) as previously described.[19] The 3′-untranslated region (UTR) of PTEN was cloned into the luciferase reporter vector psiCHECK-2 (Promega Corporation) according to the manufacturer's instruction. Briefly, HEK-293 cells were cultured in a 24-well plate the day before transfection. Cells were cotransfected with wild type (WT) or mutant (MUT) PTEN 3′-UTR reporter (100 ng), and miR-485-5p mimic (50 nM; 5′- AGAGGCUGGCCGUGAUGAAUUC-3′) or negative control (miR-NC; 5′-UUCUCCGAACGUGUCACGUTT-3′) by using lipofectamine 2000 reagent. Then relative luciferase activity was detected with a microplate reader (Molecular Devices, LLC, Sunnyvale, CA, USA). Renilla luciferase was used for normalization. All tests were performed at least three times.

2.6. Statistical analysis

All data analysis was performed on the SPSS 24.0, and measurement data were expressed as mean ± SD. Differences between groups were compared by the Chi-square test or one-way analysis of variance analysis. Pearson's correlation test was used for analyzing the relationship between miR-485-5p and different clinical variables. The receiver operating characteristic (ROC) curves with the area under the curve (AUC) was constructed to assess the discriminative ability of miR-485-5p. Survival curve analysis was performed by Kaplan-Meier method and compared by Log-rank test. Multivariable cox regression analysis was performed for the evaluation of prognostic indicators for LN. Differences were considered to be significant when $p < 0.05$.

3. RESULTS

3.1. Demographic and clinical information of the study population

The demographic and clinical data of the study population were collected and analyzed. As shown in Table 1, there was no significant difference for age and gender distribution among control, SLE, and LN groups (all $p > 0.05$). But the levels of estimated glomerular filtration rate (eGFR), serum creatinine (Scr), proteinuria, SLEDAI score, IL-1β, IL-6, and TNF-α were statistically significant among three groups, and were highest in LN patients (all $p < 0.001$).

3.2. MiR-485-5p expression is increased in lupus nephritis

According to qRT-PCR, miR-485-5p was highly expressed in SLE patients compared with the healthy controls, and LN patients had the highest expression level of miR-485-5p (Fig. 1A). Moreover, expression level of serum miR-485-5p in active LN patients was significantly higher than that in nonactive ones ($p < 0.001$, Fig. 1B).

According to the mean value of miR-485-5p expression in LN patients, all patients were divided into low miR-485-5p expression group (n = 58) and high miR-485-5p expression group (n = 58). Then the relationship between miR-485-5p expression and the clinical parameters of the patients was analyzed. As shown in Table 2, a larger proportion of patients with high miR-485-5p levels were diagnosed with active LN, suggesting that the expression level of miR-485-5p had close correlation with LN activity ($p < 0.001$). However, there was no significant correlation for miR-485-5p with age and gender ($p > 0.05$).

3.3. Diagnostic potential of serum miR-485-5p levels in systemic lupus erythematosus and lupus nephritis

To explore the diagnostic value of miR-485-5p in LN, the ROC curve was established. Fig. 2A showed that miR-485-5p could discriminate the SLE patients from the healthy patients. The AUC for miR-485-5p was 0.705. And miR-485-5p had a sensitivity of 81.5% and specificity of 60.0% at the cutoff value of 1.309. Additionally, serum miR-485-5p also showed a high diagnostic value to distinguish LN patients from SLE cases.

Table 1

Clinical characteristics of the study population

Parameters	Health (n = 50)	SLE (n = 65)	LN (n = 116)	*p*
Age	47.34 ± 14.16	45.06 ± 10.81	43.86 ± 14.28	0.308
Gender (male/female)	7/43	5/60	15/101	0.487
eGFR (mL/min/1.73m^2)	120.76 ± 7.61	117.23 ± 7.58	60.89 ± 15.18	<0.001
Scr (mg/dL)	0.82 ± 0.08	0.85 ± 0.11	1.78 ± 0.89	<0.001
Proteinuria (mg/24h)	81.04 ± 23.99	120.27 ± 12.68	523.07 ± 107.81	<0.001
SLEDAI	—	11.46 ± 3.65	16.73 ± 7.74	<0.001
IL-1β (ng/mL)	10.91 ± 2.46	50.54 ± 11.06	88.32 ± 15.48	<0.001
IL-6 (ng/mL)	10.89 ± 1.36	38.08 ± 10.90	63.51 ± 14.51	<0.001
TNF-α (ng/mL)	5.74 ± 1.85	19.39 ± 6.81	33.43 ± 9.07	<0.001

eGFR = estimated glomerular filtration rate; IL = interleukin; LN = lupus nephritis; Scr = serum creatinine; SLE = systemic lupus erythematosus; SLEDAI = SLE disease activity index.

