Candidate microRNA Biomarkers in Lupus Nephritis: A Meta-Analysis of Profiling Studies in Kidney, Blood and Urine Samples

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Abstract

Lupus nephritis (LN) is a kidney disease caused by systemic lupus erythematosus in which kidneys are attacked by the immune system. MiRNAs participate in the pathogenesis of LN as modulatory effectors in immune responses and nephrogenesis pathways. Despite the large number of miRNAs that are expressed differentially, identifying the most suitable biomarker candidate for LN is challenging. The aim of this study was to introduce a consensus panel of potential miRNA biomarkers by performing a meta-analysis of miRNA profiles in the kidney, blood, and urine samples of LN patients. A comprehensive literature review approach was considered to find LN-related miRNA expression profiles. After including the 41 eligible studies and performing the data extraction, meta-analysis was done based on the vote-counting rank strategy and as well as meta-analysis of p-value. The result of the meta-analysis was three lists of consensus miRNAs with altered expression profiles in the various tissue samples of LN patients. Of the 13 studies on kidney tissue, the meta-miRNAs were let-7a, miR-198, let-7e, miR-145, and miR-26a. In addition, meta-miRNAs of miR-199a, miR-21, miR-423, miR-1260b, miR-589, miR-150, miR-155, miR-146a, and miR-183 from 21 studies on blood samples, and miR-146a, miR-204, miR-30c, miR-3201, and miR-1273e from 11 studies on urine samples can be considered as non-invasive biomarker panels for LN. The meta-miRNAs and their related genes were subjected to functional enrichment analyses and network construction. Functional enrichment analysis confirmed the involvement of their target genes in nephropathy-related signaling pathways. Network construction showed miR-145-5p in blood and miR-155-5p in kidney, the best hub miRNAs as candidate drug targets. In conclusion using a meta-analysis approach, our study proposes three meta-miRNA panels that could be the target of further research to assess their potential as biomarkers / therapeutic targets in LN disease.

Introduction

Lupus nephritis (LN) is a severe complication of systemic lupus erythematosus (SLE), and remains a major cause of death in these patients (1, 2). The LN pathogenesis involves cell proliferation and autoantibody deposition in the glomerulus that leads to increased renal inflammatory responses, renal disruption, and renal fibrosis (3, 4). LN is frequently associated with a poor long-term prognosis. It affects up to 70% of SLE cases, of which 10–30% end up with end-stage renal failure (ESRD). Therefore, early detection of LN and care will greatly increase prognosis(5).

MicroRNAs, known as endogenous and small non-coding RNAs, regulate gene expression by targeting the RNA/proteins. Further, miRNAs are known not only to target genes but also to target significant parts of pathways. Therefore, microRNAs have been shown to play a role in the regulation of immune cell activities and inflammatory mediators. They are taking a part in the development/progression of autoimmune diseases and their altered expression can result in a change in the normal expression of their target genes (3). In terms of the biomarker potential of miRNAs, cell-free ones are valuable molecules since they are stable in body fluids and can be obtained non-invasively (7). In addition, the expression of some miRNAs might be limited to particular tissues, biological stages, or types of the disease, and such expression sometimes is detectable before the occurrence of clinical symptoms (8).

miRNAs participate in the pathogenesis of SLE as transcriptional and translational regulators and have been shown to act as modulatory effectors in immune responses, DNA methylation, and nephrogenesis pathways (9, 10). So far, different miRNA expression profiling studies have been performed to identify the dysregulated miRNAs in kidney, blood, and urine samples of LN patients. Despite the large number of miRNAs that are expressed differentially, identifying the most suitable biomarker candidate for LN is challenging due to differences in the applied profiling platforms and methods. Here, meta-analysis approaches could be highly beneficial in combining the available data aiming to maximize predictive strength, overcome contradictions and finally obtain the most consensus results (11-13).

Literature reviews on miRNAs corresponding to LN were merely narrative, and as far as we know, no meta-analysis for miRNAs involved in LN has been reported so far. In this study, we used a meta-analysis approach to identify a consensus list of dysregulated miRNAs (meta-miRNAs) as candidate biomarkers in LN. The gene targets of the meta-miRNAs were then determined, and pathways and enrichment analyses were conducted to find the physiological effects of miRNA dysregulation on LN progression. Finally, to determine the LN's most associated hub genes, a miRNA-gene regulatory network was constructed and analyzed.

Results

Including the independent studies

A total of 407 studies were identified through the first searches in PubMed and EMBASE. After screening the titles and abstracts, 234 of these studies were eliminated. Then, 48 studies were excluded after evaluating the full text according to the inclusion and exclusion criteria. Accordingly, 41 individual studies were selected for the meta-analysis. Figure 1 depicts the comprehensive workflow of the study. The overall characteristics of the included studies, as well as their detailed information, are listed in Table 1.

