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Long non-coding RNA NEAT1 and its targets (microRNA-21 and microRNA-125a) in rheumatoid arthritis: Altered expression and potential to monitor disease activity and treatment outcome

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Abstract

Background: The present study aimed to explore the association of long non-coding RNA nuclear-enriched abundant transcript 1 (lnc-NEAT1) with inflammation, disease activity, treatment outcome, and its targets (microRNA [miR]-21 and miR-125a) in patients with rheumatoid arthritis (RA).

Methods: Peripheral blood mononuclear cells were sampled from 130 RA patients at baseline, week (W) 6, and W12, as well as from 60 healthy controls (HCs) after enrollment. Meanwhile, the expressions of lnc-NEAT1, miR-21, and miR-125a were detected by reverse transcription-quantitative polymerase chain reaction.

Results: lnc-NEAT1 was elevated, but miR-21 and miR-125a were declined in RA patients compared with HCs (all $p < 0.001$); meanwhile, lnc-NEAT1 was negatively correlated with miR-21 and miR-125a (both $p < 0.05$) in RA patients. Besides, elevated lnc-NEAT1 but declined miR-21 and miR-125a were correlated with erythrocyte sedimentation rate (ESR) and C-reactive protein and the 28-joint Disease Activity-ESR score (all $p < 0.05$) in RA patients. Moreover, lnc-NEAT1 was declined from baseline to W12 in RA patients ($p < 0.001$). Additionally, lnc-NEAT1 at W12 was declined in response patients compared with non-response patients ($p = 0.006$), and also decreased in remission patients compared with non-remission patients ($p < 0.001$).

Conclusion: lnc-NEAT1 and its targets (miR-21 and miR-125a) correlate with RA risk and disease activity, and declined lnc-NEAT1 associates with better treatment outcome to some extent in RA patients, suggesting that lnc-NEAT1 might be a potential biomarker to monitor disease activity and treatment outcome in RA.

KEYWORDS

disease activity, lncRNA NEAT1, miR-21 and miR-125a, rheumatoid arthritis, treatment outcome

[Correction added on 12th November 2021, after first online publication: the university name has been changed as 'Wuxi Traditional Chinese Medicine Hospital' in affiliations.]

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1 | INTRODUCTION

Rheumatoid arthritis (RA) is an autoimmune disorder featured by persistent synovitis, systemic inflammation, and volcanic autoantibodies.[1] The morbidity of RA is approximately 0.5%–1.0% in industrialized countries, and it is more frequent in female than in male patients.[2,3] Furthermore, RA can induce irreversible joint destruction and disability, as well as an elevated risk of comorbidities (including cardiovascular disease and severe infection), which causes serious adverse effects on the quality of life among these patients.[1,2,4] Currently, the main treatments of RA include conventional disease-modifying antirheumatic drugs (DMARDs; such as methotrexate and iguratimod), biologicals (eg, tumor necrosis factor inhibitors), and glucocorticoids (including methylprednisolone and prednisolone), while the treatment outcome of a part of RA patients is relatively poor and the recurrence of RA is still frequent.[2,5,6] Thus, the exploration of reliable biomarkers to monitor disease activity and predict treatment outcome is crucial to improve the management of RA.

Long non-coding RNA nuclear paraspeckle assembly transcript 1 (lnc-NEAT1) is viewed to play an important role in regulating inflammation and autoimmunity.[7-12] For instance, it has been presented that lnc-NEAT1 is able to target microRNA (miR)-21 and miR-125a to modulate inflammation[7-9]; additionally, lnc-NEAT1 promotes the proliferation of fibroblast-like synoviocytes cells and production of inflammatory cytokines in RA *via* targeting miR-204-5p[10]; meanwhile, lnc-NEAT1 mediates inflammatory response in inflammatory bowel disease *via* regulating exosome-mediated polarization of macrophages.[11] Furthermore, lnc-NEAT1 is also engaged in autoimmune disease in the clinical field.[12] For example, lnc-NEAT1 expression is dysregulated in patients with systemic lupus erythematosus (SLE) compared to healthy populations.[12] In addition, miR-21 and miR-125a are potential diagnostic biomarkers in RA.[13] Inspired by the above-mentioned studies, we deduced that lnc-NEAT1, miR-21, and miR-125a might play an important role in clinical management of RA, while relevant research is obscure.