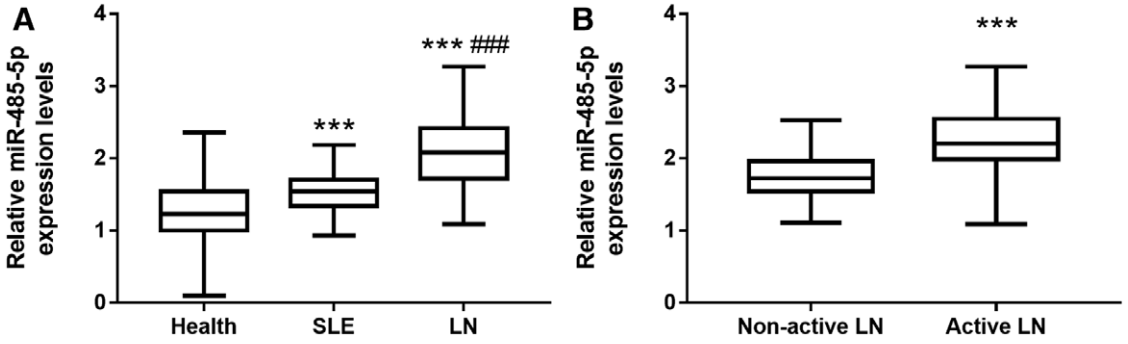

Fig. 1 The level of miR-485-5p in the serum of SLE and LN patients. A, miR-485-5p was highly expressed in SLE patients compared with the healthy controls, and LN patients had the highest expression level of miR-485-5p. ***p < 0.001, compared with healthy controls; ###p < 0.001, compared with SLE group. B, Serum expression of miR-485-5p was determined to be significantly higher in active-LN patients than that in nonactive cases. ***p < 0.001, compared with nonactive group. LN = lupus nephritis; SLE = systemic lupus erythematosus.

As shown in Fig. 2B, the AUC was 0.841, with a sensitivity of 68.1% and specificity of 90.8% at the cutoff value of 1.846.

Then we further examined whether serum miR-485-5p could distinguish active LN patients from nonactive ones. The AUC of serum miR-485-5p was 0.801 with a sensitivity of 80.60% and specificity of 75.0% at the cutoff value of 2.005 (Fig. 2C).

3.4. Effects of miR-485-5p on inflammation factor and kidney function

To explore the role of miR-485-5p in inflammation, the protein expression levels of IL-6, TNF-α, and IL-1β were detected, and the correlations between the serum expression level of miR-485-5p and inflammation factors (IL-1β, IL-6, and TNF-α) were analyzed in LN patients. It was observed that serum miR-485-5p showed positive association with the levels of IL-1β (Fig. 3A; Pearson's r = 0.6979; p < 0.001), IL-6 (Fig. 3B; Pearson's r = 0.6322; p < 0.001), TNF-α (Fig. 3C; Pearson's r = 0.7178; p < 0.001). Additionally, a positive association was also observed for serum miR-485-5p with SLEDAI score (Fig. 3D; Pearson's r = 0.5639; p < 0.001).

Moreover, we further detected the correlation of serum miR-485-5p with kidney function for LN patients. It was found that there were positive associations of serum miR-485-5p with the levels of eGFR (Fig. 3E; Pearson's r = 0.4113; p < 0.001), Scr (Fig. 3F; Pearson's r = 0.6579; p < 0.001) and proteinuria (Fig. 3G; Pearson's r = 0.5733; p < 0.001) in LN patients.

Table 2

Correlation between miR-485-5p expression levels and clinical features

Parameters	Cases (n = 116)	miR-485-5p expression		*p*
		Low (n = 58)	High (n = 58)	
Gender				0.782
Male	15	8	7	
Female	101	50	51	
Age (y)				0.190
≤30	51	22	29	
>30	65	36	29	
Activity				<0.001
Yes	80	28	52	
No	36	30	6	

3.5. Phosphatase and tensin homolog is target of miR-485-5p

The luciferase reporter assay was performed in the HEK-293 cell lines to confirm whether PTEN was the target gene of miR-485-5p. As shown in Fig. 4B, the luciferase activity of the PTEN-WT was decreased in cells transfected with miR-485-5p mimic compared with the control. On contrary, the luciferase activity of cells in PTEN-WT group was increased after transfected with miR-485-5p inhibitor. However, the effect of miR-485-5p was almost eliminated when cells were transfected with PTEN-MUT.