Table 1
Characteristics of miRNA expression profiling studies (lupus nephritis/control)

No.	Author	Year	Country	Tissue	Control type	No. of Control	No. of Case	Assay type	No. of probes	total	Up-regulated	Down-regulated
1	Costa-Reis	2015	USA	Renal biopsy	Healthy	6	12	NGS (nCounter assay (NanoString))	734	62	21	41
				Renal biopsy	Healthy	6	12	qRT-PCR	3	3	0	3
				Urine	Healthy	19	14	qRT-PCR	2	2	0	2
2	Han	2016	China	Renal biopsy	Healthy	12	14	qRT-PCR	1	1	0	1
3	Navarro-Quiroz	2017	Colombia	blood (serum)	Healthy	7	4	NGS (Illumina1)	-	24	16	8
				blood (serum)	SLE	10	4	NGS (Illumina1)	-	2	1	1
4	Yao	2016	China	Renal biopsy	Healthy	35	35	qRT-PCR (TaqMan miRNA assays)	1	1	0	1
5	Qingjuan	2016	China	blood (serum)	Healthy	20	20	qRT-PCR (TaqMan miRNA assays)	1	1	1	0
6	Solé	2015	Spain	Urine	Healthy	20	32	qRT-PCR (TaqMan miRNA assays)	1	1	0	1
7	Dai	2009	China	Renal biopsy	Healthy	3	5	Microarray (miChip)	NR	66	36	30
8	Te	2010	USA	blood (PBMC)	Healthy	26	26	Microarray (PIQORTM miRXplore)	850	21	20	1
9	Su	2018	Taiwan	blood (PBMC)	SLE	6	6	NGS (Illumina MiSeq)	-	41	15	26
10	Zhu	2017	China	blood (PBMC)	Healthy	30	88	qRT-PCR	1	1	0	1
11	Cai	2019	China	Renal biopsy	SLE	4	28	qRT-PCR	1	1	0	1
				Renal biopsy	SLE	4	17	qRT-PCR	1	1	0	1
12	Cui	2017	China	Renal biopsy	Healthy	10	30	qRT-PCR	1	1	1	0
13	Wang	2015	China	blood (serum)	Healthy	40	32	qRT-PCR	2	1	1	0
				blood (serum)	Healthy	4	4	Microarray (miScriptmiRNA PCR Array)	372	7	7	0
				blood (serum)	Healthy	12	32	qRT-PCR	2	2	0	1
				blood (serum)	Healthy	4	4	Microarray (miScriptmiRNA PCR Array)	372	21	0	21
14	Solé	2019	Spain	Urine (exosom)	Healthy	20	45	qRT-PCR	5	4	2	2
15	Navarro-Quiroz	2017	Colombia	blood (serum)	SLE&healthy	40	100	qRT-PCR	6	5	5	0

No.	Author	Year	Country	Tissue	Control type	No. of Control	No. of Case	Assay type	No. of probes	total	Up-regulated	Down-regulated
				blood (serum)	SLE	40	40	NGS (Illumina barcoded miRNA sequencing)	-	7	4	3
				blood (serum)	SLE	40	40	NGS (Illumina barcoded miRNA sequencing)	-	10	6	4
				blood (serum)	Healthy	40	20	NGS (Illumina barcoded miRNA sequencing)	-	6	6	0
				blood (serum)	Healthy	40	20	NGS (Illumina barcoded miRNA sequencing)	-	16	9	7
16	Liu	2015	China	Renal biopsy	SLE	47	51	qRT-PCR (TaqMan miRNA assays)	5	4	4	0
17	Ichii	2014	Japan	Urine	Healthy	8	13	qRT-PCR	1	1	1	0
18	Zhang	2018	China	blood (serum)	Healthy	100	101	qRT-PCR	9	3	0	3
19	Wu	2018	China	Renal biopsy	SLE	31	28	qRT-PCR	1	1	1	0
20	Cardenas-Gonzalez	2017	USA	Urine	Healthy	10	10	Microarray	365	18	16	2
				Urine	Healthy	119	99	qRT-PCR	18	16	14	2
				Urine	Healthy	86	86	qRT-PCR	12	4	2	2
				Urine	SLE	37	86	qRT-PCR	12	4	2	2
21	Hashad	2012	Egypt	blood (serum)	Healthy	48	112	qRT-PCR	1	1	0	1
				blood (serum)	SLE	48	112	qRT-PCR	1	1	0	1
22	Krasoudaki	2015	Greece	Renal biopsy	Healthy	3	12	Microarray (TLDA)	365	24	9	15
23	Lu	2012	Taiwan	blood (T cell)	Healthy	5	5	Microarray	270	7	1	6
				blood (T cell)	Healthy	22	26	qRT-PCR	7	2	1	1
24	Lu	2012	China	Renal biopsy (glomerular)	Healthy	10	42	qRT-PCR	5	3	2	1
25	Perez-Hernandez	2015	Spain	Urine (exosom)	Healthy	12	6	qRT-PCR	4	4	4	0
26	Tang	2009	China	blood (PBMC)	Healthy	29	33	qRT-PCR	1	1	0	1
27	Wang	2011	China	blood (serum)	Healthy	30	40	qRT-PCR	7	6	0	6
				Urine	Healthy	30	40	qRT-PCR	7	5	0	5
28	Wang	2010	China	blood (serum)	Healthy	30	40	qRT-PCR	2	2	0	2
				Urine	Healthy	30	40	qRT-PCR	2	1	1	0
29	Wang	2012	China	Urine (sediment)	Healthy	13	40	qRT-PCR	2	2	2	0
30	Zhou	2013	USA	Renal biopsy	SLE	9	9	Microarray (Affymetrix GeneChip miRNA arrays)	847	16	7	9

