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The correlation of plasma microRNA-200 family expressions with risk and disease severity of lupus nephritis

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Abstract. – OBJECTIVE: To investigate whether plasma microRNA-200 family expressions could serve as diagnostic biomarkers in patients with lupus nephritis (LN), and their association with disease severity.

PATIENTS AND METHODS: 101 adult LN patients and 100 adult health volunteers were recruited in our study. A blood sample was obtained from all participants, and total RNA was extracted from plasma. Real-time PCR was performed to evaluate the relative expression of microRNA (miRNA). SLE disease activity (SLEDAI) score was calculated to evaluate the overall disease severity.

RESULTS: Plasma miR-200b-5p, miR-141-5p and miR-200c-5p expressions were decreased in LN patients compared to that in health controls (HCs). Multivariate logistic regression model revealed that plasma miR-200b-5p, miR-141-5p, and miR-200c-5p expressions were independent protective factors for progression states. Receiver operating characteristic curve (ROC) was conducted to assess the diagnostic value of miR-200b-5p, miR-141-5p, and miR-200c-5p, and they revealed good diagnostic value with the area under curve (AUC) of 0.748, 0.748, and 0.723, respectively. When miR-200b-5p, miR-141-5p, and miR-200c-5p were combined with each other, the AUC increased to 0.936, suggesting a great diagnostic value for LN. Also, plasma miR-141-5p was observed to be negatively associated with serum creatinine and SLEDAI score, and the inverse correlations were found of miR-200c-5p with SLEDAI score, as well as miR-200b-3p with proteinuria.

CONCLUSIONS: Circulating miR-200b-5p, miR-141-5p, and miR-200c-5p expressions could be considered as novel and convincing diagnostic biomarkers for LN.

Key Words:
miR-200 family, Lupus nephritis, Risk, Severity, Biomarker.

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease, with the prevalence at 40 to 50[5], and usually occurs in women (female:male 9:1)[1,2]. SLE involves in almost all organs and systems including vascular, skin, kidney, joint and so on, and it is characterized by headache, skin rush, proteinuria as well as joint pain[4]. Among these affected organs and systems, kidney is one of the most frequently and seriously impaired systems. It is reported that 45% to 85% SLE patients are at risk to develop lupus nephritis (LN) during their lifetime[5,6], which has been implicated as a major cause of mortality and morbidity progressing to end-stage renal disease[7]. Renal biopsy is considered as the golden standard to diagnose and distinguish the extent of renal damage in LN patients[8], while there are still some SLE patients who could not undergo this procedure due to its invasion with high risks of several complications, including infection, and haemorrhage[9]. Hence, exploring novel and promising biomarkers for diagnosis and disease severity management of LN patients is necessary.

microRNAs (miRNAs) are a great family of small, endogenous, non-protein-coding RNAs. They could regulate the gene expression at post transcriptional level[10]. Aberrant expressions of several miRNAs could be found in the progression of several diseases, and some of them have been identified as diagnostic and/or prognostic biomarkers of various diseases, including osteosarcoma[11], abdominal aortic aneurysm[12], diabetes[13], and renal diseases[14]. Accumulating evidence has proven that differential miRNAs expressions are associated with the pathogenesis and clinicopathological manifestations in IgA nephropathy, autosomal dominant polycystic kidney disease[15], as well as LN[16-18].

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miR-200 family, consisted of five different members (miR-200a, miR-200b, miR-200c, miR-141, and miR-429), is reported to play a critical role in the pathogenesis of nephritis[19,20]. However, a few studies have been validated hither to about the correlation of plasma miR-200 family expressions with risk and disease severity of LN in clinical practice. Therefore, the purpose of this work was to investigate whether plasma miR-200 family expressions could serve as diagnostic biomarkers in patients with LN, and their association with disease severity.