Moreover, serum PTEN mRNA levels in all subjects were detected by qRT-PCR. As shown in Fig. 4C, the PTEN mRNA expression levels in SLE patients were significantly decreased compared with the healthy controls (p < 0.001). Meanwhile, the PTEN mRNA level in LN group was decreased compared with that in SLE group (p < 0.001).

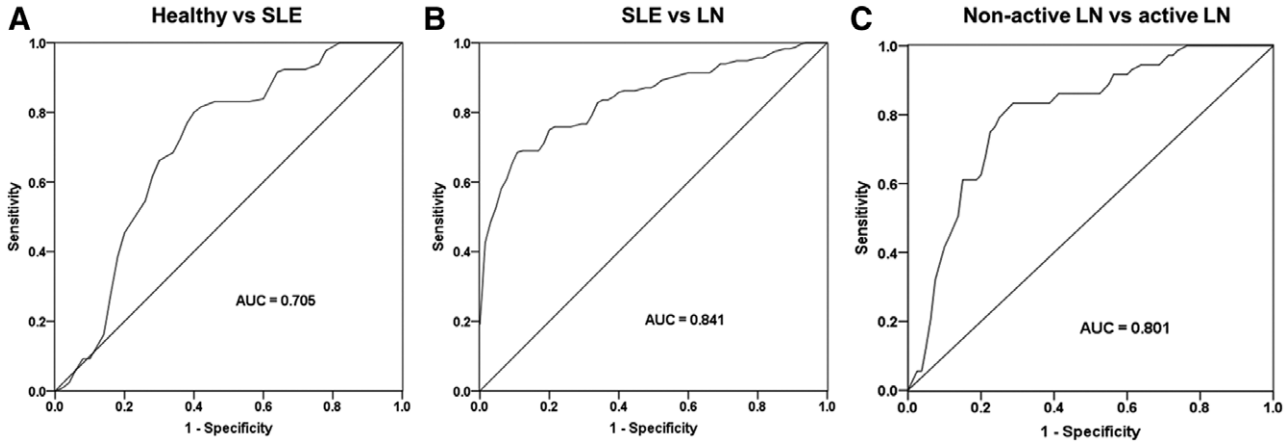

Fig. 2 ROC curve was used to identify the diagnostic value of serum miR-485-5p in different patients groups. A, Diagnostic value of miR-485-5p to distinguish SLE patients from healthy controls. B, Diagnostic value of miR-485-5p to distinguish LN patients from SLE patients. C, Diagnostic value of miR-485-5p to distinguish activity LN patients from nonactive cases. LN = lupus nephritis; ROC = receiver operating characteristic; SLE = systemic lupus erythematosus.

