Abstract. The human transcriptome is primarily composed of long non-coding RNAs (lncRNAs), which are key regulatory molecules of multiple biological processes. In the present study, the expression profiles of lncRNAs in the peripheral blood and CD4+ T cells of patients with active rheumatoid arthritis (RA) were determined. Based on the expression profiles, 493 lncRNAs and 374 mRNAs were identified to be differentially expressed in the peripheral blood of active RA patients and healthy donors. Further verification of lncRNAs was performed using reverse transcription -quantitative (RT-q) PCR analysis of peripheral blood from 5 healthy donors and 5 patients with active RA and 14 additional differentially expressed genes were identified. CD4+ T cells in peripheral blood from 12 patients with active RA and 8 healthy donors were isolated using magnetic beads and qPCR was used to assess differentially expressed lncRNAs. The results suggested that 7 lncRNAs were upregulated and 2 were downregulated. The results indicated that these 9 lncRNAs may be involved in the pathogenesis of RA. An increased ratio of Th17: T-regulatory (Treg) cells was also observed. It may be hypothesized that lncRNAs serve important roles in the differentiation of CD4+ T cells. Receiver operating characteristic curve analysis suggested that these 9 lncRNAs are of potential clinical diagnostic value for RA. Pearson correlation analysis indicated that the correlation coefficient between Ensembl transcript (ENST)00000569543 and complement C4 was 0.623 (P<0.05), and that between ENST000000420096 and anti-cyclic citrullinated peptide antibody or disease activity evaluation score, the correlation coefficient was 0.662 and 0.605, respectively (P<0.05 for each). In conclusion, the results of the present study suggest a possible role of lncRNAs in the differentiation of CD4+ T cells and the pathogenesis of RA, as well as the potential value as diagnostic biomarkers for active RA.

Introduction

Rheumatoid arthritis (RA) is a multi-systemic inflammatory autoimmune disease that causes joint swelling, pain, loss of function and stiffness in affected appendages, and significantly impacts the health and quality of life of patients. The worldwide prevalence of RA is ~0.24% and it is 2-3 times higher in females compared with that in males (1-3). Although the pathogenesis remains to be fully elucidated, it is well known that inflammatory response-mediated immune dysfunction is a key factor involved in the pathogenesis (4,5).

High-throughput sequencing is a comprehensive analysis platform for studying differentially expressed genes in multiple diseases (6,7). High-throughput sequencing studies have indicated that various lncRNAs may be associated with a variety of autoimmune diseases, including RA, systemic lupus erythematosus, Sjogren's syndrome and multiple sclerosis (8-12). LncRNAs are also hypothesized to be involved in the differentiation and activation of CD4+ T cells, which are aberrantly activated adaptive immune cells in multiple autoimmune diseases (13-15). Dysregulated differentiation of CD4+ T cells alter the balance of interactions between the CD4+ T-cell subgroups and may be an important factor leading
to the development of RA disease (16). As such, it may be possible to identify potential therapeutic targets by analyzing the differential expression of IncRNAs. In the present study, the IncRNA expression profiles in the peripheral blood and CD4+ T cells of healthy donors were compared with those of patients with active RA using high-throughput sequencing. Further verification was performed using quantitative (q)PCR and the results supported the potential involvement of specific IncRNA in CD4+ T-cell differentiation during the development of RA. These results provide novel insight into the immunological mechanisms underlying the development of RA.