No.	Author	Year	Country	Tissue	Control type	No. of Control	No. of Case	Assay type	No. of probes	total	Up-regulated	Down-regulated
31	Zheng	2017	China	Renal biopsy	Healthy	15	58	qRT-PCR	1	1	0	1
32	Wang	2017	China	Renal biopsy	Healthy	25	29	qRT-PCR	2	2	2	0
33	Li	2020	China	blood (exosom)	Healthy	18	17	qRT-PCR	3	2	2	0
				blood (exosom)	SLE	21	17	qRT-PCR	3	2	2	0
34	Nakhjavani	2019	Iran	blood (serum)	Healthy	26	26	qRT-PCR	3	2	2	0
				blood (serum)	Healthy	26	26	qRT-PCR	3	2	2	0
35	Lin	2020	China	blood (serum)	Healthy	40	92	qRT-PCR	2	2	0	2
36	Perez-Hernandez		Spain	Urine (exosom)	Healthy	20	17	qRT-PCR	1	1	1	0
		2020		Urine (exosom)	SLE	14	17	qRT-PCR	1	1	1	0
37	Etemadi	2019	Iran	blood (serum)	Healthy	26	26	qRT-PCR	2	1	1	0
38	Elessawi	2019	Egypt	blood (serum)	Healthy	20	50	qRT-PCR	1	1	1	0
				blood (serum)	SLE	48	50	qRT-PCR	1	1	1	0
39	Zhang	2019	China	blood (serum)	SLE	6	9	qRT-PCR	1	1	0	1
40	Yuan	2019	China	blood (serum)	SLE	20	21	qRT-PCR	1	1	0	1
41	You	2020	China	blood (PBMC)	Healthy	38	94	qRT-PCR	1	1	0	1

Meta analysis results

miRNA profiling for LN is usually conducted either on renal tissue, urine or blood samples. The aim of miRNA profiling in renal tissue samples is mostly identifying and studying miRNAs that are involved in the pathogenesis, while the aim of miRNA profiling in biological fluids such as blood and urine is to identify non-invasive miRNA biomarkers. Therefore, the results of the vote counting meta-analysis along with the p-value meta-analysis were presented separately for different tissue types, control types and time-course studies:

-Blood meta-miRNAs

Among the 21 studies containing 35 miRNA expression profiles obtained from blood samples, 175 DE-miRNAs were reported (Figure 2). 61 DE-miRNAs were reported in at least two studies, of which 35 miRNAs had a consistent direction (24 up-regulated and 11 down-regulated miRNAs (Tables S1 and S2)). Table S3 listed the inconsistently reported miRNAs which were 26 miRNAs.

Those with $OR > 2$ (upregulated) or $OR < 0.5$ (downregulated) in the list of inconsistent miRNAs were added to the lists of consistently reported miRNAs and the panel of up-regulated miRNAs were increased by one (Table S4). In this panel, two miRNAs (miR-199a-5p, and miR-21-5p) were reported in 5 studies, other two miRNAs (miR-423-5p, miR-1260b) were reported in 4 profiling studies, three miRNAs (miR-589-3p, miR-150-3p, miR-155-5p) were reported in 3 profiling studies and eighteen miRNAs were reported in 2 studies.

In addition, the number of down-regulated miRNAs was increased to 13 (Table S5). In this panel, one different miRNA (miR-146a-5p) was reported in seven studies, one miRNA (miR-183-5p) was reported in 4 studies, and 11 miRNAs were reported in 2 studies. Top dysregulated blood miRNAs with cut-off > 2 and their meta-analysis of p-values are listed in Table 2.

Table 2
Top dysregulated miRNAs (cut off>2) in LN studies in tissues of kidney, blood and urine.