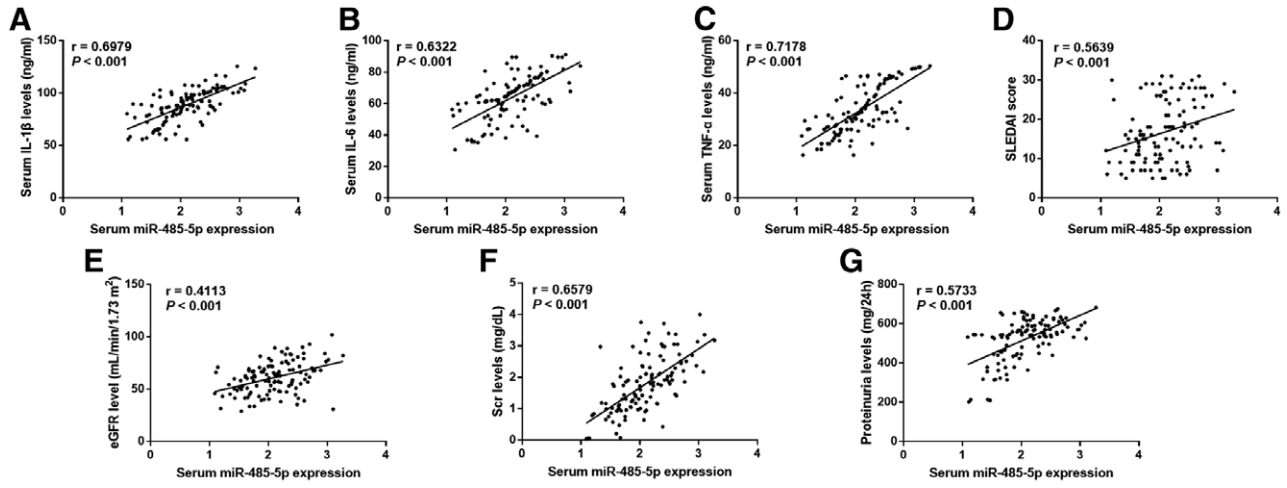

Fig. 3 The correlations of the serum expression level of miR-485-5p with inflammation factor and kidney function in LN patients. A–C, Correlation between the expression level of miR-485-5p and serum IL-1β, IL-6, and TNF-α levels. D, Correlation between the expression level of miR-485-5p and SLEDAI score. E–G, Correlation of serum miR-485-5p with the levels of eGFR, Scr, and proteinuria. IL = interleukin; LN = lupus nephritis; SLEDAI = SLE disease activity index.

To further explore the relation between miR-485-5p and PTEN, the correlation between the expression level of miR-485-5p and PTEN was analyzed in SLE and LN patients (n = 181), and a negative correlation was detected (Fig. 4D; r = –0.6191; $p < 0.001$). These results confirmed that PTEN was a target of miR-485-5p.

3.6. Prognostic value of miR-485-5p expression for the end-stage renal disease risk in lupus nephritis patients

LN patients (n = 116) were followed up for 5 years. It was found that among 116 LN patients, 19 cases developed ESRD. As Kaplan-Meier analysis results showed, more ESRD events were observed in cases with high miR-485-5p expression (log-rank $p = 0.023$, Fig. 5). Furthermore, according to the multivariable cox regression analysis results (Table 3), miR-485-5p (HR = 3.438; 95% confidence interval [CI], 1.149-10.287; $p = 0.027$) and SLEDAI (HR = 3.302; 95% CI, 1.076-10.133; $p = 0.037$) were proved to be the independent factors for ESRD risk in LN patients.

4. DISCUSSION

SLE is characterized by progressive involvement of multiple-organ systems with alternating clinical exacerbations and remissions.[16] LN is a common debilitating feature of SLE. SLE should be closely monitored for renal manifestations, early diagnosis and treatment are vital for renal preservation.[20] But the reliable and sensitive biomarkers for LN are limited, and the pathogenesis toward LN is poorly understood.

Recently, a large number of miRNAs have been identified to be aberrantly expressed in LN, such as miR-148a-3p,[21] miR-410,[22] and miR-198.[23] These dysregulated miRNAs have been highlighted for their clinical significance in diagnosis and prognosis and attracted increasing attention on their therapeutic potential in human diseases.[24,25] For example, in LN patients, circulating miR-146a is detected to be significantly decreased, and has the relative ability for the diagnosis of LN.[1] As reported by Zhang et al,[26] miRNA-124 serves as a novel diagnostic marker in human LN and plays an inhibitory effect on the growth and inflammation of renal mesangial cells by targeting TRAF6. In

