

LETTER TO EDITOR

Serum tsRNA as a novel molecular diagnostic biomarker for lupus nephritis

Dear Editor,

Systemic lupus erythematosus (SLE) is an autoimmune illness with a lifelong toll on the body. Meanwhile, approximately 30% of SLE patients develop lupus nephritis (LN).¹ However, clinical diagnosis of LN still relies on the "com-

ination" of clinical symptoms, such as all proteins in the 24-hour urine of the patient (24-hour proteinuria) and anti-double stranded DNA antibodies (anti-dsDNA).² tRNA-derived fragments (tsRNAs) are novel noncoding small RNAs (14~40 nt in length) formed by the cleavage of

TABLE 1 Statistics of clinical information of specimens

Characteristics	Training set		Validation set	
	HC	LN	HC	LN
Cases	24	24	46	73
Sex (female, percentage)	22 (91.67%)	19 (79.17%)	33 (71.74%)	68 (93.15%)
Age (mean ± SD, year)	38.92 ± 2.75	41.75 ± 3.21	32.13 ± 1.11	41.88 ± 1.64
WBC (mean ± SD, 10 ⁹ /L)	5.20 ± .34	5.38 ± .51	6.04 ± .26	6.44 ± .49
RBC (mean ± SD, 10 ¹² /L)	4.47 ± .08	3.40 ± .13	4.66 ± .06	3.48 ± .08
HGB (mean ± SD, g/L)	131.96 ± 2.68	103.42 ± 4.51	140.27 ± 2.36	100.16 ± 2.85
HCT (mean ± SD, %)	39.57 ± .76	30.48 ± 1.21	41.63 ± .60	30.59 ± .74
PLT (mean ± SD, 10 ⁹ /L)	256.96 ± 10.72	161.29 ± 18.56	254.55 ± 10.18	172.35 ± 11.07
Ne (mean ± SD, 10 ⁹ /L)	3.03 ± .26	3.91 ± .39	3.64 ± .20	5.10 ± .44
Ly (mean ± SD, 10 ⁹ /L)	1.75 ± .09	1.00 ± .14	1.90 ± .08	.92 ± .06
Mo (mean ± SD, 10 ⁹ /L)	.28 ± .02	.41 ± .05	.33 ± .02	.39 ± .04
eGFR (mean ± SD)	127.77 ± 5.11	117.95 ± 11.70	132.99 ± 3.40	110.73 ± 6.68
Anti-dsDNA (mean ± SD, IU/L)	N/A	297.91 ± 88.60	.91 ± .46	291.77 ± 41.79
Proteinuria (mean ± SD, mg/L)	N/A	1987.69 ± 572.46	N/A	2055.17 ± 338.14
CRP (mean ± SD, mg/L)	N/A	24.02 ± 6.71	N/A	13.45 ± 2.24
IgG (mean ± SD, g/L)	N/A	12.47 ± 1.15	N/A	13.30 ± .88
C3 (mean ± SD, g/L)	N/A	.75 ± .07	N/A	.69 ± .04
C4 (mean ± SD, g/L)	N/A	.14 ± .02	N/A	.12 ± .01
Anti-β ₂ -GP I (mean ± SD, RU/mL)	N/A	4.73 ± 1.07	N/A	8.50 ± 3.99
ANuA (positive, percentage)	N/A	7 (29.2%)	N/A	19 (26.0%)
ARPA (positive, percentage)	N/A	8 (33.3%)	N/A	11 (15.1%)
AHA (positive, percentage)	N/A	8 (33.3%)	N/A	26 (35.6%)
ASMA (positive, percentage)	N/A	5 (20.8%)	N/A	15 (20.5%)
SLEDAI-2K (mean ± SD)	N/A	11.21 ± .75	N/A	9.49 ± .60

Abbreviations: AHA, anti-dsDNA, anti-double stranded DNA antibody; anti-histone antibody, Anti-β₂-GP I, anti-beta 2 glycoprotein 1 antibody, ANuA, anti-nuclear antibody; ARPA, anti-ribosomal p protein antibody; ASMA, anti-Smith antibody; C3, complement C3; C4, complement C4; CRP, C-reactive protein; eGFR, glomerular filtration rate; HC, healthy control; HCT, haematocrit; HGB, haemoglobin; IgG, immunoglobulin G; LN, lupus nephritis; Ly, lymphocyte; Mo, monocyte; N/A, not available; Ne, neutrophils; PLT, platelet; RBC, red blood cell; SD, standard deviation; SLEDAI-2K, Systemic Lupus Erythematosus Disease Activity Index 2000; WBC, white blood cell.