Materials and methods

**Patients and specimens.** A total of 19 patients with RA (16 females and 3 males; mean age, 53 years) who visited the Department of Rheumatology and Immunology of Jiangsu Province Hospital of Chinese Medicine (Nanjing, China) between March 2016 and June 2016 were recruited. Patients were recruited if they met the criteria for the diagnosis of RA set out by the American College of Rheumatology in 1987 (17). The patients recruited were outpatients or hospitalized patients. Peripheral blood was collected and first used to detect RA-related indicators prior to administration of immunosuppressive therapy. Erythroid sedimentation rate (ESR) was measured using the Westergren method. An ESR >20 mm/h was considered positive (18). Rheumatoid factor (RF) and C-reactive protein (CRP) were measured by immunonephelometry, RF >15 U/ml and CRP >8 mg/l were considered positive (19,20). Anti-cyclic citrullinated peptide (anti-CCP) antibody levels were measured by chemiluminescence microparticle immunoassay, anti-CCP titers <5.0 RU/ml were judged as negative, and ≥5.0 RU/ml as positive (21). The Disease Activity Score 28 (DAS28) were then used to assess RA disease activity. For each patient in the validation cohort, the disease severity was assessed according to the DAS28 (22). A total of 10 healthy donors (8 females and 2 males; mean age, 50 years) with no history of autoimmune diseases or administration of immunosuppressive therapy were used as the control group. The present study was approved by the Institutional Review Board (IRB) of the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (Nanjing, China; affiliated to Nanjing University of Chinese Medicine; approval no. 2016NL-KS14).

**RNA extraction.** Total RNA from peripheral blood was extracted using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. Subsequently, total RNA was evaluated by 1% agarose gel electrophoresis and observation under ultraviolet transmitted light, quantified using a NanoDrop ND-1000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.).

**High-throughput sequencing analysis.** High-throughput sequencing was performed using an Arraystar v.3.0 (Arraystar, Inc.). Genome-wide analysis of IncRNA and mRNA expression profiles in peripheral blood samples from two patients with RA and two healthy donors was performed using Arraystar Human IncRNA Microarray version 3.0. In brief, RNA extraction and evaluation were performed as described above. Sample labeling (Labeling Kit Agilent p n 5190-0442; Agilent Technologies, Inc.) and array hybridization was performed according to the Agilent One-Color Microarray-Based Gene Expression Analysis protocol (Agilent Technologies, Inc.). Specific experimental process: mRNA was purified from total RNA using a mRNA isolation kit after the removal of ribosomal RNA (mRNA-ONLY™ Eukaryotic mRNA Isolation kit; Epicentre; Illumina, Inc.). Subsequently, each sample was amplified and transcribed into fluorescent cRNA (complementary RNA) using a mixture of oligo (dt) and random primers (Arraystar Flash RNA Labeling kit; Arraystar, Inc.). The labeled cRNAs were purified using an RNeasy Mini kit (Qiagen). The concentration and specific activity of the labeled cRNAs (pmol cyanine 3/µg cRNA) were measured using a NanoDrop-1000 (Thermo Fisher Scientific, Inc.). A total of 1 µg of each labeled cRNA was fragmented by adding 5 µl 10X Blocking Agent (Agilent Technologies, Inc.) and 1 µl of 25X Fragmentation Buffer. The mixture was heated at 60°C for 30 min, after which 25 µl 2x GE Hybridization buffer (Agilent Technologies, Inc.) was added to dilute the labeled cRNA. A total of 50 µl of hybridization solution was dispensed into a gasket slide and assembled to obtain the IncRNA microarray slide. The slides were incubated at 65°C in an Agilent Hybridization oven for 17 h. The hybridized arrays were washed, fixed and scanned with the Agilent DNA Microarray Scanner (cat. no. G2505C; Agilent Technologies, Inc.).

**CD4+ T-cell isolation and total RNA extraction.** CD4+ T cells in the peripheral blood of each participant were separated by gradient centrifugation at 400 x g for 30 min at 4°C and enriched using magnetic beads. In brief, an equivalent volume of PBS was added to the peripheral blood and the sample was agitated until it was evenly distributed. Subsequently, the mixture was added to the lymphocyte separation solution, followed by centrifugation at 400 x g for 30 min at 4°C to obtain peripheral blood mononuclear cells. CD4+ T cells were isolated using a BD IMag™ cell separation protocol using the following reagents: Anti-human CD4 particles-dm CD (cat. no. 557767) and IMag buffer (cat. no. 552362; both from BD Biosciences). Separated CD4+ T cells were frozen in pre-chilled RNase-free vials for 5 min in liquid nitrogen and stored at -78°C prior to RNA extraction.