Therefore, the present study aimed to investigate the association of lnc-NEAT1, miR-21, and miR-125a with RA risk and activity, then to explore the variation of lnc-NEAT1 during treatment and its relation to patients' outcome in RA.

2 | METHODS

2.1 | Subjects

This study was approved by the Ethics Committee of Wuxi Traditional Chinese Medicine Hospital. A total of 130 patients with active RA who were treated in our hospital between September 2017 and April 2020 were included in this study. Patients were enrolled in the study based on the following criteria: (1) diagnosed as RA in accordance with the 2010 RA classification criteria[14]; (2) aged older than 18 years; (3) had active disease that indicated by a 28-joint Disease Activity Score (DAS) based on erythrocyte sedimentation rate (DAS28-ESR) score more than 3.2; and (4) able to understand the study and volunteer to participate in this study. The patients were ineligible for enrollment if they had any of the following conditions: (1) concomitant with other autoimmune diseases, inflammatory diseases, or hematological diseases; (2) active infections; (3) accompanied by tumors; (4) severe liver and kidney diseases; (5) unable to complete regular follow-up (which was evaluated by investigators); (6) in pregnancy or lactating. In addition to RA patients, meanwhile, 60 healthy subjects with age and gender matched to RA patients, who spontaneously came to the hospital for a medical examination, were also included in this study as healthy controls (HCs). In order to match the age and gender of HCs to the RA patients, the gender ratio of HCs was limited as 4:1 (female vs. male), and the age of HCs was limited within 40–70 years. Besides, eligible HCs were required to have no abnormality reported in the medical examination form and no history of autoimmune diseases or malignancies. All subjects provided the written informed consent.

2.2 | Baseline examination and assessment

The age, gender, body mass index (BMI), and disease duration of RA patients were documented after basic examination. The levels of rheumatoid factor (RF), anti-citrullinated protein antibodies (ACPA), erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP) were recorded after the laboratory test. The tender joint count (TJC), swollen joint count (SJC), Health Assessment Questionnaire Disability Index (HAQ-DI) score, and DAS28-ESR score were also recorded after disease activity assessment.

2.3 | Treatment and evaluation

Depending on the disease condition and personal willingness, some patients chose to receive biologics-based regimen (such as tumor necrosis factor inhibitor or interleukin-6 inhibitor) with or without combination of conventional disease-modifying antirheumatic drug (cDMARD), while some patients chose to receive monotherapy or combination therapy of cDMARDs. The clinical response and remission were evaluated at week 6 (W6) and week 12 (W12) after initiation of treatment. The clinical response was defined as a decline of 1.2 points in DAS28 from the baseline,[15] and the clinical remission was defined as DAS28 < 2.6 points.[14]

2.4 | Sample collection and detection

Whole blood samples of RA patients at baseline and HCs were collected. After collection, the samples were immediately processed by gradient density centrifugation to separate peripheral blood mononuclear cells (PBMCs). The expressions of lnc-NEAT1, miR-21, and

miR-125a in the PBMCs were detected by reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Moreover, to further evaluate the changes of lnc-NEAT1 in RA patients during the treatment, the whole blood samples of RA patients were also collected at W6 and W12 after initiation of treatment, respectively, and the expression of lnc-NEAT1 in the PBMCs of RA patients was determined by RT-qPCR as well.