Patients and Methods

Participants

101 adult LN patients from Department of TCM in Tongren Hospital, Shanghai Jiao Tong University School of Medicine, between January 2016 and October 2016, were enrolled in this case control study. The inclusion criteria were as follow: age above 18 years; diagnosed with SLE according to 1997 revised American College of Rheumatology criteria for SLE[21]; con[illegible] nephritis by renal biopsy. In addition, 1[illegible] health volunteers were recruited as healt[illegible]n-trols (HC), whose age and gender were ma[illegible] to the LN patients. HCs with history of rheu[illegible] toid diseases, severe infection, mal[illegible]nant tumo[illegible] severe hepatic or renal dysfun[illegible] exclud-ed. This investigation was a[illegible]ved b[illegible] Ethics Committee of Tongren H[illegible]tal, Sh[illegible]ai Jiao Tong University Schoo[illegible]edici[illegible] patients and HCs sign[illegible] the[illegible] consents.

Samples

Blood sampl[illegible] btained from[illegible] particip-ants and pla[illegible] was[illegible]d subsequently. Total RNA in pla[illegible]ma was then[illegible]d using the miR-NA Isola[illegible] kit (Sigma-Ald[illegible]t. Louis, MO, USA) a[illegible]ding t[illegible] manufacturer[illegible]s protocol. RNA conc[illegible]tion an[illegible]rity were detected by spectro-ph[illegible]ter (T[illegible]no Fish[illegible] Waltham, MA, USA).

miRNA [illegible]min[illegible]n by Real-time [illegible]erase Chain Reaction (PCR)

R[illegible] was su[illegible]quently reversely transcribed by [illegible] Step PrimerScript miRNA cDNA Syn-th[illegible]aRa, Otsu, Shiga, Japan) and [illegible]titative analysis of 9 candidate miRNAs of [illegible]200 family was performed using SYBR Pre-[illegible] TaqTM II (TaKaRa, Otsu, Shiga, Japan). Exp[illegible]ions of miRNAs were normalized by the expression of U6 as internal reference, and were calculated utilizing the $2^{-\Delta\Delta t}$ method.

Assessment of Disease Seve[illegible]

SLE disease activity index[illegible]DAI) score was calculated to evaluate the [illegible] disease severity. Serum creatine, [illegible]imated [illegible]rula[illegible] filtration rate (eGFR), blo[illegible]rea nitroge[illegible] and 24 hours proteinu[illegible]ere de[illegible]rmined t[illegible] sess the severity of r[illegible]itis.

Statistical An[illegible]sis

Statistical a[illegible]sis was p[illegible]me[illegible]ng R 2.1 software. D[illegible]was mainly p[illegible]d as mean value ± s[illegible]viation, med[illegible] (1/4 to 3/4 quarter[illegible]e) o[illegible]t (percentage). Compar-ison between two g[illegible] was determined by Ma[illegible]ney test or [illegible]st. The values of [illegible]As to predict LN states were evaluated by [illegible]variate logisti[illegible]regression analysis, and then [illegible]factors with [illegible]value below 0.1 were further [illegible]cted by mul[illegible]iate logistic regression anal-y[illegible]eceiver [illegible]erating Characteristic (ROC) curv[illegible] to assess the diagnostic value of independent predictive miRNAs for LN. Correla-[illegible]n was determined by Spearman test. $p < 0.05$ [illegible]dered significant.

Results

Characteristics

No difference of mean age ($p = 0.312$) and gender ($p = 0.451$) between LN patients and HCs was found (Table I). The median values of SLE duration and LN duration were 8.3 (5.7-11.9) years and 4.3 (2.5-6.8) years respectively. In addition, the median values of serum creatinine, eGFR and BUN were 1.0 (0.8-1.2) mg/dL, 97.3 (82.1-114.5) mL/min/1.73 m^2, and 5.1(3.6-6.3) mmol/L respectively. The median levels of white blood cell (WBC), platelets, total bilirubin (TBIL), total bile acid (TBA), alanine aminotransferase (ALT), aspartate aminotransferase (AST), C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) were 4.3 (3.4-6.0) × 10^9/L, 206 (126-348) × 10^9/L, 17.5 (13.4-25.2) umol/L, 20.3 (11.0-32.5) I/L, 37.7 (29.0-50.0) u/L, 45.1 (33.6-63.7) u/L, 6.8 (5.2-8.9) mg/L and 31 (20-46) mm/H, respectively. The numbers of patients with neurological disorder, arthritis, myocarditis, alopecia, erythra, ulcer, pleurisy, serositis, vasculitis, fever, thrombocytopenia, leukopenia, haematuria, proteinuria and cylindruria were 5 (5%), 33 (33%), 11 (11%),

Table I. Demographic and clinical characteristic of LN patients and HC.

