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ORIGINAL RESEARCH

MicroRNA-203 Expression as Potential Biomarker for Lupus Nephritis

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Introduction and Purpose: Lupus nephritis (LN) is the main cause of morbidity and mortality in systemic lupus erythematosus (SLE) patients, therefore the discovery of new biomarkers, which are reliable for the diagnosis of NL is necessary. Various studies have reported alteration of some miRNAs expression in LN, that considered as biomarkers and/or therapeutic targets in LN. MicroRNA-203 has been associated with the development of nephritis in SLE patients, playing an important role in the initiation and progression of the disease, but research on circulating miRNA-203 expression in LN in clinical practice is still very limited. The aim of this study was to prove the role of microRNA-203 in LN.

Patients and Methods: Serum was obtained from 40 participants consisting of 20 SLE patients and 20 LN patients. The diagnostic of SLE and LN was based on the ACR 1997 criteria. MicroRNA-203 expression was determined by real-time Polymerase Chain Reaction (PCR). Statistical analysis was performed with Mann–Whitney test.

Results: The expression of miRNA-203 in the SLE group was 1.66 (0.00–8.64) and in the NL group was 5.18 (0.25–49.84). There were significant differences in microRNA-203 expression between SLE and LN patients (p=0.003).

Conclusion: MicroRNA-203 expression might be associated with nephritis manifestations in SLE patients.

Keywords: microRNA-203, systemic lupus erythematosus, lupus nephritis

Introduction

The kidney is the main target organ in systemic lupus erythematosus (SLE). Approximately 50% of SLE patients have manifestations of lupus nephritis (LN), which is a major cause of morbidity and mortality in SLE patients.[1] Patients with active LN have a poor long-term prognosis and approximately 30% will progress to end-stage renal disease (ESRD) requiring dialysis or kidney transplantation.[2] Early detection of NL is essential for early initiation of treatment so that it is expected to improve therapeutic outcomes. Therefore, it is necessary to find new reliable non-invasive biomarkers that can effectively differentiate between LN and SLE.[3]

Micro RNAs are a subclass of endogenous RNA molecules involved in post-transcriptional regulation of protein-coding genes.[2,4] Micro RNAs play an important role in cell biology and disease development, by restricting mRNA translation and/or accelerating its degradation, resulting in the restriction of specific protein synthesis to certain target proteins.[5] Various investigations have reported altered miRNA expression profiles in LN patients and different miRNAs have been introduced as biomarkers and/or therapeutic targets in LN. Roointan et al performed a meta-analysis of miRNA profiles in the LN patients, from 13 studies on kidney tissue, 21 studies on blood samples, and 11 studies on urine samples. They found that let-7a, miR-198, let-7e, miR-145, and miR-26a (from kidney tissue); miR-199a, miR-21,

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miR-423, miR-1260b, miR-589, miR-150, miR-155, miR-146a, miR-183 (from blood samples); miR-146a, miR-204, miR-30c, miR-3201, miR-1273e (from urine) involved in nephropathy-related signaling pathways in LN.[6]

Abnormal expression and function of miRNA-203 has been linked to the occurrence of various autoimmune diseases. Altered expression of miRNA-203 was found in the serum of patients with SLE. MicroRNA-203 has been associated with the development of nephritis in SLE patients, playing an important role in the initiation and progression of the disease.[7] Studies conducted to assess the correlation of circulating miRNA-203 expression with the diagnosis of LN in clinical practice are still very limited.[7] The aim of this study was to prove the role of microRNA-203 in LN.

Materials and Methods

Study Design

This was observational analytic study with cross sectional design.

Study Subjects

Serum was obtained from 40 participants consisting of 20 SLE patients and 20 NL patients. The diagnosis criteria of SLE and NL were determined based on the 1997 ACR criteria. The diagnosis of NL is established if persistent proteinuria is found, >0.5 g/day or >3+, or cellular sediment: red blood cells, hemoglobin, granular/mixed tubules and/or renal biopsy shows NL class II, III, IV or V.