**Reverse transcription (RT)-qPCR.** Of the differentially expressed IncRNAs, 21 were selected (Table SI) according to the following criteria: i) ≥10-fold up- or downregulation in expression; ii) the sequence of the IncRNA was in the database (LncRNA Disease; http://cmbi.bjmu.edu.cn/lncrnadisease) and was closely associated with RA. For RT reactions, SuperScript™ III Reverse Transcriptase (Invitrogen; Thermo Fisher Scientific, Inc.) was used. qPCR was performed using specific primers, the sequences of which are provided in Table SII. The thermocycling conditions were as follows: PCR assay was set at an initial denaturation step at 95°C for 10 min, followed by 40 cycles of 95°C for 10 sec and 60°C for 1 min. The ViiA 7 Real-time PCR System (Applied Biosystems; Thermo Fisher Scientific, Inc.) was used for PCR amplification. PCR amplification was performed in triplicate. The results were quantified using the 2^-ΔΔCq method (23).

**Flow cytometry and ELISA.** Samples were washed with PBS and fixed with immobilization reagent (BD Biosciences). Type
17 T-helper (Th17) cells were stained with allophycocyanine mouse anti-human CD4 (cat. no. 561840; BD Biosciences) for 20 min at 37°C, phycoerythrin (PE) mouse anti-human interleukin (IL)-17A (cat. no. 580487; BD Biosciences) for 30 min at 4°C, PerCP-Cy™ 5.5 mouse anti-human CD25 (cat. no. 580503; BD Biosciences) for 20 min at 4°C, PE mouse anti-human CD127 (cat. no. 561028; BD Biosciences) for for 20 min at 4°C. A total of 1x10^6 of cells were resuspended in 1% BSA-PBS and the volume of fluorescent antibodies added to cells were as listed below: CD4: 1 µl; IL-17A: 2 µl; CD25: 2 µl and CD127: 2 µl. After incubation with the antibodies, cells were analyzed using flow cytometry on a BD FACSCalibur (BD Biosciences).

ELISA kits (cat. nos. m1037279 and m1028599; Shanghai Enzyme Link) were used to detect the levels of IL-17 and transforming growth factor (TGF)-β in human serum (1:5 dilution), according to the manufacturer's instructions.

**Volcano plots and hierarchical cluster analyses.** Microarray data were log-transformed and subjected to quantile normalization. After elimination of unreliable transcripts, the remaining data of IncRNA and mRNA expression were further analyzed. The transcripts were distributed according to statistical significance (y-axis) and magnitude of change (log2 ratio of RA group/normal control group; x-axis). Volcano plots were used for visualizing genes that were differentially expressed between the two groups. Hierarchical cluster analysis was performed to display RNA expression profiles between different samples.

**Bioinformatics analysis.** Kyoto Encyclopedia of Genes and Genomes (KEGG; www.genome.ad.jp/kegg) database and Gene Ontology (GO; www.geneontology.org) analysis were used to explore the potential role of differentially expressed mRNA in biological pathways, including biological process, cellular component and molecular function.

**Co-expression network.** Co-expression analysis was performed based on the Pearson correlation coefficient between the coding and non-coding gene transcripts. Pearson correlation coefficient ≥0.90, P<0.01 and false discovery rate <0.01 were used as thresholds and a coding-non-coding network was plotted using Cytoscape version 2.8.2 (http://cytoscape.org).

**Statistical analysis.** Data were analyzed using GraphPad Prism version 5.0 (GraphPad Software, Inc.). A non-parametric Mann-Whitney U-test was used to compare gene expression between two groups. Spearman rank correlation analyses were performed and coefficients were determined to assess the correlation between expression levels of IncRNAs and clinical features. P<0.05 (bilateral) was considered to indicate a statistically significant difference.