2.5 | RT-qPCR

In brief, total RNA was extracted by TRIzol™ Reagent (Thermo Fisher Scientific) and reversely transcribed by PrimeScript™ RT Reagent Kit (Perfect Real Time; Takara). Meanwhile, qPCR was performed by QuantiNova SYBR Green PCR Kit (Qiagen), and the conditions for PCR were as follows: PCR initial activation step: 95°C for 2 min; and 2-step cycling: denaturation at 95°C for 5 s and combined annealing/extension at 60°C for 10 s. Furthermore, the relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method; GAPDH was used as internal reference of lnc-NEAT1 calculation, and U6 was used as internal reference of miRNA calculation. The primers in RT-qPCR were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd, which are displayed in Table S1.

2.6 | Statistical analysis

SPSS 26.0 (IBM Corp) and GraphPad Prism 7.01 (GraphPad Software Inc) were respectively applied for data analysis and graph construction. Comparison between two groups was determined by the Wilcoxon sum rank test. Correlation between variables was estimated by Spearman's rank correlation test. Comparison of repeated-measures data was analyzed by Friedman's test for repeated measures. A p value <0.05 indicated statistical significance.

3 | RESULTS

3.1 | Characteristics of RA patients

Among 130 included RA patients, the mean age was 54.5 ± 9.8 years; meanwhile, there were 106 (81.5%) women and 24 (18.5%) men. Furthermore, the median (interquartile range [IQR]) of disease duration was 2.5 (1.1–5.2) years. In addition, there were 105 (80.8%) patients with RF positive and 78 (60.0%) patients with ACPA positive. Besides, the mean value of TJC and SJC was 7.0 ± 3.0 and 5.7 ± 2.9, respectively. Moreover, the median value of ESR and CRP was 31.0 (19.8–46.5) mm/h and 23.3 (11.6–42.8) mg/L, respectively. Additionally, the mean value of DAS28-ESR score and HAQ-DI score was respectively 5.0 ± 0.7 and 1.2 ± 0.3. Regarding current treatment, 29 (22.3%) patients received biologics-based regimen and 101 (77.7%) patients received cDMARDs (Table 1).

TABLE 1 Characteristics of RA patients

Items	RA patients (N = 130)
Age (years), mean ± SD	54.5 ± 9.8
Gender, No. (%)	
Female	106 (81.5)
Male	24 (18.5)
BMI (kg/m^2), mean ± SD	22.7 ± 3.0
Disease duration (years), median (IQR)	2.5 (1.1–5.2)
RF positive, No. (%)	105 (80.8)
ACPA positive, No. (%)	78 (60.0)
Tender joint count, mean ± SD	7.0 ± 3.0
Swollen joint count, mean ± SD	5.7 ± 2.9
ESR (mm/h), median (IQR)	31.0 (19.8–46.5)
CRP (mg/L), median (IQR)	23.3 (11.6–42.8)
DAS28-ESR score, mean ± SD	5.0 ± 0.7
HAQ-DI score, mean ± SD	1.2 ± 0.3
Current treatment, No. (%)	
Biologics-based regimen	29 (22.3)
cDMARD (monotherapy or combination)	101 (77.7)

Abbreviations: ACPA, anti-citrullinated protein autoantibody; BMI, body mass index; cDMARD, conventional disease-modifying antirheumatic drug; CRP, C-reactive protein; DAS28, 28-joint Disease Activity; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire Disability Index; IQR, interquartile range; RA, rheumatoid arthritis; RF, rheumatoid factor; SD, standard deviation.

3.2 | Comparison of lnc-NEAT1, miR-21, and miR-125a between RA patients and HCs

lnc-NEAT1 was elevated in RA patients (median [IQR]: 2.847 [1.764–3.460]) compared with HCs (median [IQR]: 0.997 [0.700–1.474]; $p < 0.001$; Figure 1A). Furthermore, miR-21 was declined in RA patients (median [IQR]: 0.487 [0.334–0.801]) compared with HCs (median [IQR]: 0.993 [0.689–1.163]; $p < 0.001$; Figure 1B). Moreover, miR-125a was also decreased in RA patients (median [IQR]: 0.384 [0.258–0.670]) compared with HCs (median [IQR]: 0.991 [0.707–1.408]; $p < 0.001$; Figure 1C). Meanwhile, negative correlation was found in lnc-NEAT1 with miR-21 ($r = -0.239$, $p = 0.006$; Figure 2A) and miR-125a ($r = -0.401$, $p < 0.001$) in RA patients (Figure 2B).