Pararmeters	LN patients	HC	
Number (N)	101	100	
Age (years)	34.20 ± 6.35	33.30 ± 6.13	0.312
Gender (F)	85 (84%)	81 (81%)	0.4514
SLE duration (years)	8.3 (5.7-11.9)	–	
LN duration (years)	4.3 (2.5-6.8)	–	
Serum creatinine (mg/dL)	1.0 (0.8-1.2)	–	
eGFR (mL/min/1.73 mx)	97.3 (82.1-114.5)	–	–
BUN (μmoL/L)	5.1 (3.6-6.3)	–	–
WBC (× 10^9/L)	4.3 (3.4-6.0)		
Platelets (× 10^9/L)	206 (126-348)	–	
TBIL (μmol/L)	17.5 (13.4-25.2)	–	–
TBA (I/L)	20.3 (11.0-32.5)	–	–
ALT (μ/L)	37.7 (29.0-50.0)	–	–
AST (μ/L)	45.1 (33.6-63.7)	–	–
CRP (mg/L)	6.8 (5.2-8.9)		
ESR (mm/H)	31 (20-46)		
Anti-dsDNA (IU/mL)	325.5 (241-408.5)	–	–
Serum C3 (mg/dL)	83.9 (67.65-104.4)	–	–
Serum C4 (mg/dL)	14 (9-21.25)	–	–
Proteinuria (g/24h)	3.3 (1.4-5.1)	–	–
Neurological disorder (n/%)	5 (5%)		–
Arthritis (n/%)	33 (33%)		–
Myocarditis (n/%)	11 (11%)		–
Alopecia (n/%)	23 (23%)	–	–
Erythra (n/%)	31 (31%)	–	–
Ulcer (n/%)	10 (1	–	–
Pleurisy (n/%)	7 (	–	–
Serositis (n/%)	8 (8	–	–
Vasculitis (n/%)	4 (4%	–	–
Fever (n/%)	47 (47%	–	–
Thrombocytopenia (n/%)	21 (21%)	–	–
Leukopenia (n/%)	35 (35%)	–	–
Hematuria (n/%)	(26%)	–	–
Proteinuria (n/%)	81%)	–	–
Cylindruria (n/%)	43%)	–	–
SLEDAI score		–	–
Histologic class			
Class II	21 (21%)	–	–
Class III	29 (29%)	–	–
Class IV	35%)	–	–
Class V	)	–	–
Class V+III	3 5%)	–	–
Class V+IV	5 (5%)	–	–

Data distrib was descripted by lue ± standard deviation or median (1/4 to 3/4 quarter value) and count (percentage); Significa the comparison was d rmined by Mann-Whitney test or Chi-squared test. LN, lupus nephritis; HC, health control d, bloo nitrogen; eGFR, estimated glomerular filtration rate; WBC, white blood cell; TBIL, total bilirubin; TBA bile ac T, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; ESR, erythrocyte sed n r EDAI d, systemic lupus erythematosus disease activity index; $p < 0.05$ was considered significant.