**Results**

Differentially expressed IncRNAs and mRNAs in the peripheral blood of patients with RA. Aberrantly expressed mRNAs and IncRNAs associated with RA were identified based on the following criteria: ≥2-fold upregulation or <2-fold downregulation in expression and P<0.05. To visualize the differentially expressed IncRNAs and mRNAs, scatter plots (Fig. 1A and B) and volcano plots (Fig. 1C and D) were analyzed to further explore the differences in peripheral blood between patients with active RA and normal people. A total of 493 differentially expressed IncRNAs and 374 mRNAs were identified in the peripheral blood of patients with RA, of which 275 IncRNAs and 193 mRNAs were upregulated and the remaining ones were downregulated. The results of hierarchical clustering analysis showed distinguishable IncRNA and mRNA expression profiles between patients with active RA and normal people. (Fig. 1E and F).

**Classification of IncRNAs.** Among the 275 significantly upregulated IncRNAs, 116 were associated with intergenic interaction, 53 were natural antisense, 39 were intronic antisense, 36 were exon sense overlapping, 12 were bidirectional and 12 were other types of interactions. Of the 208 significantly downregulated IncRNAs, 108 of these were associated with intergenic interactions, 26 were natural antisense, 21 were intronic antisense, 45 were exon sense overlapping, 4 were bidirectional and 14 were other types of interactions (Fig. 1G and H).

**qPCR validation of IncRNA in peripheral blood.** Further identification was performed with qPCR on peripheral blood plasma of 5 patients and 5 healthy individuals. Based on these results, 7 of the 11 upregulated IncRNAs and 7 of the 10 downregulated IncRNAs obtained by chip were confirmed as differentially expressed (Fig. 2).

**Pathway and GO analyses.** As IncRNAs are non-coding RNAs that are not translated and the majority of them are structurally similar to mRNA, their function may be reflected in the associated mRNAs. Pathway and GO enrichment analysis of differentially expressed mRNAs further demonstrated the regulatory role of IncRNAs (Fig. 3). Pathway analysis indicated that differentially expressed mRNAs in peripheral blood from patients with RA were associated with multiple signaling pathways, including mitogen-activated protein kinase (MAPK) and P53 (Fig. 3G and H). These mRNAs were further characterized as being involved in the regulation of processes associated with cell growth and immune regulation (Fig. 3A-F). Of note, the MAPK and P53 signaling pathways are involved in the differentiation of CD4+ T cells, which serves an important role in the pathogenesis of RA.
qPCR validation of lncRNA in CD4⁺ T cells. To further investigate the alternative expression of lncRNAs in CD4⁺ T cells from patients with RA, peripheral blood was collected from 12 patients with RA and 8 healthy individuals. CD4⁺ T cells were isolated with magnetic beads. Based on the qPCR results, one of the less differentially expressed lncRNAs were excluded for analysis in CD4⁺ T cells and finally, a total of 13 lncRNAs (those previously identified in plasma) were validated. The results suggested that the differential expression of 7 of the lncRNAs upregulated in plasma was consistent with that observed in CD4⁺ T lymphocytes. Furthermore, two of the lncRNAs downregulated in plasma were also observed to be downregulated in CD4⁺ T lymphocytes (Fig. 4) differentiation of CD4⁺ T cells in patients with RA. Differentiation of CD4⁺
Figure 3. Enrichment analysis of GO terms and pathways of the differentially expressed mRNAs. (A) Top 10 GO terms in the categories CC for upregulated mRNA; (B) Top 10 GO terms in the categories CC for downregulated mRNA; (C) Top 10 GO terms in the categories BP for upregulated mRNA; (D) Top 10 GO terms in the categories BP for downregulated mRNA; (E) Top 10 GO terms in the categories MF for upregulated mRNA; (F) Top 10 GO terms in the categories MF for downregulated mRNA. (G and H) Top 10 pathways that exhibited significant differences between the patients with RA and healthy controls. From left to right, the upregulated and downregulated coding genes are displayed. Sig, significant; DE, differentially expressed; GO, gene ontology; CC, cellular component; MF, molecular function; BP, biological process; RA, rheumatoid arthritis; MAPK, mitogen-activated protein kinase.
T cells from patients with RA was observed. The ratio of Th17/T-regulatory (Treg) cells was upregulated in patients with active RA by flow cytometry (Fig. 5A and B). IL-17 and TGF-β are important cytokines that are considered as regulatory sensors in RA (24,25). The results also suggested an increase in the levels of IL-17 and a decrease in the release of TGF-β in patients with RA, although there was no significant difference in TGF-β compared to the normal control group (Fig. 5C; Table I), demonstrating a difference in the differentiation of CD4+ T cells.