3.3 | Correlation of lnc-NEAT1, miR-21, and miR-125a with clinical characteristics in RA patients

lnc-NEAT1 was positively correlated with TJC ($r = 0.231$, $p = 0.008$), ESR ($r = 0.270$, $p = 0.002$), CRP ($r = 0.309$, $p < 0.001$), and DAS28-ESR score ($r = 0.307$, $p < 0.001$). Moreover, miR-21 was negatively correlated with disease duration ($r = -0.185$, $p = 0.035$), ESR ($r = -0.203$, $p = 0.020$), CRP ($r = -0.191$, $p = 0.030$), and DAS28-ESR score ($r = -0.229$, $p = 0.009$). Furthermore, miR-125a was negatively correlated with TJC ($r = -0.233$, $p = 0.008$), SJC ($r = -0.237$,

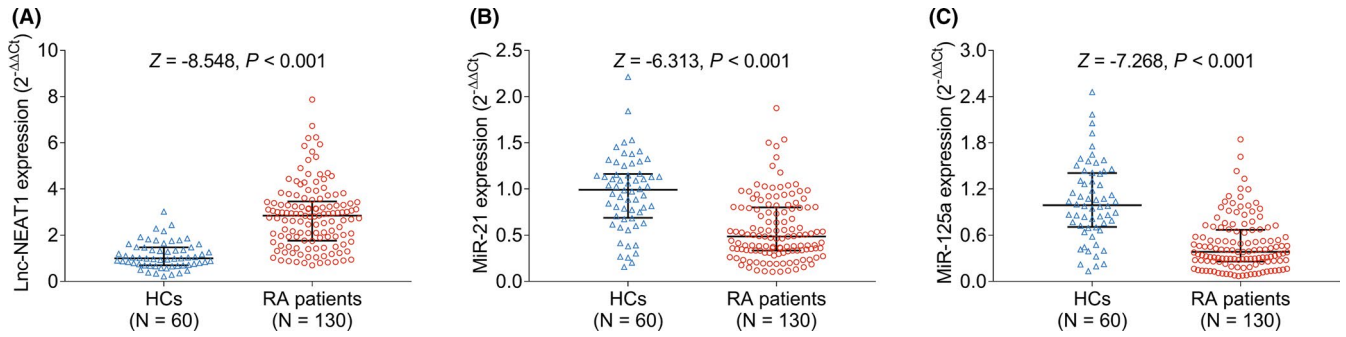

FIGURE 1 lnc-NEAT1, miR-21, and miR-125a in RA patients and HCs. Comparison of lnc-NEAT1 (A), miR-21 (B), and miR-125a (C) between RA patients and HCs. RA, rheumatoid arthritis; lnc-NEAT1, long non-coding RNA nuclear-enriched abundant transcript 1; miR, microRNA; and HCs, healthy controls

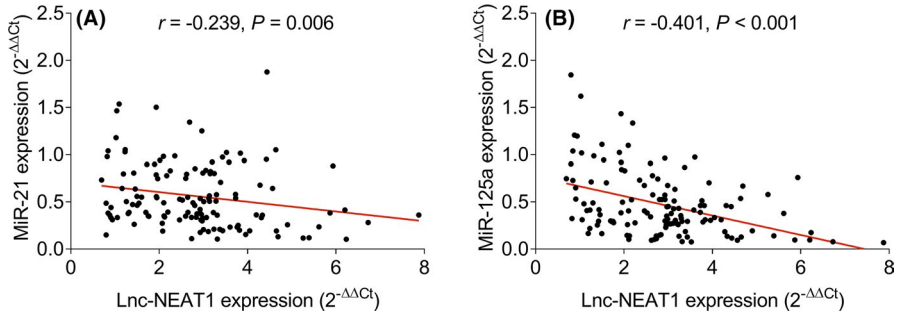

FIGURE 2 Association of lnc-NEAT1 with miR-21 and miR-125a. Correlation of lnc-NEAT1 with miR-21 (A) and miR-125a (B) in RA patients. lnc-NEAT1, long non-coding RNA nuclear-enriched abundant transcript 1; miR, microRNA; and RA, rheumatoid arthritis