Blood Collection

Venous blood samples of 5–10 mL were collected into non-anticoagulant tubes and left for 1–2 hours at room temperature until the blood coagulated and clot retraction occurred. Samples were centrifuged at 1500–3000 rpm for 15–20 minutes, then serum was collected and divided into aliquots of ±1-1.5 mL in sterile tubes. Samples were frozen at −80°C until needed for analysis.

Measurement of Micro RNA-203 Expression

Micro RNA-203 was examined using real-time quantitative polymerase chain reaction (PCR) method using reverse transcriptase reagents PrimeScript (Takara, Dalian, China) and miScript (Qiagen, Hilden, Germany), according to the manufacturer's protocol. Real-time qPCR was performed using SYBR Green Master Mix (Takara, Dalian, China) (primer 5′-GUGAAAUGUUUAGGACCACUAG-3′), according to the manufacturer's protocol. The PCR equipment used was Bioneer, Bioneer Corporation, Korea. The data were analyzed through the comparative threshold cycle (Ct) method. Hsa-U6 was use as a housekeeping gene to normalized the expression of miRNA-203. The relative quantification of serum miRNAs was calculated using the equation: amount of target miRNA expression = $2^{-\Delta\Delta Ct}$.

Statistics

Statistical analysis was performed using IBM SPSS Statistics 25. Analysis of expression differences between the NL and SLE subject groups was assessed using the Mann–Whitney test.

Results and Discussion

Forty subjects were involved in this study, who were divided into 2 groups, LN and SLE. The demographics of both groups are shown in Table 1. This results showed that all subjects were female, in accordance with references which

Table 1 Study Demographics

Characteristics	Lupus (n=20)	Nephritis Lupus (n=20)
Age (years)	23 (18–46)	29 (18–56)
Gender		
Male	0 (0%)	0 (0%)
Female	20 (100%)	20 (100%)

show that the incidence of SLE in women is much higher than in men, with a ratio of 1: 9 between male and female patients.[8,9] Despite the variation of SLE across all age groups, the prevalence is higher in the 15–45 years age group,[8–10] in line with this study which showed that the subjects had an age range of 18–56 years, and there was no significant difference between the SLE and LN groups.

Based on the 1997 ACR criteria, the diagnosis of LN is made if persistent proteinuria is found, >0.5 g/day or >3+, or cellular sediment: red blood cells, hemoglobin, granular/mixed tubules. A creatinine/protein ratio in urine at >0.5 g can replace 24-hour proteinuria measurements, and active urine sediment (>5 red blood cells/LPB, >5 leukocytes/LPB without infection, or cellular cylinders of red blood cells/leukocytes) can replace cellular sediment. Renal biopsy shows features of immune complex-mediated glomerulonephritis.[11] The urinalysis result of both groups are shown in Table 2. There were significant differences on statistic analysis of proteinuria and hematuria parameters with p value 0.000 and 0.001, respectively. In this study, proteinuria, as well as hematuria, both had significant differences between the SLE and LN groups. Proteinuria and hematuria in LN showed as glomerulonephritis manifestation, that primarily caused by a type 3 hypersensitivity reaction, which results in the formation of immune complexes. Autoantibody binds to self-antigen, which forms an antigen-antibody complex (immune complex). These immune complexes deposit on the mesangium, subendothelial, and/or subepithelial space near the glomerular basement membrane of the kidney. This leads to an inflammatory response with the onset of lupus nephritis, in which the complement pathway is activated with a resultant influx of neutrophils and other inflammatory cells.[2,12]