%), 31 (10%), 7 (7%), 8 (8%), 4 (4% (47%), 1%), 35 (35%), 26 (26%), 82 (81% d 43 (43%), respectively. Moreover, the m SLEDAI score was 10.8 (9.2- in LN patients. As to histological class, the bers of patients with Class II, Class III, Class s V, Class V+III and Class V+IV were 21 ( ), 29 (29%), 35 (35%), 8 (8%), 3 (3%) and 5 (5%), respectively. The other clinical and biochemical features of LN patients were presented in Table I.

miR-200 Family Expressions in LN Patients and HCs

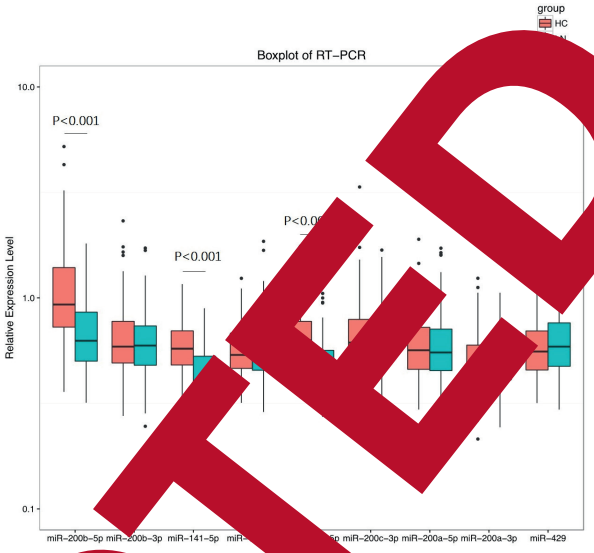
The comparison of miR-200 family expressions in LN patients and HCs were analyzed

(Figure 1). Plasma miR-200b-5p expression was decreased in LN patients compared with that in HCs ($p < 0.001$), and the levels of miR-200c-5p and miR-141-5p were also found to be lower in LN patients than HCs (both $p < 0.001$).

Diagnostic Value of miR-200b-5p, miR-141-5p and miR-200c-5p for LN

Univariate logistic analysis exhibited that plasma miR-200b-5p expression was related to lower risk of LN with odds ratio (OR) 0.049, 95% CI 0.017-0.143, $p < 0.001$, so were the plasma miR-141-5p (OR: 0.004, 95% CI 0.001-0.022, $p < 0.001$) and miR-200c-5p (OR: 0.021, 95% CI 0.005-0.096, $p < 0.001$) levels (Table II). All factors with a p-value < 0.1 in univariate logistic model were further analyzed by multivariate logistic regression model, which indicated lower plasma miR-200b-5p, miR-141-5p and miR-200c-5p expressions were independent factors for predicting LN states with OR 0.004, 95% CI 0.001-0.025, $p < 0.001$, OR 0.001, 95% CI 0.000-0.004, $p < 0.001$ and OR 0.001, 95% CI 0.000-0.011, $p < 0.001$ respectively.

miR-200b-5p, miR-141-5p and miR-200c-5p were subsequently analyzed by ROC cur[illegible] to assess the value in LN diagnosis. All mi[illegible] 5p, miR-141-5p and miR-200c-5p revealed [illegible]od diagnostic value with area under curve (A[illegible]) of 0.748, 0.748, 0.723, respectively (Figure [illegible]). While combined each other, the AUC raised [illegible] 0.873, 0.854 and 0.847, respe[illegible]Moreover, the combination of miR-200[illegible], miR[illegible]-5p and miR-200c-5p disclosed an [illegible] greate[illegible]gnostic value for LN with AUC [illegible]36 (95[illegible]



Fi[illegible]omparison of expre[illegible]ns of candidate miRNAs [illegible] patients and HCs. Comparison between two groups [illegible] determined by [illegible]coxon rank sum test. $p < 0.05$ was [illegible]idered signific[illegible]LN, lupus nephritis; HCs, health [illegible]ols.

0.968). Sensitivity and specificity were 80% and [illegible]3%, respectively, at best cut-off point, which de[illegible]e point that sensitivity plus specificity [illegible]the maximum value.