TABLE 2 Correlation of lnc-NEAT1, miR-21, and miR-125a with clinical characteristics in RA patients

	lnc-NEAT1		MiR–21		MiR–125a	
Items	***r***	***p* value**	***r***	***p* value**	***r***	***p* value**
Disease duration	0.146	0.098	−0.185	0.035	0.070	0.428
Tender joint count	0.231	0.008	−0.136	0.123	−0.233	0.008
Swollen joint count	0.153	0.081	−0.086	0.329	−0.237	0.007
ESR	0.270	0.002	−0.203	0.020	−0.225	0.010
CRP	0.309	<0.001	−0.191	0.030	−0.250	0.004
DAS28-ESR score	0.307	<0.001	−0.229	0.009	−0.320	<0.001
HAQ-DI score	0.122	0.165	−0.049	0.582	−0.126	0.152

Abbreviations: CRP, C-reactive protein; DAS28, the 28-joint Disease Activity; ESR, erythrocyte sedimentation rate; HAQ-DI, the Health Assessment Questionnaire Disability Index; lnc-NEAT1, long non-coding RNA nuclear-enriched abundant transcript 1; miR, microRNA; RA, rheumatoid arthritis.

$p = 0.007$), ESR ($r = -0.225$, $p = 0.010$), CRP ($r = -0.250$, $p = 0.004$), and DAS28-ESR score ($r = -0.320$, $p < 0.001$; Table 2).

3.4 | Change of lnc-NEAT1 during treatment in RA patients

In order to explore the longitudinal change of lnc-NEAT1 in RA patients during treatment, the expression of lnc-NEAT1 was evaluated at baseline, W6, and W12. Furthermore, it was found that lnc-NEAT1 was declined with time in patients during treatment ($p < 0.001$; Figure 3). Furthermore, lnc-NEAT1 was declined from baseline to W12 in patients with biologics-based regimen and cDMARD (both $p < 0.001$; Figure S1).

3.5 | Comparison of lnc-NEAT, miR-21, and miR-125a in RA patients with different treatment outcomes

In RA patients, the clinical response rate was 0.0%, 16.2%, and 40.0% at baseline, W6, and W12, respectively (Figure 4A); meanwhile, the

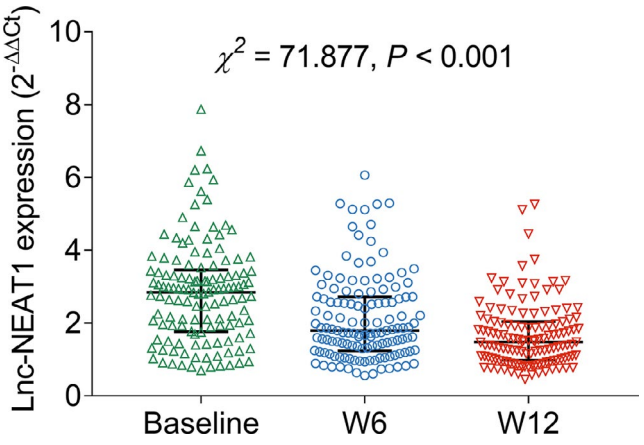

FIGURE 3 Longitudinal change of lnc-NEAT1 during RA treatment. Changes of lnc-NEAT1 at baseline, W6, and W12. lnc-NEAT1, long non-coding RNA nuclear-enriched abundant transcript 1; W, week; and RA, rheumatoid arthritis