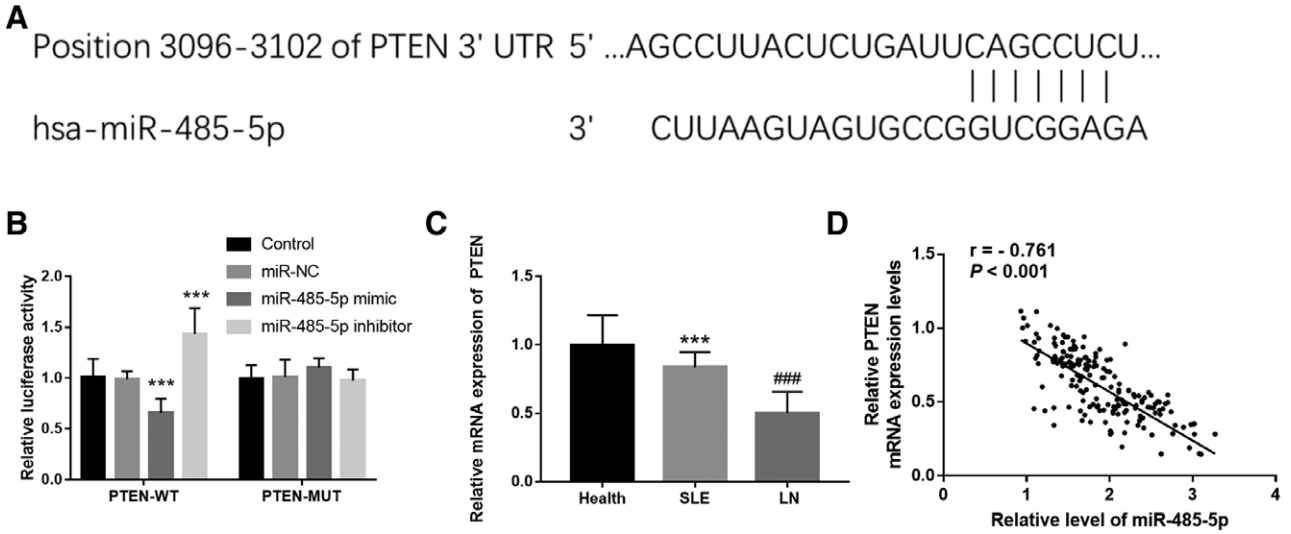

Fig. 4 Validation of PTEN as a direct target of miR-485-5p. A, The putative miR-485-5p binding site in the 3′-UTR of PTEN was shown. B, The relative luciferase activity was determined by luciferase reporter assay in cells cotransfected with miR-485-5p mimic, miR-485-5p inhibitor or miR-NC, and wild or mutant reporter vectors (PTEN-WT or PTEN-MUT). C, The PTEN mRNA expression levels in the serum of subjects were performed. ***$p < 0.001$, compared with control group. ###$p < 0.001$, compared with SLE group. D, Correlation between the expression level of miR-485-5p and mRNA expression of PTEN in all patients. miR = microRNA; PTEN = phosphatase and tensin homolog; PTEN-MUT = mutant PTEN 3′-untranslated region; PTEN-WT = wild type PTEN 3′-untranslated region; SLE = systemic lupus erythematosus; UTR = untranslated region.

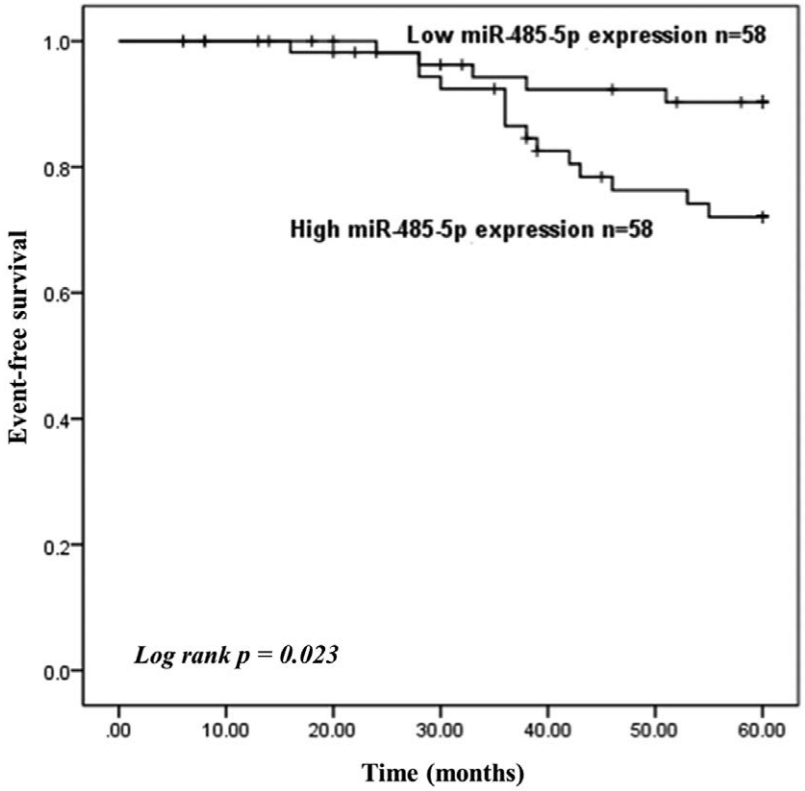

Fig. 5 Survival curve analysis of miR-485-5p impact on ESRD progression. ESRD = end-stage renal disease.

this study, serum expression of miR-485-5p was identified to be significantly increased in SLE and LN patients compared with healthy control, and the miR-485-5p expression levels in activity LN patients were clearly higher than that in nonactivity ones. These results indicated that the miR-485-5p expression levels were related to the progression of SLE and might be associated with the activity of LN in SLE.