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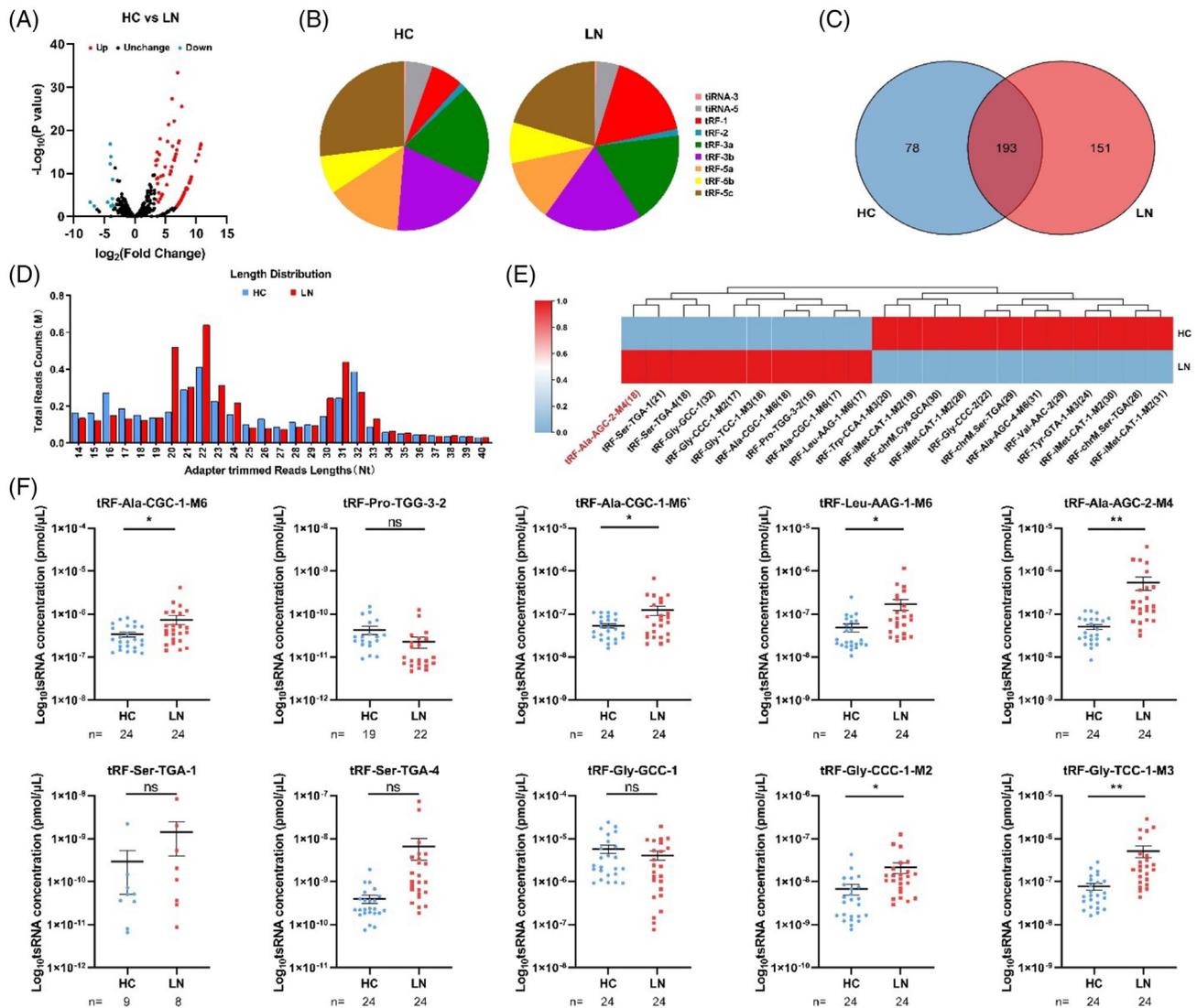