Correlation of miR-200 Family Expression With Disease Severity in LN Patients

Plasma miR-200b-5p was observed to be negatively associated with BUN (R = -0.233, $p = 0.020$), but no correlation with SLEDAL score (R

Table II. Univariate [illegible]ltivariate logist[illegible]sion analysis of miRNAs for predicting LN states.

m[illegible]A	Univariate logistic				Multivariate logistic (stepwise)			
		95% CI				95% CI		
	OR	Lower	Higher	*p*-value	OR	Lower	Higher	*p*-value
hs[illegible]200b.5	0.049	0.017	0.143	< 0.001	0.004	0.001	0.025	< 0.001
hsa.[illegible]	0.75[illegible]	0.266	2.146	0.599	–	–	–	–
hsa.miR[illegible]	0[illegible]	0.001	0.022	< 0.001	0.000	0.000	0.004	< 0.001
[illegible]iR.141[illegible]	[illegible]	0.565	6.325	0.302	–	–	–	–
[illegible] 200c.5[illegible]	[illegible]021	0.005	0.096	< 0.001	0.001	0.000	0.011	< 0.001
hsa.[illegible]200c.3p	[illegible].000	0.681	1.469	0.998	–	–	–	–
hsa[illegible]	1.478	0.539	4.058	0.448	–	–	–	–
h[illegible]	1.397	0.418	4.663	0.587	–	–	–	–
[illegible]miR.429	1.790	0.581	5.507	0.310	–	–	–	–

[illegible]lue of miRNAs to predict LN states were tested by univariate and multivariate logistic regression model. The OR, 95% CI [illegible]alue represent especially. $p < 0.05$ was considered significant. LN, lupus nephritis; OR, odds ratio; 95% CI, 95% confid[illegible]e interval.

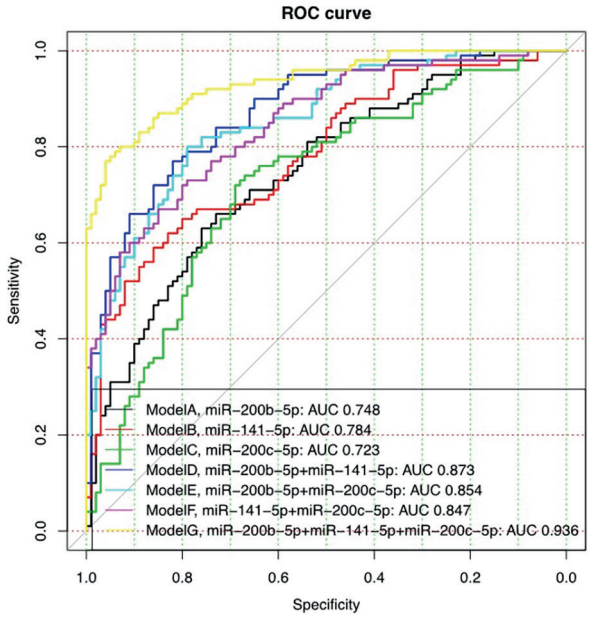


Figure 2. ROC curves of miR-200b-5p, miR-141-5p and miR-200c-5p for LN. ROC curve was conducted to evaluate the predictive value of miR-200b-5p, miR-141-5p and miR-200c-5p for LN. ROC curves, receiver operating characteristic curves; LN, lupus nephritis.

= -0.075, $p = 0.456$) (Table III). Also, the ... se correlations were found of miR-141-5p wit... rum creatinine (R = -0212, $p = 0.035$) and S...

DAI score (R = -0.364, $p < 0.001$). miR-141-3p expression was negatively correlated wi... score (R = -0.401, $p < 0.001$). In a... on, hi... 200c-5p was negatively correlate... th SLEDAI score (R = -0.308, $p = 0.002$)... while, miR-200a-3p decreased with increasing ... inuria (R = -0.336, $p = 0.001$), but ... reased ... igher SLEDAI score (R = 0.2... = 0.040). ... expression was positi... correl... d with ... (R = 0.197, $p = 0.050$... d prote... ia (R = 0.251, $p = 0.012$).

Discussio...

miRN... ysreg... n is often related to the renal lesion, but wh... iRNA could be biomar... predict the ... nd disease severity c..., remains to be elucidated. In this study, ... analyzed the ...rrelation of plasma miR-200 ...ily expressi... with risk and disease se...y of LN. W... und that plasma miR-200b-5... -200c-5... d miR-141 expressions were deci... patients compared to HCs. In addition, these three miRNAs were found to be ...dependently factors for predicting LN states. ...re, the combination of miR-200b-5p, ...5p and miR-200c-5p disclosed a great diagnostic value for LN with AUC as high as

Table III. The correlation analysis ... ression lev... miRNAs and characteristic of LN patients.