Emerging evidence has reported that miR-485-5p was dysregulated in various diseases, such as osteoporosis,[25] colorectal cancer,[27] and ischemia–reperfusion injury.[28] Its clinical value in

Table 3

Multivariate cox regression analysis for the event-free survival of LN patients

Parameters	Multivariate cox regression analysis		
	HR	95% CI	*p*
MiR-485-5p	3.438	1.149-10.287	0.027*
Age	1.889	0.629-5.677	0.257
Gender	2.898	0.744-11.296	0.125
Scr	1.276	0.481-3.382	0.624
eGFR	1.025	0.378-2.779	0.961
Proteinuria	2.693	0.835-8.686	0.097
IL-1β	1.095	0.351-3.421	0.875
IL-6	2.560	0.879-7.454	0.085
TNF-α	2.251	0.739-6.853	0.153
SLEDAI	3.302	1.076-10.133	0.037*
PTEN	0.269	0.073-1.000	0.050

*$p < 0.05$.

eGFR = estimated glomerular filtration rate; HR = hazard ratio; IL = interleukin; LN = lupus nephritis; PTEN = phosphatase and tensin homolog; Scr = serum creatinine; SLEDAI = SLE disease activity index; TNF = tumor necrosis factor.

diagnosis and prognosis attracted the researcher's attention. For instance, miR-485-5p is identified to be upregulated in patients with osteoporosis by comparing with health cases, and miR-485-5p could be a potential therapeutic strategy for the treatment of osteoporosis.[25] Wang et al[29] reported that miR-485-5p was lowly expressed in breast cancer, and overexpression of miR-485-5p could inhibit cell viability and invasion and migration of breast cancer cell line MCF-7 and promote apoptosis. Considering the dysregulation of miR-485-5p in the serum of SLE patients, we further explored its diagnostic value for SLE and LN. The ROC curve analysis results indicated that serum miR-485-5p was a promising biomarker for the early diagnosis of SLE, and it can distinguish LN patients from simple SLE cases. Furthermore, according to the kidney condition, the LN patients were further divided into nonactive and active LN patients. It was found that serum miR-485-5p level was increased in active LN patients, and could distinguish active LN patients from nonactive cases. These results demonstrated that serum miR-485-5p is a potential biomarker for the early diagnosis of LN in SLE cases, and it might be associated with the LN severity.

Considering the dysregulation of miR-485-5p between active and nonactive LN patients, we further assessed the role of miR-485-5p in kidney function in LN patients. It is well known that eGFR, Scr, and proteinuria levels are indicators reflecting the kidney function.[30] In the present study, Pearson's correlation analysis results indicated that serum miR-485-5p showed a close positive correlation with the levels of eGFR, Scr, and proteinuria in LN patients, revealing that miR-485-5p was linked to the kidney function in LN patients. Additionally, we further studied the correlation between the serum expression level of miR-485-5p and inflammation factor in LN patients. It was found that there was a positive association for miR-485-5p with the inflammatory cytokine release. Consistently, in a study of osteoarthritis, miR-485-5p is proved to promote the production of inflammatory factors on the cartilage surface, demonstrating its crucial role in inflammatory response.[31] Additionally, a positive association was also detected for miR-485-5p level with SLEDAI score. These findings indicated that serum miR-485-5p might be associated with SLE severity and kidney function status.

PTEN, a potent tumor suppressor gene, is a phosphatase that negatively regulates the PI3K pathway.[32,33] PTEN has been identified as a key regulator of B cell function and plays an important role in the development of the immune complex disease, including SLE.[34] Additionally, as Liu et al reported, PTEN was downregulated in the serum of LN patients, and it may participant in the regulatory role of miR-148a-3p in LN progression.[21] In the current study, a dual-luciferase assay showed that PTEN was a direct target of miR-485-5p. A marked reduction in the mRNA expression of PTEN was found in both SLE and LN patients compared with that in healthy controls, and the mRNA expression level of PTEN in active LN group was significantly lower than that in the nonactivity group, the results were consistent with the previous evidence. Furthermore, correlation analysis confirmed that miR-485-5p was negatively correlated with the mRNA level of PTEN. Therefore, we concluded that PTEN might be involved in the regulatory role of miR-485-5p in the pathogenesis of LN.

Additionally, all LN patients were followed up for 5 years to explore the predictive value of miR-485-5p for LN. The Kaplan-Meier analysis results indicated that more ESRD events were observed in cases in high miR-485-5p expression, and serum miR-485-5p could identify cases at higher risk of developing future ESRD in LN patients. Furthermore, multivariable cox regression analysis results indicated that miR-485-5p was an independent factor for ESRD risk in LN patients. The current study provides evidence for the potential diagnostic value of serum miR-485-5p for LN and its predictive significance for the risk of future ESRD for LN patients. However, there are several limitations to our study. First, our study population is relatively small, future studies with a larger population are needed to confirm the present findings. Besides, the baseline clinical information of the LN patients during the 5-year follow-up period was not collected in our study, it will be essential for a better understanding of the role of miR-485-5p in LN. Additionally, a group of nephritis without SLE is useful to explore whether the role of miR-485-5p in kidney function is associated with the existence of SLE.