clinical remission rate was 0.0%, 3.1%, and 13.1% at baseline, W6, and W12, respectively (Figure 4B). Furthermore, lnc-NEAT1 was declined with time in both response patients (n = 52) and non-response patients (n = 78; both $p < 0.001$; Figure 4C, D). Moreover, lnc-NEAT1 was also decreased with time in remission patients (n = 17) and non-remission patients (n = 113; both $p < 0.001$; Figure 4E, F). In addition, lnc-NEAT1 at W12 was declined in response patients compared with non-response patients ($p = 0.006$), but lnc-NEAT1 at baseline ($p = 0.388$) and W6 ($p = 0.430$) showed no difference between these two-group patients (Figure 4G-I). Meanwhile, lnc-NEAT1 at W12 was also decreased in remission patients compared with non-remission patients ($p < 0.001$), but lnc-NEAT1 at baseline ($p = 0.403$) and W6 ($p = 0.061$) showed no difference between these two-group patients (Figure 4J-L). In addition, only miR-125a at baseline was declined in response patients compared with non-response patients at W12 ($p = 0.030$; Figure S2A-D).

Furthermore, higher lnc-NEAT1 at W12 was independently correlated with poor clinical response at W12 ($p = 0.017$, OR = 0.381) and clinical remission at W12 ($p = 0.004$, OR = 0.002; Tables S2 and S3).

4 | DISCUSSION

In the present study, we found a variety of interesting findings: (1) lnc-NEAT1 was elevated, while miR-21 and miR-125a were declined in RA patients compared with HCs; furthermore, lnc-NEAT1 was negatively correlated with miR-21 and miR-125a in RA patients; (2) elevated lnc-NEAT1 but declined miR-21 and miR-125a were correlated with inflammation and disease activity in RA patients; and (3) lnc-NEAT1 was gradually declined with time during treatment; meanwhile, decreased lnc-NEAT1 was correlated with better treatment outcome to some extent in RA patients.

In the clinical perspective, lnc-NEAT1, miR-21, and miR-125a might serve as potential diagnostic biomarkers in autoimmune disorders.[12,16,17] For instance, lnc-NEAT1 is abnormally elevated in SLE patients compared with healthy volunteers[12]; furthermore, miR-21 is downregulated in ulcerative colitis patients relative to normal populations[16]; moreover, it has been presented that miR-125a is declined in patients with Graves' disease compared with HCs.[17] In our study, we found that lnc-NEAT1 was elevated, while miR-21 and miR-125a were declined in RA patients compared with HCs. The possible explanations might be that: (a) lnc-NEAT1 could accelerate the occurrence of RA through modulating the miR-23a/murine double minute-2 (MDM2)/sirtuin 6 axis[18]; (b) decreased miR-21 could accelerate immune response *via* several signaling pathways, such as phosphate and tension homology deleted on chromosome 10 and phosphatidylinositol 3 kinase pathways, which would be involved in the pathogenesis of RA[19]; (c) declined miR-125a might be able to promote the secretion of inflammatory cytokines (such as IL-6 and IL-1β) and increase immune response through elevating nuclear factor kappa-B (NF-κB) pathway, which could lead to the occurrence of RA.[20] Taken together, lnc-NEAT1 was increased, while miR-21 and miR-125a were decreased in RA patients compared with HCs. Furthermore, we also found that lnc-NEAT1 was negatively correlated with miR-21 and miR-125a, which might be explained by that lnc-NEAT1 could target miR-21 and miR-125a in immunological disease (such as allergic rhinitis), the similar targeting manner might also exist in RA.[7]