Spearman	Se... creatini...	eGFR	BUN	Proteinuria	SLEDAI score
hsa-miR-200b-5p		0.075	**-0.233**	0.150	0.075
p-value		0.458	**0.020**	0.136	0.455
hsa-miR-200b-3p	-0.1...	0.134	0.049	**-0.300**	-0.075
p-value	0.057	0.182	0.626	**0.003**	0.456
hsa-miR-141-5p	**-0.212**	0.001	0.135	-0.066	**-0.364**
p-value	**0.035**	0.995	0.182	0.808	**0.000**
hsa-miR-14...	...048	0.111	0.061	-0.177	**-0.401**
p-value		0.273	0.548	0.078	**0.000**
hsa-mi... c-5p	-0...29	-0.034	0.130	0.166	**-0.308**
p-val...	0.775	0.735	0.199	0.100	**0.002**
hs... -200c-...	-0.133	0.068	-0.108	0.115	-0.116
p-v...	0.187	0.502	0.286	0.256	0.250
hsa-mi...	0.051	0.048	0.088	0.120	0.160
p-...lue	0.612	0.637	0.384	0.237	0.112
...R-200a...	0.012	0.189	0.139	**-0.336**	**0.206**
p-v...	0.906	0.060	0.168	**0.001**	**0.040**
hsa... 429	0.038	0.090	**0.197**	**0.251**	0.179
p...	0.706	0.374	**0.050**	**0.012**	0.074

...correlation between the expression level of miR-200 family and characteristic of LN patients were tested by the spearman ...ients. The spearman coefficents and p-value were present. LN, lupus nephritis; BUN, blood urea nitrogen; eGFR, e... glomerular filtration rate; SLEDAI scored, systemic lupus erythematosus disease activity index; $p < 0.05$ was consi... significant.

0.936. The negative correlations were found in miR-200b-5p expression with BUN, miR-141-5p expression with serum creatinine and SLEDAI score, as well as miR-200c-5p expression with SLEDAI score.

LN arises from the accumulation of autologous immune complex to induct renal inflammatory and fibrotic processes[22-24]. MiRNAs belong to non-coding RNAs and consist of approximately 19 to 25 nucleotides, and they could inhibit the protein translation or degrade the messenger RNA by binding to the 3'untranslated region (3'UTR) of target messenger RNAs[25]. These miRNAs have been identified as critical roles in variety of physiological processes (such as cell development and cell differentiation) and pathological processes (such as oncogenic activity or tumor suppression)[26,27]. The dysregulated miRNAs expressions have been reported in various autoimmunity disorders, including SLE, LN and psoriasis[28-30]. Among these, miRNAs were discovered to affect renal fibrosis and excretion of inflammatory cytokines by targeting several genes and pathways, including type I interferon pathway, phosphate and tensi[illegible] homology deleted on chromosome ten ([illegible] kallikrein-related peptidase 4 (KLK4) an[illegible] suppressor of cytokine signaling 1 (SOCS1)[8,[illegible]]. According to recent studies, miR-146a repres[illegible] nuclear factor kappa B (NF-kB) transcription activity and inflammatory fact[illegible]s during LN prognosis, including int[illegible]kin ([illegible]6, IL-8 and tumor necrosis factor [illegible]F)-α[35]. [illegible]-371-5p directly regulates the [illegible] ind[illegible] 1α to inhibit human [illegible] proliferation and promotes apo[illegible] in L[illegible] tissues[36]. Meanwhile, miR-[illegible] was revea[illegible] improve LN disease con[illegible] targeting typ[illegible] interferon pathway, [illegible] the [illegible]ulation of miR-422a induces L[illegible] developme[illegible] reducing KLK4[37]. Therefor[illegible]se researches [illegible] several miRNAs c[illegible] play crucial roles in the development and [illegible]ession [illegible]N.