FIGURE 1 Identification of serum differential expression of tsRNA between patients with lupus nephritis (LN) and healthy controls (HC). (A) Volcano plot of differentially expressed tsRNAs. The tsRNAs represented by red (upregulation) or blue dots (downregulation) illustrated a fold change greater than 10 and a P value less than .01 between the two compared groups, and black dots represented tsRNAs with no differential expression. (B) Species distribution profiles of tsRNAs between LN patients and healthy controls. (C) Venn distribution of tsRNAs in LN groups and healthy controls. (D) Length distribution profiles of tsRNAs between LN patients and healthy controls. (E) Hierarchical clustering indicates the differences in tsRNA expression profiles between the two groups. (F) RT-qPCR verification of 10 differentially expressed tsRNAs in the serum of the LN group and HC group. Statistical significance was determined by unpaired two-tailed t test (* $P < .05$, ** $P < .01$, ns: no significant difference)

mature tRNAs or their precursors under the action of enzymes or stress.³ tsRNAs have earlier biogenesis than most RNA and protein synthesis and are abundant in serum (> 50%) than miRNAs.^{4,5} Hence, we researched and identified novel serum tsRNA molecular marker for the diagnosis of LN, with prospects to become noninvasive liquid biopsy tool for LN diagnosis.

The sera of LN patients and healthy controls (HCs) were acquired at “Drum Tower Hospital affiliated with Nanjing University School of medicine” (Table 1). Thoroughly following the 1997 American Society of Rheumatology

(ACR), guidelines, signed consent forms from patients and approval from ethics committee (ID: 2020-327-01) were obtained. Serum RNA was extracted with TRIZOL and treated with a rtStart™ tRF and tRNA pretreatment kit by Arraystar Inc., USA, before sequencing. cDNA was constructed prior to quantitative reverse transcription polymerase chain reaction (RT-qPCR).⁶ When mapping the sequencing results, only one mismatch was allowed, and the differentially expressed tsRNAs (fold change > 10, P value < .01) in LN were selected for subsequent exploration (Figure 1A; and Table S1). tsRNAs of the tRF-1 type