A previous study has illustrated that lnc-NEAT1 is positively correlated with disease activity in SLE patients[21]; furthermore, another study shows that miR-125a negatively correlates with disease severity in patients with Crohn's disease.[22] However, the correlation of lnc-NEAT1 with miR-21 and miR-125a, as well as their correlation with clinical characteristics in RA, is rarely reported. Thus, we conducted the present study and found that in RA patients, increased lnc-NEAT1 but declined miR-21 and miR-125a were correlated with inflammation and disease activity in RA patients. The possible reasons might be that: (a) elevated lnc-NEAT1 and decreased miR-21 and miR-125a could promote inflammatory cytokines and immune response, which consequently resulted in elevated inflammation in RA patients[7,19,20,23]; and (b) increased lnc-NEAT1 and declined miR-21 and miR-125a might inhibit new bone formation and accelerate bone degradation through regulating several signaling pathways (such as Janus kinase 2/signal transducer, activator of transcription-3, and E26 transformation-specific-1 pathways), which could lead to high disease activity in RA patients.[24-26] Therefore, elevated lnc-NEAT1 but declined miR-21 and miR-125a were correlated with inflammation and disease activity in RA.

Several studies have illustrated that lnc-NEAT1 is dysregulated in autoimmune diseases, such as Sjögren's syndrome, SLE, RA, and multiple sclerosis.[12,18,27,28] However, few studies illustrate a longitudinal change of lnc-NEAT1 during treatment and its correlation with outcome in RA patients. Thus, we evaluated the expression of lnc-NEAT1 at distinct time points in RA patients with different treatment outcomes. Surprisingly, we discovered that: (1) lnc-NEAT1 was declined with time in RA patients during treatments, which could be explained by that biologics-based regimen,