MicroRNA-203 is an important member of miRNAs and is located on human chromosome 14q32.33. Abnormal expression and function of miRNA-203 have been linked to the occurrence of various autoimmune disease.[7] Abnormal function or expression of miRNA-203 is correlated with the occurrence of various autoimmune diseases such as oral lichen planus (OLP), rheumatoid arthritis (RA) and psoriasis.[7,13] Altered expression of miRNA-203 was found in the serum of patients with SLE. Research by Li et al showed that miRNA-203 expression was significantly decreased in the plasma of SLE patients compared to healthy controls, with lower expression in active versus inactive SLE patients. The decrease in miRNA-203 expression correlated with erythrocyte sedimentation rate, C-reactive protein (CRP), anti-dsDNA antibody, complement, and systemic lupus erythematosus disease activity index (SLEDAI) scores.[7] Research by Zhang et al also showed that miRNA-203 in the serum of active LN patients was significantly downregulated compared to inactive NL patients and healthy controls. Receiver operating characteristic (ROC) analysis showed that decreased miRNA-203 was a significant diagnostic biomarker for active LN patients, with an area under curve (AUC) of

Table 2 Urinalysis Results

Characteristics	Lupus (n=20)	Nephritis Lupus (n=20)	*p* value
Proteinuria			
Negative	19	3	0.000*
Trace	0	2	
+1	0	8	
+2	0	6	
+3	1	1	
Hematuria (Blood)			
Negative	14	2	0.001*
Trace	0	2	
+1	1	9	
+2	2	5	
+3	3	2	
Leukocyturia			
Negative	12	14	0.508*
Trace	2	3	
+1	6	3	

Note: *Chi-Square test.

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0.974, sensitivity of 85.79%, and specificity of 89.40%, suggest that miRNA-203 could be a candidate diagnostic biomarker for LN.[13]

Dysregulation of miRNA-203 expression in SLE patients is an important aspect of the pathophysiology of this disease, which is closely related to the underlying genetic targeting mechanisms. miRNA-203 exerts its regulatory influence on a spectrum of genetic targets, mainly including the mitogen-activated protein kinase (MAPK) signaling cascade, as well as the cytokine receptor pathway and various genetic signaling networks involved in focal adhesion and cellular tight junction dynamics.[14] Carlsen et al evaluate the specificity of expression patterns of cell-free circulating microRNAs in SLE and found higher expression of miR-142–3p, significant decrease in the expression of miR-20a and miR-92a (and a trend toward decreased expression of miR-203) in SLE relative to healthy control subjects.[14,15] Liu et al found that the 1–10th nucleotide sequence in the 5′- base of miR-203, is complementary to the 3′- UTR of TLR4 mRNA, confirming that the TLR4 is a miRNA-203 target gene, which can directly regulate TLR4 transcription and translation through binding to the 3′-UTR region of TLR4. Overexpression of microRNA-203 significantly decreased the levels of TLR4 mRNA and protein. These results suggest that miRNA-203 may be involved in inflammation via TLR4.[16,17] Zhou et al found that miR-203 effectively inhibited the synthesis and release of inflammatory factors TNF-α and IL-12 via targeting TLR4 expression in inhibit maturation of dendritic cells, thus exerting negative regulation on innate immunity.[18] Research by Zhang showed that circulating miR-203 expression was positively correlated with the serum concentrations of C3 and C4, and negatively correlated with the serum expression of IL-1β, IL-6, and TNF-α in active LN patients.[13] Previous study by Luo et al revealed that the expression level of miR-203-3p in renal tissue of LN mice was significantly decreased, while the expression of triggering receptor expressed on myeloid cells 1 (TREM1) protein was significantly increased. Overexpression of miR-203-3p significantly inhibited the levels of TNF-α, IL-1β, IL-6, and the expression of TREM1 protein in renal tubular epithelial cells of LN mice, confirmed that TREM1 was the target gene of miR-203-3p. Overexpression of miR-203-3p also promoted the cell proliferation, inhibited its apoptosis, and up-regulated the expression of Bcl-2 protein, while down-regulated the proteins expression of Bax, TGF-β1 and p-p38MAPK.[19]