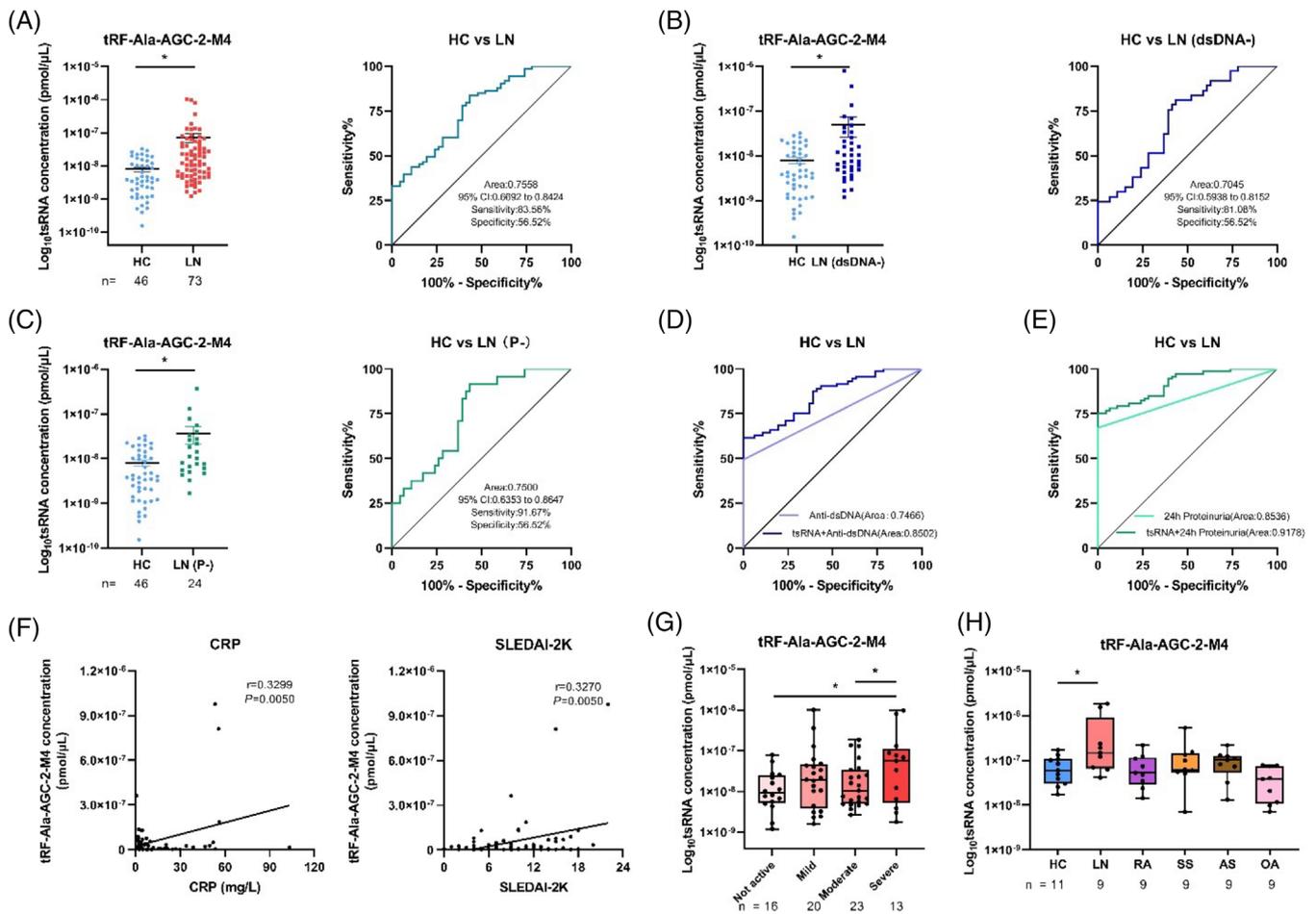


FIGURE 2 Diagnostic value of tRF-Ala-AGC-2-M4 in clinical applications. (A) Diagnostic value of tRF-Ala-AGC-2-M4 in the LN group and HC group. (B) Diagnostic value of tRF-Ala-AGC-2-M4 in anti-dsDNA negative (dsDNA-) LN disease individuals and the HC group. (C) Diagnostic value of tRF-Ala-AGC-2-M4 in 24-hour proteinuria-negative (P-) LN disease individuals and the HC group. (D) ROC combined diagnostic analysis of tRF-Ala-AGC-2-M4 and anti-dsDNA in the LN group and HC group. (E) ROC combined diagnostic analysis of tRF-Ala-AGC-2-M4 and 24-hour proteinuria in the LN group and healthy controls. (F) Analysis of the correlation between tRF-Ala-AGC-2-M4 and CRP and SLEDAI-2K. (G) Differential analysis of tRF-Ala-AGC-2-M4 in different LN severity groups. (H) Differential expression analysis of tRF-Ala-AGC-2-M4 in different autoimmune diseases. Statistical significance was determined by unpaired two-tailed *t* test ($*P < 0.05$). SPSS binary logistic regression was used to predict the probability of joint diagnosis. Statistical correlation was determined by linear regression and Pearson correlation. AS, ankylosing spondylitis; HC, healthy control; LN, lupus nephritis; OA, osteoarthritis; RA, rheumatoid arthritis; ROC, receiver operating characteristics; SS, Sjogren's syndrome

were more abundant in LN serum (Figure 1B), which may be associated with systemic damage and preference of splice sites of angiopoietins in lupus patients.⁷ Venn analysis showed that 193 tsRNAs were present simultaneously in both groups (Figure 1C). The length range of tsRNA fragments was enriched at bases 20-23 nt and 30-32 nt (Figure 1D). Finally, we cluster analysed the 10 highly and 12 lowly expressed tsRNAs in LN (Figure 1A) and selected the highly expressed tsRNAs for marker screening (Figure 1E; and Tables S2, S3). RT-qPCR confirmed over-expression of tRF-Ala-AGC-2-M4 and tRF-Gly-TCC-1-M3 in the LN compared to HC (training set, Figure 1F) using agarose gel electrophoresis and sequencing of T vector clones to confirm the specificity of RT-qPCR (Figure

S5). A standard curve of quantitative CT values was transformed for tsRNA concentration (Figure S1).

tRF-His-GTG-1 has elevated levels in SLE patients but not in LN patients; hence, it can be used to distinguish LN patients from SLE patients.⁸ In this work, the total number of reads per million for tRF-Ala-AGC-2-M4 in the serum of LN patients was higher than that of HC patients (Table S2); RT-qPCR confirmed that tRF-Ala-AGC-2-M4 was exuberantly expressed in LN patients than in HC patients ($P < .01$), with area under the curve (AUC) = .7558 (validation set, Figure 2A). However, tRF-Gly-TCC-1-M3 expression was not significantly different between HC and LN (Figure S2). For LN clinical diagnosis, renal biopsy is the gold standard but is not suitable for dynamic

other autoimmune diseases, we verified the specificity of tRF-Ala-AGC-2-M4 in LN (Figure 2H; and Table S7). These results indicate that serum tRF-Ala-AGC-2-M4 has a certain clinical discrimination ability and can be used as a potential diagnostic marker for LN.