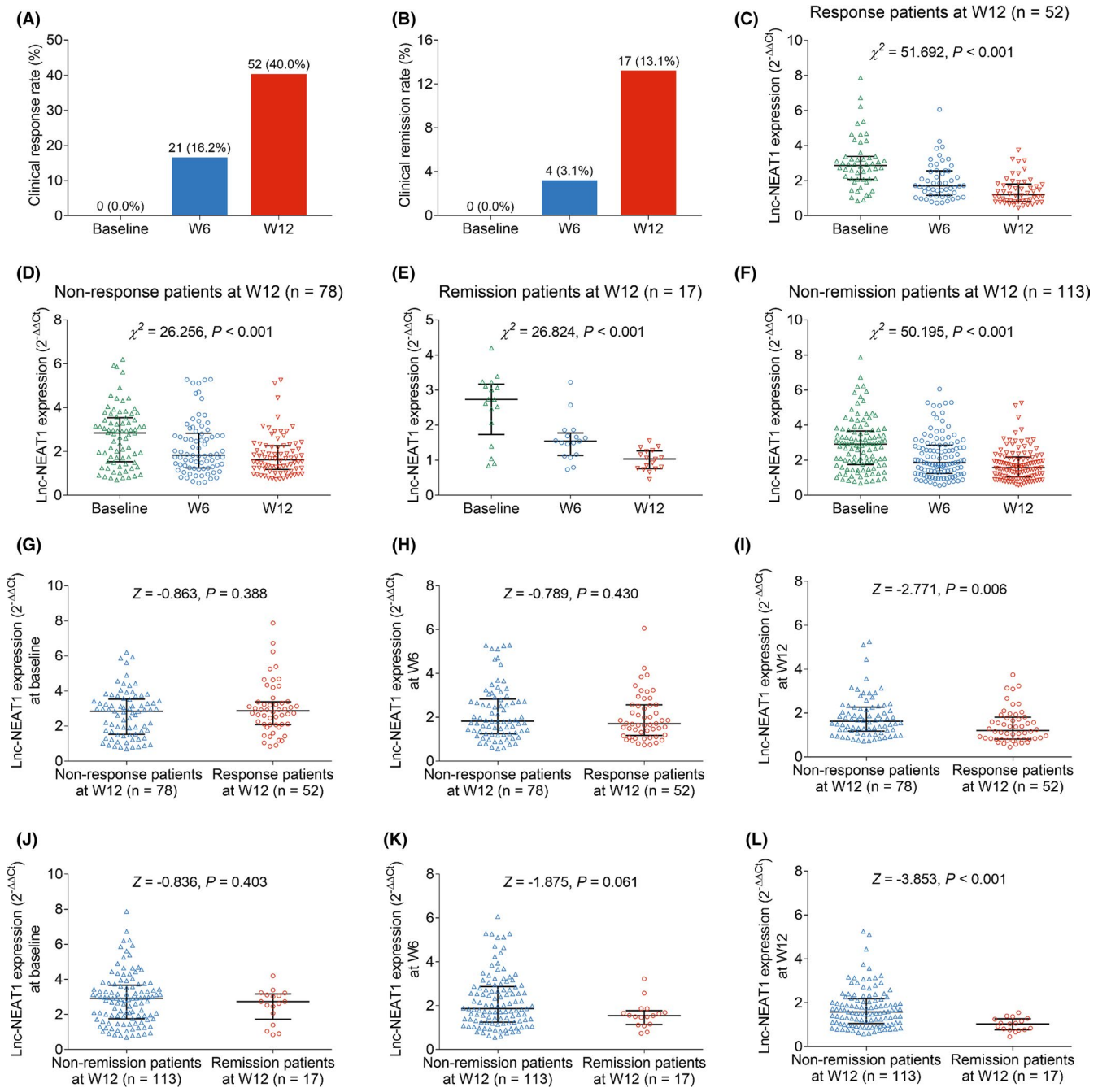

FIGURE 4 Correlation of lnc-NEAT1 with treatment outcome in RA patients. Clinical response rate (A) and clinical remission rate (B) in RA patients during treatment; lnc-NEAT1 at different time points in response patients (C), non-response patients (D), remission patients (E), and non-remission patients (F); comparison of lnc-NEAT1 at baseline (G), W6 (H), and W12 (I) between response patients and non-response patients; comparison of lnc-NEAT1 at baseline (J), W6 (K), and W12 (L) between remission patients and non-remission patients. RA, rheumatoid arthritis; lnc-NEAT1, long non-coding RNA nuclear-enriched abundant transcript 1; and W, week

and cDMARDs could decrease inflammation in RA patients[29]; meanwhile, lnc-NEAT1 could positively regulate inflammation in RA *via* targeting miR-204-5p through NF-κB pathway,[4,10] indicating lnc-NEAT1 might be a potential biomarker to reflect inflammation in RA. Thus, lnc-NEAT1 was decreased with time in RA patients during treatment; and (2) declined lncRNA NEAT1 was more noticeable in response patients (*vs*. non-response patients) and remission patients (*vs*. non-remission patients), the possible reason might be that patients who achieved clinical response and remission indicated a relative lower inflammation and disease activity compared with non-response and non-remission patients; meanwhile, lnc-NEAT1 positively regulated inflammation and its declined expression correlated with inflammation and disease activity (above-mentioned).[10,23] Thus, decreased lnc-NEAT1 was correlated with better treatment outcomes to some extent in RA patients.

There were several limitations in the present study: (1) the precise mechanism of lnc-NEAT1 through targeting miR-21 and miR-125a in the pathogenesis and progression of RA could be explored in the further study; (2) the follow-up period was relatively short; hence, the longer-term longitudinal change of lnc-NEAT1 and its association with treatment outcome in RA patients could be completed in the future; (3) the enrolled patients were from a single center, which might result in less generalizability of our observations; (4) change of HAQ-DI score could be explored in the further study; and (5) the change of miR-21 and miR-125a after therapy in patients treated by biologics-based regimen or cDMARD could be conducted in the future.

In conclusion, lnc-NEAT1 is elevated while its targets (miR-21 and miR-125a) are decreased in RA patients, and they are all correlated with inflammation and disease activity; meanwhile, declined lnc-NEAT1 is correlated with better treatment outcome to some extent in RA patients, suggesting that lnc-NEAT1 might be a potential biomarker to monitor disease activity and treatment outcome in RA.

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CONFLICT OF INTEREST

The authors declared that there was no conflict of interest.

DATA AVAILABILITY STATEMENT

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

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