tissue	Up/Down regulated	miRNA		Studies with the same direction (reference)	No. of samples	Mean of FC (min._max.)
blood	Up-regulated	miR-199a-5p	5	Elessawi(2019),Elessawi(2019),Navarro-Quiroz(2017),Navarro-Quiroz(2016),Te(2010)	311	2.53 (1.5_4.29)
		miR-21-5p	5	Li(2020),Li(2020),Nakhjavani(2019),Nakhjavani(2019),Nakhjavani(2019)	229	5 (2_ 7)
		miR-423-5p	4	Nakhjavani(2019),Nakhjavani(2019),Su(2018),Navarro-Quiroz(2016)	196	5.85 (1.35_12)
		miR-1260b	4	Navarro-Quiroz(2017),Navarro-Quiroz(2016),Navarro-Quiroz(2016),Wang(2015)	159	6.5 (3.11_9.85)
		miR-589-3p	3	Navarro-Quiroz(2017),Navarro-Quiroz(2016),Navarro-Quiroz(2016)	231	9.28 (3.92_14.93)
		miR-150-3p	3	Navarro-Quiroz(2016),Navarro-Quiroz(2016),Te(2010)	212	13.3 (2.47_22.63)
		miR-155-5p	3	Li(2020),Li(2020),Te(2010)	125	2.68 (1.5_5)
	Down-regulated	miR-146a-5p	7	Su(2018),Zhu(2017),Wang(2015),Hashad(2012),Hashad(2012),Wang(2010),Tang(2009)	590	0.27(0.02_0.72)
		miR-183-5p	4	Lin(2020),Navarro-Quiroz(2017),Navarro-Quiroz(2016),Navarro-Quiroz(2016)	283	0.25(0.09_0.5)
urine	Up-regulated	miR-146a-5p	5	Perez-Hernandez(2020),Perez-Hernandez(2020),Perez-Hernandez(2015),Wang(2012),Wang(2010)	209	26.96 (1.2_103)
		miR-204-5p	4	Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017)	533	4.57 (1.88_7.27)
		miR-30c-5p	4	Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017)	533	2.99 (1.59_4.38)
	Down-regulated	miR-3201	4	Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017)	533	0.49(0.08_0.89)
		miR-1273e	4	Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017),Cardenas-Gonzalez(2017)	533	0.6(0.26_0.93)
kidney	Up-regulated	let-7a-5p	4	Liu(2015),Liu(2015),Dai(2009),Dai(2009)	212	3.13 (1.1_5.24)
		miR-198	4	Cui(2017),Krasoudaki(2015),Lu(2012),Dai(2009)	115	7.07 (2.34_12)
		let-7e-5p	3	Liu(2015),Liu(2015),Dai(2009)	204	2.73 (1.5_3.68)
	Down-regulated	miR-145-5p	3	Cai(2019),Cai(2019),Costa-Reis(2015)	71	0.43(0.58_0.25)
		miR-26a-5p	3	Krasoudaki(2015),Costa-Reis(2015),Costa-Reis(2015)	51	0.31(0.42_0.1)

-Urine meta-miRNAs

Thirty-four DE-miRNAs were reported in 14 expression profiles of urine samples (Figure 2). Among the 19 DE-miRNAs that were reported in at least two studies, 17 miRNAs had a consistent direction, 15 were reported to be upregulated (Table S6), and 2 were reported as downregulated (Table S7). Of the two inconsistent miRNAs listed in Table S8, none of them can cross the OR threshold. In the panel of up-regulated miRNAs (Table S9), miR-146a-5p, miR-204-5p, and miR-30c-5p were reported in more than three studies. In this panel, 12 miRNAs were reported in only 2 studies. In the panel of down-regulated miRNAs (Table S10), there were only two different miRNAs in 4 studies (miR-3201, miR-1273e). Top dysregulated urinary miRNAs with cut-off>2 of LN studies their meta-analysis of p-values are listed in Table 2.

-Renal tissue meta-miRNAs

One hundred and forty-two DE-miRNAs were reported in 15 miRNA expression profiles of renal tissue samples (Figure 2). Among the 33 DE-miRNAs that were reported in at least two studies, 23 miRNAs had consistent direction, (6 upregulated and 17 downregulated miRNAs (Table S11 and S12)). Inconsistent miRNAs (10 miRNAs) are listed in Table S13.

In the panel of up-regulated miRNAs, let-7a-5p and miR-198 were reported as upregulated miRNAs in 4 studies. In this list, upregulation of let-7e-5p and 5 other miRNAs also were reported in three and two studies, respectively (Table S14). Among 17 down-regulated miRNAs, two (miR-145-5p, miR-26a-5p) and fifteen miRNAs were reported in 3 and 2 studies, respectively (Table S15). Top dysregulated renal miRNAs with cut-off>2 and their meta-analysis of p-values are listed in Table 2.

-Meta-miRNAs in time-course studies in blood

Early diagnosis of chronic kidney diseases (CKDs) provides an opportunity to implement therapies aiming for hindering the disease progression (17). Therefore, a meta-analysis of miRNA profiling time-course studies in biofluids can be beneficial for determining miRNA biomarkers in the early stages compared to the late stages of the disease. Four of the 41 studies with 8 profiles investigated the expression patterns of blood miRNAs in the early and late stages of LN in a time-course manner. The dysregulated miRNAs in these studies were classified into two stages of early and late and the VCR method was performed for each group independently (Tables S16-18). Among all, miR-150-3p, miR-423-5p, miR-130b-3p, and miR-1260b were dysregulated blood miRNAs in the early stages of DN and can be considered as non-invasive early diagnostic biomarkers.