In conclusion, our study suggests the potential clinical value of miR-485-5p for the early diagnosis of LN patients. MiR-485-5p has potential predictive value for ESRD risk in LN patients.

REFERENCES

1. Zhu Y, Xue Z, Di L. Regulation of MiR-146a and TRAF6 in the diagnose of lupus nephritis. *Med Sci Monit* 2017;**23**:2550–7.
2. La Paglia GMC, Leone MC, Lepri G, Vagelli R, Valentini E, Alunno A, et al. One year in review 2017: systemic lupus erythematosus. *Clin Exp Rheumatol* 2017;**35**:551–61.
3. Ocampo-Piraquive V, Nieto-Aristizábal I, Cañas CA, Tobón GJ. Mortality in systemic lupus erythematosus: causes, predictors and interventions. *Expert Rev Clin Immunol* 2018;**14**:1043–53.
4. Han X, Wang Y, Zhang X, Qin Y, Qu B, Wu L, et al. MicroRNA-130b ameliorates murine lupus nephritis through targeting the type I interferon pathway on renal mesangial cells. *Arthritis Rheumatol* 2016;**68**:2232–43.
5. Trotter K, Clark MR, Liarski VM. Overview of pathophysiology and treatment of human lupus nephritis. *Curr Opin Rheumatol* 2016;**28**:460–7.
6. Yap DY, Chan TM. Lupus nephritis in Asia: clinical features and management. *Kidney Dis (Basel)* 2015;**1**:100–9.
7. Sheng ZX, Yao H, Cai ZY. The role of miR-146b-5p in TLR4 pathway of glomerular mesangial cells with lupus nephritis. *Eur Rev Med Pharmacol Sci* 2018;**22**:1737–43.
8. Moroni G, Raffiotta F, Ponticelli C. Remission and withdrawal of therapy in lupus nephritis. *J Nephrol* 2016;**29**:559–65.
9. Besouw MT, Vande Walle JG, Ilias MI, Raes AM, Prytula AA, Claeys L, et al. Pediatric lupus nephritis presenting with terminal renal failure. *Acta Clin Belg* 2016;**71**:455–7.
10. de Zubiria Salgado A, Herrera-Diaz C. Lupus nephritis: an overview of recent findings. *Autoimmune Dis* 2012;**2012**:849684.
11. Parikh SV, Almaani S, Brodsky S, Rovin BH. Update on lupus nephritis: core curriculum 2020. *Am J Kidney Dis* 2020;**76**:265–81.
12. Bagavant H, Fu SM. Pathogenesis of kidney disease in systemic lupus erythematosus. *Curr Opin Rheumatol* 2009;**21**:489–94.