Construction of the coding-non-coding gene co-expression networks. Functional prediction of lncRNAs was performed based on the function of the co-expressed mRNAs. A co-expression network for patients with RA was constructed based on the correlation analysis between differentially expressed lncRNAs and mRNAs. A total of 9 central lncRNAs were selected for the co-expression networks (uc021xin.1, ENST00000420096, ENST00000563752, ENST00000572491, ENST000000569543, ENST000000563752,ENST00000572491,ENST00000563752,ENST00000569543,ENST000000570118), which were verified using qPCR (Fig. 6). The results indicated that C-C motif chemokine ligand (CCL)19, CD74 and adaptor-related protein complex 3 subunit δ1 were correlated with lncRNAs detected in the network, which were also closely associated with T-cell differentiation. RT-qPCR further validated CCL19 as an upregulated signaling factor in patients with RA (Fig. 7).

Diagnostic value of lncRNA for RA. ROC curves were used to assess the potential diagnostic value of these 9 lncRNAs in RA. The areas under the ROC curves of ENST00000420096, ENST00000563752, ENST00000572491, ENST000000569543, ENST000000570118, NR_039985, NR_038238, NR_027148, NR_039985 and uc021xin.1 were 0.9792, 0.9479, 0.9896, 1.0000, 0.9167, 0.8750, 0.8333, 0.8958 and 0.8438, respectively (Table II, Fig. 8).

Correlation analysis between lncRNA expression and clinical indicators of RA. ESR, CRP, RF and CCP antibodies are conventional indicators for clinical diagnosis of RA (26). DAS28 is an internationally recognized method for determining RA activity. Complement 3 (C3) and C4 are involved in RA autoimmune disorders and inflammatory pathological
processes (27). Pearson correlation analysis was used to analyze the correlation between target lncRNAs and ESR, CRP, RF, anti-CCP antibody and DAS28. The correlation coefficient between ENST00000569543 and C4 was 0.623 (P<0.05) and the correlation coefficient between ENST00000420096 and anti-CCP antibody or the DAS 28 score was 0.662 and 0.605, respectively (P<0.05; Table III).

Discussion

With the development of high-throughput gene sequencing technology, numerous lncRNAs have been identified and demonstrated to exert biological functions through interactions with other cellular macromolecules (28). LncRNAs are expressed in various types of immune cells and participate in the differentiation and activation of these cells (29). RA is an autoimmune disorder, with CD4+ T cells differentiating into various Th-cell subsets that are thought to be deeply involved in the pathogenesis of RA (30). However, only few studies have assessed lncRNAs associated with CD4+ T-cell differentiation in peripheral blood of patients with active RA (8,31). In the present study, the expression of lncRNAs in CD4+ T cells from peripheral blood of patients with active RA was assessed to provide novel insight into the pathogenesis of RA.
Based on the microarray data, 493 lncRNAs and 374 differentially expressed mRNAs were identified. LncRNA functional annotations are still not fully available; however, the majority of the mRNAs identified are well annotated. Pathway and GO enrichment analysis of significantly differentially expressed mRNAs revealed that these mRNAs in the peripheral blood of patients with RA were primarily involved in the regulation of processes associated with cell growth and immune regulation. Further analysis demonstrated that these differentially expressed mRNAs were associated with the...
The present study was supported by the National Natural Science Foundation of China (grant nos. 81573869 and 81673937).