[illegible]00 fa[illegible] locate[illegible] on chromosome 1 and 12 as [illegible]-own t[illegible]me seeding sequence, [illegible] to b[illegible]ghly preserved in verte[illegible]as ma[illegible]uced in epithelial tissue[36]. Rec[illegible] data[38-4[illegible]] [illegible]ose that aberrant expression of [illegible]200 has been related to a wide range of [illegible]uding inflammation and fibrotic [illegible]ders. Their overexpression could: (1) tar[illegible] inhibitor of kappa light polypeptide gene e[illegible]r in B-cells kinase beta (IKBKB) to inhi[illegible] IL-8, repressing inflammatory responses in uterine leiomyoma[39]; (2) regulate NF-kB to suppress the proinflammatory IL[illegible] controlling inflammation in lung c[illegible]; (3) associated with interstitial fibro[illegible] and tubular atrophy through affecting epith[illegible]esenchymal transition (EMT) mechanism in [illegible] kidney diseases[38,41,42]. Therefore, t[illegible]e prev[illegible]udies suggest that miR-200 fa[illegible] may inhib[illegible] progression through [illegible]ate sev[illegible]al genes [illegible] pathways.

Furthermore, a[illegible]esti[illegible] work[16] [illegible]closes that 66 dif[illegible]nti[illegible]pressed [illegible]RNAs (DEMs) are [illegible]rmed [illegible]ro[illegible]y, which could be p[illegible]ial diagnost[illegible] prognostic biomarke[illegible] patients. A[illegible]er previous study[43] [illegible]uses [illegible]croarray to assess DEMs in LN patients from [illegible]ean America and Africa[illegible]rica, which [illegible]ates that there are 1[illegible]EMs in both racial groups. Top 5 DEMS [illegible]ording to *p*-va[illegible]e, including hsa-miR-371-5P, [illegible]-miR-423-5P[illegible]a-miR-638, hsa-miR-1224-[illegible]nd hsa-mi[illegible]3, are associated with LN, w[illegible]might b[illegible]omarkers to distinguish the prog[illegible] in accordance with these previous studies, we found that plasma miR-200b-5p, miR-200c-5p and miR-141-5p levels were [illegible]d in LN patients, and the combination [illegible]easu[illegible]ment disclosed a great diagnostic value of LN status. Possible reasons are that miR-200b, miR-200c and miR-141 might promote renal inflammation and kidney fibrosis through regulating several genes and signal pathways, including IKBKB, NF-kB and EMT[38-41,44].

As to the role of miR-200 family expression in LN patients, a previous study[45] reveals that miR-200a expression was negatively correlated with proteinuria and SLEDAI score, while miR-200b and miR-200c expression were positively associated with GFR and platelet count. However, this previous work only enrolled 40 LN patients, a relatively small sample size. In our study, a total of 101 patients with LN were retrospectively reviewed. In line with this previous research[42], the results of our investigation found that plasma miR-141-5p expression was negatively associated with serum creatinine and SLEDAI score. The correlations of miR-200c-5p expression with SLEDAI score, as well as miR-200b-3p expression with proteinuria, were found. These might result from that miR-200 family could target several genes and pathways to inhibit inflammatory responses and kidney fibrosis, thereby decreasing renal damage and SLEDAI score. However, we did not observe the correlation between plasma

miR-200a and cliniopathological features in LN patients, including eGFR, proteinuria and SLEDAI score. This might result from different study design, as well as different inclusion and exclusion criteria.

Nevertheless, certain limitations still existed in this study. Firstly, the sample size was relative small, further studies with more LN patients are necessary to be carried out. Secondly, the difference of candidate miRNAs was determined between LN patients and HCs, but the expression in SLE patients without nephritis was not performed. Thirdly, the precise mechanisms of miR-200 family in pathogenesis of LN were not evaluated in our paper.

Conclusions

We showed that circulating miR-200b-5p, miR-141-5p and miR-200c-5p expressions could be served as novel and convincing diagnostic biomarkers for LN.

Conflict of Interest

The Authors declare that they have no conflict of in[illegible]ts.

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