-Meta-miRNAs in different control types

The evaluated studies were different in terms of the nature of the control group. Indeed, 48 profiles (from 37 studies) compared LN subjects with healthy individuals; whereas, 16 other profiles (from 14 studies) compared LN with SLE control groups who have not progressed to nephropathy. Among studies that compared miRNA profiles in the LN groups versus healthy controls, 254 dysregulated miRNAs were reported. After applying both VCR criteria, the number of miRNAs were decreased up to 103 and finally 76 miRNAs (43 up-regulated and 33 down-regulated) (Tables S19-S23). In the panel of up-regulated miRNAs containing 43 miRNAs, miR-21-5p were reported in 6 studies, and miR-423-5p, miR-199a-5p, miR-1260b, and miR-198 were reported in 4 studies. In addition, 9 and 19 miRNAs were reported in 3 and 2 studies, respectively. Among 33 down-regulated miRNAs, miR-26a-5p and miR-183-5p were reported in 5 and 4 studies, respectively. There were also other down-regulated miRNAs including 5 and 23 miRNAs reported in 3 and 2 studies, respectively.

In the case of LN versus SLE controls, 83 dysregulated miRNAs were determined of which 10 and finally 6 (3 up-regulated and 3 down-regulated) miRNAs were voted after applying the two criteria (Tables S24-28). In this group, the panel of up-regulated miRNAs contained 3 miRNAs one (miR-375-3p) was reported in 3 studies and two were reported in 2 studies. In addition, 3 down-regulated miRNAs have been characterized in 2 studies.

-Meta-miRNAs in total studies

In total, 289 dysregulated miRNAs were reported in all the 44 miRNA expression profiling studies. 126 miRNAs (38.5%) were reported in at least two studies (figure 2) that 81 miRNAs (54%) were with a consistent direction (OR>2 (46 up-regulated) or OR<0.5 (35 down-regulated)).

More importantly, no miRNA was reported as dysregulated in the same direction between different tissues of the kidney, blood, and urine. Also, no common miRNAs were reported between two control subtypes (SLE groups and healthy groups). Therefore, to prevent heterogeneity, panels of tissue subgroups have not been grouped and bioinformatics investigations were performed on miRNA panels with cut-off>2 for kidney and blood tissue separately.

Construction of LN meta-miRNAs-mRNAs network

We extracted the targets of blood and kidney LN-miRNAs with cut-off>2 and ascertained their validation using miRTarBase. MIENTURNET web tool was used to analyze the interaction between blood and kidney LN-miRNAs and their target genes. Bar graphs were created for ten top genes with the most interactions with LN-miRNAs (Figure 3A and 4A, respectively). In blood, a total of 362 key genes were found to be regulated by more than 2 LN-miRNAs. The top genes with the highest interactions with LN-miRNAs (5-3) included SMAD4, NFKB1, SOCS6, OSBPL1A, and MSH2. In addition, the analysis indicated that a total of 424 key genes were regulated by more than 2 LN-miRNAs in kidney tissue. Here, ZNF566, SPTBN1, MYC, HMGA2, EZH2, COX1, and ABHD17C, were the top genes with the most interactions with LN-miRNAs (4-3).

After analysis of the constructed network between LN-miRNAs obtained from blood and kidney tissue and target genes (Figures 3B and 4B) miR-155, miR-21, miR-146a, and miR-199a were recognized as the top blood LN-miRNAs with the highest degree of centralities (66-28) (Table 3). About the kidney LN-miRNAs, miR-145, let-7a, miR-26a, and let-7e were recognized as top miRNAs with the highest degree of centralities (27-14) (Table 3).

Table 3
The network properties between blood and kidney Meta-miRNAs with target genes.

Node	Degree	Closeness	Betweenness	Eccentricity	AverageShortestPathLength
Blood Meta-miRNAs					
hsa-miR-155-5p	66	0.498207885	0.583129372	3	1.992857143
hsa-miR-21-5p	49	0.438485804	0.397004817	5	2.264285714
hsa-miR-146a-5p	33	0.396011396	0.252142751	5	2.507142857
hsa-miR-199a-5p	28	0.385041551	0.196462835	5	2.578571429
hsa-miR-183-5p	9	0.348370927	0.027881312	5	2.85
hsa-miR-150-3p	4	0.296375267	0.029355349	5	3.35
hsa-miR-1260b	2	0.309576837	0.014388489	5	3.207142857
Kidney Meta-miRNAs					
hsa-miR-145-5p	27	0.495652174	0.484569908	4	1.982758621
hsa-let-7a-5p	25	0.487179487	0.417690133	3	2.017241379
hsa-miR-26a-5p	21	0.456	0.281867616	3	2.155172414
hsa-let-7e-5p	14	0.404255319	0.161737005	4	2.431034483
hsa-miR-198	1	0.280788177	0	5	3.5

The impact of LN meta-miRNAs on cellular pathways and biological processes

-meta-miRNAs in blood

Genes related to the blood LN-miRNAs were mostly enriched in the nucleus and cytoplasm (Figure 3c). In terms of molecular function, the enriched terms were "transcription factor activity" and "protein serine/threonine kinase activity" (Figure 3D). "Signal transduction", "cell communication", and "regulation of nucleic acid metabolism" were the most significantly associated biological process for these genes (Figure 3E). Enriched Reactome pathways were most significantly associated with the innate immune system, cellular senescence, gene expression, cellular response to stress, disease of signal transduction, and various cell signaling pathways (WNT signaling, PTK6 signaling, SMAD2/3 signaling, TGF-beta signaling, TCF dependent signaling, and activin signaling) (Figure 3F).