Primo et al showed that miR-203 is a modulator of cytokine signaling with the ability to both accelerate and suppress an innate immune response. Upregulation of miR-203 facilitates repression of immunosuppressive genes and plays a potential part in disease progression. The pro-inflammatory cytokines tumor necrosis factor α (TNFα) and interleukin-24 (IL-24) are direct targets of miR-203 in a keratinocyte cell line and primary keratinocytes, exerting negative regulatory effects on immunity. This finding showed that miR-203 serves to fine-tune, or balance, cytokine signaling by down-regulating members of the SOCS gene family as well as pro-inflammatory cytokines, possibly indicating that enhanced production of miR-203 in psoriatic skin could be part of an anti-inflammatory response.[20] Stumpfova et al found that miR-203 was specifically expressed in tolerogenic dendritic cells (tDCs), and its expression was increased during the process of immature dendritic cells (imDCs) differentiation towards tDCs cells under the induction of IL-10 and TGF-β. Dendritic cells (DCs) have a role to modulate the balance between innate and adaptive immunity. Basically, DCs adjust T lymphocytes either to activate or suppress a specific immune response in the body. Fully matured activated DCs (aDCs) produce high levels of proinflammatory cytokines such as IL-6, IL-12, and IFN-*γ*, upregulate coreceptors CD80/CD86. On the other hand, tDCs perpetuate a steady state characterized by antigen presentation without T cell activation. In cell-to-cell interactions, tDCs convert naïve T cells to regulatory T lymphocytes, induce anergy in autoreactive T cells.[21]

The concentration of miRNA-203 in the lupus group was 1.66 (0.00–8.64) and in the lupus nephritis group was 5.18 (0.25–49.84). Statistical analysis showed a significant difference in the concentration of miRNA-203 in SLE and LN patients (p=0,003) (Table 3). However, in contrast to previous studies, the results of this study showed that miRNA-203

Table 3 miRNA-203 Results

Characteristics	Lupus* (n=20)	Nephritis Lupus* (n=20)	P value
miRNA-203 expression	1.66 (0.00–8.64)	5.18 (0.25–49.84)	0.003**

Notes: *Data presented as [Median, (Min-Max)]. **Mann–Whitney test.

expression was higher in the LN group compared to SLE. In line with Primo et al, upregulation of miR-203 in LN group might be intended to repress immunosuppressive genes and plays anti-inflammatory response to improve disease progression. Increase of miRNA-203 could increase differentiation imDCs towards tDCs, convert naïve T cells to regulatory T lymphocytes, induce anergy in autoreactive T cells to minimize proinflammatory condition.

The limitations of this study are the variability of the subject's characteristic, which include environmental factors, patient background (genetic factor), history of previous illnesses, duration of illness, type of medication, and length of treatment. Fewer subjects (n = 20, each group) could also affect the results of this study. No assessment of disease activity in this study, so it cannot describe the role of miRNA-203 in the active and chronic phase of the disease.