13. Hamam R, Ali AM, Alsaleh KA, Kassem M, Alfayez M, Aldahmash A, et al. microRNA expression profiling on individual breast cancer patients identifies novel panel of circulating microRNA for early detection. *Sci Rep* 2016;**6**:25997.
14. Song J, Zhao L, Li Y. Comprehensive bioinformatics analysis of mRNA expression profiles and identification of a miRNA-mRNA network associated with lupus nephritis. *Lupus* 2020;**29**:854–61.
15. Khoshmirsafa M, Kianmehr N, Falak R, Mowla SJ, Seif F, Mirzaei B, et al. Elevated expression of miR-21 and miR-155 in peripheral blood mononuclear cells as potential biomarkers for lupus nephritis. *Int J Rheum Dis* 2019;**22**:458–67.
16. Navarro-Quiroz E, Pacheco-Lugo L, Navarro-Quiroz R, Lorenzi H, España-Puccini P, Díaz-Olmos Y, et al. Profiling analysis of circulating microRNA in peripheral blood of patients with class IV lupus nephritis. *PLoS One* 2017;**12**:e0187973.
17. Hartman EAR, van Royen-Kerkhof A, Jacobs JWG, Welsing PMJ, Fritsch-Stork RDE. Performance of the 2012 Systemic Lupus International Collaborating Clinics classification criteria versus the 1997 American College of Rheumatology classification criteria in adult and juvenile systemic lupus erythematosus. A systematic review and meta-analysis. *Autoimmun Rev* 2018;**17**:316–22.
18. Weening JJ, D'Agati VD, Schwartz MM, Seshan SV, Alpers CE, Appel GB, et al; International Society of Nephrology Working Group on the Classification of Lupus Nephritis; Renal Pathology Society Working Group on the Classification of Lupus Nephritis. The classification of glomerulonephritis in systemic lupus erythematosus revisited. *Kidney Int* 2004;**65**:521–30.
19. Wu S, Wang J, Li F. Dysregulation of PTEN caused by the underexpression of microRNA-130b is associated with the severity of lupus nephritis. *Mol Med Rep* 2018;**17**:7966–72.
20. Zhu H, Mi W, Luo H, Chen T, Liu S, Raman I, et al. Whole-genome transcription and DNA methylation analysis of peripheral blood mononuclear cells identified aberrant gene regulation pathways in systemic lupus erythematosus. *Arthritis Res Ther* 2016;**18**:162.
21. Liu Q, Feng X, Zhang W, Wu C, Kang P, Li H, et al. miR-148a-3p overexpression contributes to glomerular cell proliferation by targeting PTEN in lupus nephritis. *Am J Physiol Cell Physiol* 2016;**310**: C470–8.
22. Liu D, Zhang N, Zhang J, Zhao H, Wang X. miR-410 suppresses the expression of interleukin-6 as well as renal fibrosis in the pathogenesis of lupus nephritis. *Clin Exp Pharmacol Physiol* 2016;**43**:616–25.
23. Cui D, Zhu D, Ren H, Lin J, Lai W, Huang Q, et al. MicroRNA-198 contributes to lupus nephritis progression by inhibition of phosphatase and tensin homology deleted on chromosome ten expression. *Mol Med Rep* 2017;**16**:7813–20.
24. Nalewajska M, Gurazda K, Styczynska-Kowalska E, Marchelek-Mysliwiec M, Pawlik A, Dziedziejko V. The role of microRNAs in selected forms of glomerulonephritis. *Int J Mol Sci* 2019; **20**: 5050.
25. Zhang SY, Gao F, Peng CG, Zheng CJ, Wu MF. miR-485-5p promotes osteoporosis via targeting Osterix. *Eur Rev Med Pharmacol Sci* 2018;**22**:4792–9.
26. Zhang L, Zhang X, Si F. MicroRNA-124 represents a novel diagnostic marker in human lupus nephritis and plays an inhibitory effect on the growth and inflammation of renal mesangial cells by targeting TRAF6. *Int J Clin Exp Pathol* 2019;**12**:1578–88.
27. Pan Y, Qin J, Sun H, Xu T, Wang S, He B. MiR-485-5p as a potential biomarker and tumor suppressor in human colorectal cancer. *Biomark Med* 2020;**14**:239–48.
28. Chen X, Zhang S, Shi P, Su Y, Zhang D, Li N. MiR-485-5p promotes neuron survival through mediating Rac1/Notch2 signaling pathway after cerebral ischemia/reperfusion. *Curr Neurovasc Res* 2020;**17**:259–66.
29. Wang X, Zhou X, Zeng F, Wu X, Li H. miR-485-5p inhibits the progression of breast cancer cells by negatively regulating MUC1. *Breast Cancer* 2020;**27**:765–75.
30. Katsuyama E, Miyawaki Y, Sada KE, Asano Y, Hayashi K, Yamamura Y, et al. Association of explanatory histological findings and urinary protein and serum creatinine levels at renal biopsy in lupus nephritis: a cross-sectional study. *BMC Nephrol* 2020;**21**:208.
31. Chen HO, Zhang L, Tang ZY, Gong ZM. MiR-485-5p promotes the development of osteoarthritis by inhibiting cartilage differentiation in BMSCs. *Eur Rev Med Pharmacol Sci* 2018;**22**:3294–302.
32. Yahiaoui A, Meadows SA, Sorensen RA, Cui ZH, Keegan KS, Brockett R, et al. PI3Kδ inhibitor idelalisib in combination with BTK inhibitor ONO/GS-4059 in diffuse large B cell lymphoma with acquired resistance to PI3Kδ and BTK inhibitors. *PLoS One* 2017;**12**:e0171221.
33. Liu L, Huang L, He J, Cai S, Weng Y, Huang S, et al. PTEN inhibits non-small cell lung cancer cell growth by promoting G0/G1 arrest and cell apoptosis. *Oncol Lett* 2019;**17**:1333–40.
34. Al-Mayouf SM, AlTassan RS, AlOwain MA. Systemic lupus erythematosus in a girl with PTEN variant and transaldolase deficiency: a novel phenotype. *Clin Rheumatol* 2020;**39**:3511–5.