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on
reasonable request. All of the data from the microarray trials are stored in the Gene Expression Omnibus database (dataset no. GSE134087, https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE134087).

Authors’ contributions
XZ and LZ conceived and designed the study. ML, KM and JW performed the experiments. ML and ZF analyzed and interpreted the experimental data. ML and KM wrote the manuscript. XZ, LZ and ZF reviewed and edited the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate
All patients signed informed consent forms and the study was approved by the IRB of the Affiliated Hospital of Nanjing University of Traditional Chinese Medicine (Nanjing, China; no. 2016NL-KS14).

Patient consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

References
1. Smolen JS, Aletaha D and McInnes IB: Rheumatoid arthritis. Lancet 388: 2023-2038, 2016.
2. Scott DL, Wolfe F and Huizinga TW: Rheumatoid arthritis. Lancet 376: 1094-1108, 2010.
3. Davis JM 3rd: Rheumatoid arthritis: A severe disease that preventive approaches would greatly benefit. Clin Ther 41: 1240-1245, 2019.
4. Derksen VFAM, Huizinga TWJ and van der Woude D: The role of autoantibodies in the pathophysiology of rheumatoid arthritis. Semin Immunopathol 39: 437-446, 2017.
5. Alam J, Jantun I and Bukhari SNA: Rheumatoid arthritis: Recent advances on its etiology, role of cytokines and pharmacotherapy. Biomed Pharmacother 92: 615-633, 2017.
6. Goldfeder RL, Wall DP, Khoury MJ, Ioannidis JPA and Davis JM 3rd: Rheumatoid arthritis: A severe disease that preventive approaches would greatly benefit. Clin Ther 41: 1240-1245, 2019.
7. Luo Q, Xu C, Li X, Zeng L, Ye J, Guo Y, Huang Z and Li J: The roles of p53 and NF-κB in regulating Th17/Treg cell differentiation. Cell Immunol 352: 104589, 2019.
8. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH and Luthra HS: The American rheumatism association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 31: 315-324, 1988.
9. Paradowska A, Maślińiski W, Grzybowska-Kowalczyk A and Lacki J: The function of interleukin 17 in the pathogenesis of rheumatoid arthritis. Arch Immunol Ther Exp (Warsz) 55: 100-105, 2017.
10. Liu H, Yao S, Dann SM, Qin H, Elson CO and Cong Y: ERK differentially regulates Th17- and Treg-cell development and contributes to the pathogenesis of colitis. Eur J Immunol 43: 2555-2566, 2013.
35. Zhang H, Xu CF, Ren C, Wu TT, Dong N and Yao YM: Novel role of p53 in septic immunosuppression: Involvement in loss and dysfunction of CD4+ T lymphocytes. Cell Physiol Biochem 51: 452-469, 2018.

36. Asquith DL, Bryce SA and Nibbs RJ: Targeting cell migration in rheumatoid arthritis. Curr Opin Rheumatol 27: 204-211, 2015.

37. Hähnlein JS, Nadafi R, de Jong T, Ramwadhdoebe TH, Semmelink JF, Maijer KI, Zijlstra IA, Maas M, Gerlag DM, Geijtenbeek TBH, et al: Impaired lymph node stromal cell function during the earliest phases of rheumatoid arthritis. Arthritis Res Ther 20: 35, 2018.

38. Radstake TR, van der Voort R, ten Brummelhuis M, de Waal Malefijt M, Looman M, Figdor CG, van den Berg WB, Barrera P and Adema GJ: Increased expression of CCL18, CCL19, and CCL17 by dendritic cells from patients with rheumatoid arthritis, and regulation by Fc gamma receptors. Ann Rheum Dis 64: 359-367, 2005.