-meta-miRNAs in kidney

GO and pathway enrichment analyses were performed for the target genes of the kidney LN-miRNAs. Based on the results, the genes were mostly enriched in the nucleus and cytoplasm (Figure 4C) and mostly act as "auxiliary transport protein", "receptor signaling complex scaffold", and "extracellular matrix structural constituent" (Figure 4D). In terms of biological processes, the genes were mostly enriched in the "Signal transduction" and "cell communication" processes (Figure 4E). The Reactome pathway analysis showed that cellular senescence, gene expression, cellular response to stress, generic transcription pathway, cell cycle, and disease of signal transduction were the most significant pathways regulated by LN-miRNAs (Figure 4F). There was a high degree of similarity in the enrichment results of both groups (blood and kidney LN-miRNAs).

Discussion

Recently, miRNAs have been of great attention as important players in the pathology and nephrogenesis of SLE (9, 10). Therefore, the identification of miRNAs with altered expression profiles in LN could be a valuable approach to find novel risk factors, therapeutic targets, and biomarkers for this disease. However, one challenge in the interpretation of miRNA expression profiles is the lack of consistency among the results of different studies. Such inconsistency might be due to variations in the study designs, the expression platforms, the population, the sample preparation methods, genetic, environmental, and clinicopathological conditions. In addition, gender and ethnic differences can be other factors that cause heterogeneous patterns in miRNA expression profiles (18, 19). Carrying out a meta-analysis on pooled raw miRNA expression profiles is a scientifically validated approach to overcome this challenge. However, it is also a complicated method because of the unavailability of the most of raw data for data normalization across different platforms.

In the present study, information of the experiments was obtained independently and then reassembled. We employed the VCR strategy to identify the consensus list of altered miRNAs in LN condition. The VCR is a simple meta-analysis method applied for combining differentially expressed profiles coming from individual studies (20-22). In addition, for the validation of the combined results, using meta-analysis of p values, the statistical significance of the differential expression of each meta-miRNA was calculated.

Among the 13 LN studies conducted on kidney tissue samples, 142 DE-miRNAs were reported, and 33 DE-miRNAs were validated by VCR. The expression of only 5 miRNAs was changed in more than two profiling studies, let-7a-5p, miR-198, and let-7e-5p were the up-regulated ones, and miR-145-5p and miR-26a-5p were the downregulated ones. miR-145, let-7a, miR-26a, and let-7e were recognized as hub molecules in the constructed miRNA-gene network. miRNA profiling in renal tissue is mostly for studying miRNAs involved in LN pathogenesis. For example, in juvenile lupus nephritis (JLN), lower expression of miR-145 in renal

vascular cells was shown to provoke renal vascular lesions through inducing the proliferation and migration of vascular smooth muscle cells, inhibiting apoptosis, decreasing α -smooth muscle actin (α -SM-actin) level, and increasing osteopontin (OPN) expression (23). Another study also suggested the role of the miR-145 in phenotype switching of the vascular smooth muscle cells in the renal interstitium and suggested the therapeutic potential of miR-145 after transfection of this miR into the cells and observing the recovery of renal vascular injury (24). MiR-26a has been shown to regulate different cellular processes, including differentiation, growth, apoptosis, and metastasis. This miRNA was also shown to be involved in the homeostasis of podocyte and mesangial cells and play a pivotal role in the formation of the podocyte's cytoskeleton. Based on previous experiments, decreased level of this miRNA is associated with kidney diseases (25).

The involvement of let-7a in the pathogenesis of LN is through its upregulation and inhibition of TNFAIP3 expression and suppression of NF- κ B signaling (26). The proper function of vascular endothelial cells, controlling cell proliferation, apoptosis, and cell adhesion are some known functions of let-7e miRNA. Moreover, let-7e also participates in the regulation of inflammatory responses via a complex signaling network composed of I κ B β and Inc-MK167IP-3. Upregulation of let-7e can activate NF- κ B and consequently the expression levels of inflammatory and adhesion molecules in the cells.(27).

The biofluid miRNAs are stable in samples even after multiple freeze-thaw cycles and are detectable by minimal or non-invasive techniques (28). Also, it is shown that the expression of biofluid miRNAs are specific in different tissues and disease states (29). Among the 21 LN studies of blood tissue samples, 175 DE-miRNAs were reported and 61 DE-miRNAs were validated by the VCR method. Only 9 of the miRNAs were changed in more than two profiling studies, 7 of which (miR-199a, miR-21, miR-423, miR-1260b, miR-589, miR-150, miR-155) were upregulated, and 2 (miR-146a and miR-183) were downregulated. All these spotted blood-related miRNAs can be considered as potential non-invasive biomarkers for the diagnosis of LN. Furthermore, among them, miR-155, miR-21, miR-146a, miR-199a have the highest degree property in network and are hot for more attention.