Structural prediction showed that tRF-Ala-AGC-2-M4 originates after cleavage at the T-loop of tRNA-Ala-AGC-1, which is of the tRF-3a type (Figure 3A). Meanwhile, the coexpression network of 123 highly expressed target genes of tRF-Ala-AGC-2-M4 was mapped (Figure 3B). Gene Ontology (GO) analysis showed that biological, cellular and molecular functions were enriched in “Response to endoplasmic reticulum stress” (GO0034976), “Focal adhesion” (GO0005925) and “Cell adhesion molecule binding” (GO0050839), respectively (Figure 3C). The LN patient body is in a state of “tension,” while tsRNAs also have a stress-induced production pathway. Kyoto Encyclopedia of Genes and Genomes (KEGG) probing confirmed the pathway assignment in the “Huntington disease” (hsa05016) signalling pathway. More importantly, the wiki pathway was enriched in the “Nephrotic syndrome” (WP4758) signalling pathway. Meanwhile, these 10 highly expressed tsRNAs in LN serum were enriched in the above pathway (Figure S4). LN is a form of glomerulonephritis, and massive autoantibody involvement of the kidney induces LN, which is closely related to secondary nephrotic syndrome.¹⁰ Bioinformatics tools validated that tRF-Ala-AGC-2-M4 can be crucial for the development of LN.

In summary, our study is the first to report the superior diagnostic ability of tRF-Ala-AGC-2-M4 and confirms that tsRNA markers are specifically highly expressed in LN serum and validated by sequencing and RT-qPCR of serum tsRNAs from patients with LN. Further clinical evaluation showed that tRF-Ala-AGC-2-M4 has good diagnostic value for LN and is closely related to the severity of LN. Finally, bioinformatics tools also predicted the involvement of tRF-Ala-AGC-2-M4 in the nephrotic syndrome signalling pathway. These results may provide new insights for accomplishing the goal of early diagnosis of LN and can be of value for making treatment decisions.

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CONFLICT OF INTEREST

All authors affirm no conflict of interest.

Xiaoshan Zhang^{1,2} 

Ping Yang²

Adeel Khan³

Dongjie Xu^{1,2}

Shanshan Chen⁴

Junbin Zhai²

Bin Zhang^{1,2}

Tao Xiong¹

Yanbo Wang⁵

Zhiyang Li²

¹College of Life Science, Yangtze University, Jingzhou, China

²Department of Clinical Laboratory, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

³State Key Laboratory of Bioelectronics, School of Biological Science and Medical Engineering, National Demonstration Center for Experimental Biomedical Engineering Education, Southeast University, Nanjing, China

⁴Department of Rheumatic Immunology, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, China

⁵State Key Laboratory of Pharmaceutical Biotechnology and Department of Physiology, Jiangsu Engineering Research Center for MicroRNA Biology and Biotechnology, School of Life Sciences, NJU Advanced Institute of Life Sciences (NAILS), Nanjing University, Nanjing, China

Correspondence

Tao Xiong, No. 88, Jingmi Road, College of Life Science, Yangtze University, Jingzhou, Hubei 434025, China.

Email: 476223616@qq.com

Yanbo Wang, No. 123, Xianlin Road, School of Life Sciences, Nanjing University, Nanjing 210023, China.

Email: ybwang@nju.edu.cn

Zhiyang Li, No. 321, Zhongshan Road, Department of Clinical Laboratory, The Affiliated Drum Tower Hospital of Nanjing University Medical School, Nanjing, Jiangsu, 210008, China.

Email: lizhiyangcn@qq.com

Xiaoshan Zhang and Ping Yang have contributed equally to this work.

ORCID

Xiaoshan Zhang  <https://orcid.org/0000-0001-8763-2139>

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