Conclusion

MicroRNA-203 concentration might be associated with nephritis in lupus patients.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Almaani S, Meara A, Rovin BH. Update on Lupus Nephritis. *Clin J Am Soc Nephrol CJASN*. 2017;12(5):825–835. doi:10.2215/CJN.05780616
2. Wallace DJ, Hahn BH. Preface. In: Wallace DJ, Hahn BH, editors. *Dubois' Lupus Erythematosus and Related Syndromes*. *Ninth Edition)*. Elsevier; 2019.
3. Alduraibi FK, Tsokos GC. Lupus Nephritis Biomarkers: a Critical Review. *Int J mol Sci*. 2024;25(2):805. doi:10.3390/ijms25020805
4. Luo S, Liu Y, Liang G, et al. The role of microRNA-1246 in the regulation of B cell activation and the pathogenesis of systemic lupus erythematosus. *Clin Clin Epigenet*. 2015;7(1):24. doi:10.1186/s13148-015-0063-7
5. Chen F, Shi B, Liu W, et al. Circulating exosomal microRNAs as biomarkers of lupus nephritis. *Front Immunol*. 2023;14:1326836.
6. Roointan A, Gholaminejad A, Shojaie B, et al. Candidate MicroRNA biomarkers in lupus nephritis: a meta-analysis of profiling studies in kidney, blood and urine samples. *Mol Diagn Ther*. 2023;27(2):141–158. doi:10.1007/s40291-022-00627-w
7. Li W, Titov AA, Morel L. An update on lupus animal models. *Curr Opin Rheumatol*. 2017;29(5):434–441. doi:10.1097/BOR.0000000000000412
8. Danchenko N, Satia JA, Anthony MS. Epidemiology of systemic lupus erythematosus: a comparison of worldwide disease burden. *Lupus*. 2006;15 (5):308–318. doi:10.1191/0961203306lu2305xx
9. Fatoye F, Gebrye T, Mbada C. Global and regional prevalence and incidence of systemic lupus erythematosus in low-and-middle income countries: a systematic review and meta-analysis. *Rheumatol Int*. 2022;42(12):2097–2107. doi:10.1007/s00296-022-05183-4
10. McMurray RW, May W. Sex hormones and systemic lupus erythematosus: review and meta-analysis. *Arthritis Rheum*. 2003;48(8):2100–2110. doi:10.1002/art.11105
11. Hahn BH, McMahon M, Wilkinson A, et al. American college of rheumatology guidelines for screening, case definition, treatment and management of lupus nephritis. *Arthritis Care Res*. 2012;64(6):797–808. doi:10.1002/acr.21664
12. Musa R, Brent LH, Qurie A. Lupus nephritis. In: *StatPearls*. StatPearls Publishing. 2024.
13. Zhang L, Zhang X. Downregulated miR-203 attenuates IL-β, IL-6, and TNF-α activation in TRAF6-treated human renal mesangial and tubular epithelial cells. *Int J Clin Exp Pathol*. 2020;13(2):324–331.
14. Carlsen AL, Schetter AJ, Nielsen CT, et al. Circulating microRNA expression profiles associated with systemic lupus erythematosus. *Arthritis Rheum*. 2013;65(5):1324–1334. doi:10.1002/art.37890
15. Zhang H, Huang X, Ye L, et al. B cell-related circulating microRNAs with the potential value of biomarkers in the differential diagnosis, and distinguishment between the disease activity and lupus nephritis for systemic lupus erythematosus. *Front Immunol*. 2018;9:1473.
16. Liu ZM, Zheng HY, Chen LH, et al. Low expression of miR-203 promoted diabetic nephropathy via increasing TLR4. *Eur Rev Med Pharmacol Sci*. 2018;22(17):5627–5634. doi:10.26355/eurrev_201809_15828
17. Ng PC, Chan KYY, Leung KT, et al. Comparative MiRNA expressional profiles and molecular networks in human small bowel tissues of necrotizing enterocolitis and spontaneous intestinal perforation. *PLoS One*. 2015;10(8):e0135737. doi:10.1371/journal.pone.0135737
18. Zhou M, Chen J, Zhou L, et al. Pancreatic cancer derived exosomes regulate the expression of TLR4 in dendritic cells via miR-203. *Cell Immunol*. 2014;292(1–2):65–69. doi:10.1016/j.cellimm.2014.09.004
19. Huichen L, Danhui H, Ji Z. Effect of miR-203-3p targeted TREM1 gene on the regulation of TGF-β1/p38MAPK signaling pathway on the proliferation and apoptosis of renal tubular epithelial cells in lupus nephritis mice. *J Shandong Univ Health Sci*. 2021;59(3):18–25.
20. Primo MN, Bak RO, Schibler B, et al. Regulation of pro-inflammatory cytokines TNFα and IL24 by microRNA-203 in primary keratinocytes. *Cytokine*. 2012;60(3):741–748. doi:10.1016/j.cyto.2012.07.031
21. Stumpfova Z, Hezova R, Meli AC, et al. MicroRNA profiling of activated and tolerogenic human dendritic cells. *Mediators Inflamm*. 2014;2014:259689. doi:10.1155/2014/259689