Of all, 4 studies investigated the expression patterns of miRNAs in blood samples of LN patients in a time-course manner. miR-150, miR-423, miR-130b, and miR-1260b were the dysregulated miRNAs in the early stages of LN and could be considered as non-invasive biomarkers for the detection of disease in its early stages.

miR-155 was shown to play a significant role in the regulation of inflammatory processes in LN, where its overexpression results in a reduction in the levels of CXCR5, a receptor of chemokine CXCL13. Reducing the proliferation rate of human renal mesangial cells, as well as phosphorylation of ERK and TGF- β 1 production, are other effects of miR-155 upregulation in the pathological states (30). miR-21 can target several genes in the JAK-STAT pathway, which regulate inflammatory processes. Moreover, the anti-inflammatory effects of miR-21 through inhibition of IL12 and PDCD4 in primary macrophages have recently been reported (31). In addition, both miR-150, and miR-21 was shown to cause renal fibrogenesis by increasing the synthesis of profibrotic molecules in LN (32). Reducing the expression of the antifibrotic protein suppressor of cytokine signaling 1 (SOCS1) and consequently, up-regulation of profibrotic proteins in both proximal tubular and mesangial cells in LN are other outcomes of miR-150 dysregulation (33). Other miRNAs including miR-589, miR-199a, miR-423-5p, miR-130b-3p and miR1260b were shown to be involved in different pathways that finally lead to the promotion of inflammatory responses and induction of fibrosis in LN (34-36). miR-1260b was shown to promote cell invasion through Wnt/ β -catenin signaling pathway and it is one of the miRNAs found in the plasma of patients with class IV lupus nephritis. Therefore, this miRNA could also be a good marker for early diagnosis of the disease process and reaching the final stages of the disease (37, 38).

After analyzing the 11 LN studies conducted on urine samples, 34 DE-miRNAs were reported and 19 DE-miRNAs were validated by the VCR method. Among these, only 5 miRNAs including miR-146a, miR-204, miR-30c, miR-3201, and miR-1273e were changed in more than two profiling studies and therefore can be considered as biomarkers in urine samples of LN patients.

The involvement of these miRNAs in inflammatory responses was previously shown. For instance, a positive correlation was observed between the miR-204 expression and upregulation of target molecules involved in the inflammatory responses (39). Based on a recent report, miR-30c is among the top 10 highly expressed miRNAs in urinary extracellular vesicles of patients with different nephropathies. This miRNA has been shown to play a role in different cellular processes like chromatin modification, gene expression, double-strand RNA (dsRNA) and mRNA metabolic processes, cell cycle, and posttranscriptional regulation (40-42). In LN patients, down-regulation of miR-3201 and miR-1273e is related to endocapillary glomerular inflammation (43).

In our study, miR-146a was the only dysregulated miRNA in both the bio-fluid samples. This miRNA showed an upregulation pattern in the LN-urine samples while a downregulation pattern in the LN-blood samples. It seems that the urinary level of miR-146a is related to kidney tissue damages rather than plasma filtration. Downregulation of this miRNA in renal tissues and peripheral blood mononuclear cells (PBMCs) of LN patients was shown to be negatively correlated with TRAF6 expression and therefore its involvement in NF- κ B activity and inflammatory responses(9). A study also has shown that serum miR-146a level is lower in patients with SLE in comparison with healthy people, which negatively correlated with proteinuria, SLE disease activity index, and renal function; whereas, urinary level of miR-146a in patients with SLE was higher than that in the healthy control group (44).

By comparing the obtained results from healthy vs. LN and SLE vs. LN profiles, it could be assumed that other than the secondary effects of nephropathy like kidney fibrosis, most of the LN-related miRNAs could be under the effects of the SLE condition. Another assumption is the potential of the presented LN-meta-miRNAs in differentiating between LN and other nephropathies.

According to enrichment analysis, LN-meta-miRNAs can target a range of genes, and it is highly important to highlight targets that focus on LN pathological pathways inducing abnormal kidney structure or function such as mesangial cell hypertrophy and proliferation (rarely apoptosis) endothelial-to-mesenchymal transition and podocyte apoptosis, and detachment from the glomerular basement membrane. As well, our Reactome analysis on blood and kidney LN-meta-miRNAs, similarly revealed the target genes of these miRNAs in both tissues collect and organize very rich networks in the innate immune system, cellular senescence, cell cycle, gene expression, cellular response to stress and disease of signal transduction that many studies have shown the role of these pathways in LN (45-47). Furthermore, various cell signaling pathway related to nephropathy such as WNT signaling (48, 49), PTK6 signaling (50), SMAD2/3

signaling (51, 52), TGF-beta signaling (51, 53), TCF dependent signaling by WNT (54), and activin signaling (55) enriched for meta-miRNAs. This further highlight the importance of these miRNAs as potential biomarkers or therapeutic candidates for clinical application.

Conclusions

In the last few years, various studies have introduced different miRNAs as biomarkers for LN diagnosis. However, still, there is no comprehensive list of potential miRNA biomarkers for this purpose. Applying a meta-analysis approach, the present study introduced three panels of dysregulated microRNAs in different samples of LN patients. These meta-miRNAs could be targets of more investigations regarding their biomarker/drug target potentials in the diagnosis/treatment of LN disease.

Materials And Methods

Search strategies

A systematic and comprehensive review of the literature was conducted to find LN miRNA expression profiling studies published up to December 2020. We used the MESH and Emtree search headings ("lupus nephritis" OR "Lupus Glomerulonephritis") AND (miRNA OR microRNA) to search electronic databases, including Medline (PubMed) and Embase databases.

Study selection

The inclusion criteria to check the eligibility of studies were: (1) they had to be miRNA expression profiling studies on LN patients; (2) they had to compare LN and health or SLE control samples; (3) they reported the relative miRNAs expression via miRNA microarray, NGS or qRT-PCR; (4) they had to report cut-off criteria of dysregulated miRNAs; (5) they had to report sample sizes; (6) they had to report fold changes (even if non-explicit).

Studies were excluded from this meta-analysis if they failed to meet one or more of the following criteria: (1) the inclusion criteria were not met, (2) no findings of significance were published or could be determined, (3) cell lines and animal models were used for studies, and (4) non-original paper, such as conference abstracts, letters, and review

Data extraction

The collected information from the full text and supplementary details of the eligible studies was categorized into separated data groups as following: first author, date of publication, country of study, type of studied tissues, type of control, sample sizes, type of miRNA expression profiling assay, number of probes, the list of up-regulated and down-regulated miRNAs, fold changes in miRNA expression, p-value of changes and cut-off criteria of dysregulated miRNAs. Version 21 of miRBase was used to standardize all miRNA names. miRNAs that were introduced neither -3p nor -5p in miRBase were named according to literature. We assessed data quality at each step of data extraction and 25% of the data was re-reviewed randomly.

Meta-analysis

Each study that compared miRNA expression in renal tissues, blood, urine, and normal samples provided a list of differentially expressed miRNAs (DE-miRNA). Griffith (14) and Chan (15) have developed a vote counting strategy for ranking potential molecular biomarkers, which is used for the meta-analysis. The identified dysregulated miRNAs by each study were ranked based on the order of the following items: 1) the number of studies with consistently DE-miRNA and the majority of them with a consistent direction of change; 2) the overall number of samples; and 3) the average fold change. Furthermore, for maintaining DE-miRNAs with a high repetition in studies and a very low inconsistency, we used a parameter called OR (odds ratio), which is defined as the ratio of odds of up-regulation of a miRNA to odds of down-regulation of same miRNA. Then in the list of inconsistent miRNAs, those with OR>2 (Up-regulated) or OR<0.5 (Down-regulated) were added to lists of consistently reported miRNAs to construction of meta-miRNAs panels for LN in each of tissues. Statistical Product and Service Solutions (SPSS 21.0 for windows) was used for all the ranking and comparisons.

In addition, the significance of the differential expression in all studies for each of the meta-miRNAs was calculated using meta-analysis of p-values, which was performed by Fisher's test with stata 11.

Network construction and functional enrichment analysis for meta-miRNAs

The top miRNAs from the meta-analysis lists of blood and kidney samples (with cut-off>2) were considered as meta-miRNAs, and their validated target genes were obtained from the miRTarBase database. The MIENTURNET web tool was used for interaction analysis and network construction between LN-miRNAs and target genes (16). Enrichment analyses of LN-miRNAs targets in Go terms, and Reactome pathways were carried out, and p-values for each pathway were obtained. Also, the association between those pathways affected by LN-miRNAs and LN pathways was assessed.

Declarations

Competing interests

The authors report no conflict of interest.

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Authors' contributions

AR and AG contributed equally in study concept, design, data acquisition, data analysis and manuscript drafting. BS and KH contributed in data acquisition and revision of the manuscript and YG contributed in revision of the manuscript. All authors read and approved the final manuscript.

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Figures

Figure 1

Flow diagram of the process of study selection depicting identification, screening, eligibility extraction and inclusion steps.

Figure 2

Workflow and information of the conducted meta-analysis. Red arrows are indicating criterion 1 (dysregulated miRNAs that reported in at least two expression profiling studies) and the blue arrows are indicating criterion 2 (dysregulated miRNAs with OR>2 (Up-regulated) or OR<0.5(Down-regulated)). Top up or down-regulated miRNAs are shown in each subgroup section.

Figure 3

Bioinformatic analysis on blood Meta-miRNAs. A: Bar graph of the number of interactions of the 10 top genes with Meta-miRNAs. B: The interaction networks between Meta-miRNAs and target genes. The Pink square represents miRNAs, and the green nodes represent the target gene. C: Topmost enriched cellular component, D: molecular function, E: biological process GO terms and F: Reactome pathways for the Meta-miRNAs.

Figure 4

Bioinformatic analysis on kidney Meta-miRNAs. A: Bar graph of the number of interactions of the 10 top genes with Meta-miRNAs. B: The interaction networks between Meta-miRNAs and target genes. The Pink square represents miRNAs, and the green nodes represent the target gene. C: Topmost enriched cellular component, D: molecular function, E: biological process GO terms and F: Reactome pathways for the Meta-miRNAs.

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