39. Förster R, Davalos-Misslitz AC and Rot A: CCR7 and its ligands: Balancing immunity and tolerance. Nat Rev Immunol 8: 362-371, 2008.

40. Hauser MA and Legler DF: Common and biased signaling pathways of the chemokine receptor CCR7 elicited by its ligands CCL19 and CCL21 in leukocytes. J Leukoc Biol 99: 869-882, 2016.

41. Hu Z, Li Y, Van Nieuwenhuijze A, Selden HJ, Jarrett AM, Sorace AG, Yankeelov TE, Liston A and Ehrlich LIR: CCR7 modulates the generation of thymic dendritic cell T cells by altering the composition of the thymic dendritic cell compartment. Cell Rep 21: 168-180, 2017.

42. Shi LJ, Li JH, Cen XM, Yang NP, Yin G and Xie QB: CCL19/IL-1beta positive feedback loop involved in the progress of inflammation in rheumatoid arthritis. Sichuan Da Xue Xue Bao Yi Xue Ban 46: 272-275, 2015 (In Chinese).

43. Viereck Jand Thum T: Circulating noncoding RNAs as biomarkers of cardiovascular disease and injury. Circ Res 120: 381-399, 2017.

44. Dang X, Lian L and Wu D: The diagnostic value and pathogenetic role of IncRNA-ATB in patients with osteoarthritis. Cell Mol Biol Lett 23: 55, 2018.

45. Fan CN, Ma L and Liu N: Systematic analysis of IncRNA-miRNA-mRNA competing endogenous RNA network identifies four-IncRNA signature as a prognostic biomarker for breast cancer. J Transl Med 16: 264, 2018.

46. Knoll M, Lodish HF and Sun L: Long non-coding RNAs as regulators of the endocrine system. Nat Rev Endocrinol 11: 151-160, 2015.

47. Quinn JJ and Chang HY: Unique features of long non-coding RNA biogenesis and function. Nat Rev Genet 17: 47-62, 2016.

48. Zhang Y, Yu YZ, Sun N, Liu JH, Chen FF, Guan XL, Li A, Wang F, Zhao QF, Wang HY, et al: Long noncoding RNA expression profile in fibroblast-like synoviocytes from patients with rheumatoid arthritis. Arthritis Res Ther 18: 227, 2016.

49. Linden A: Measuring diagnostic and predictive accuracy in disease management: An introduction to receiver operating characteristic (ROC) analysis. J Eval Clin Pract 12: 132-139, 2006.

50. Cook NR: Statistical evaluation of prognostic versus diagnostic models: Beyond the ROC curve. Clin Chem 54: 17-23, 2008.

51. Nishimura K, Sugiyama D, Kogata Y, Tsuji G, Nakazawa T, Kawano S, Saigo K, Morinobu A, Koshiba M, Kunz KM, et al: Meta-analysis: Diagnostic accuracy of anti-cyclic citrullinated peptide antibody and rheumatoid factor for rheumatoid arthritis. Ann Intern Med 146: 797-808, 2007.

52. Anderson JK, Zimmerman L, Caplan L and Michaud K: Measures of rheumatoid arthritis disease activity: Patient (PtGA) and provider (PrGA) global assessment of disease activity, disease activity score (DAS) and disease activity score with 28-joint counts (DAS28), simplified disease activity index (SDAI), clinical disease activity index (CDAI), patient activity score (PAS) and patient activity score-II (PASII), routine assessment of patient index data (RAPID), rheumatoid arthritis disease activity index (RADA1) and rheumatoid arthritis disease activity index-5 (RADA1-5), chronic arthritis systemic index (CASII), patient-based disease activity score with ESR (PDAS1) and patient-based disease activity score without ESR (PDAS2), and mean overall index for rheumatoid arthritis (MOI-RA). Arthritis Care Res (Hoboken) 63 (Suppl 11): SI4-S36, 2011.

53. van Riel PL and Renskers L: The disease activity score (DAS) and the disease activity score using 28 joint counts (DAS28) in the management of rheumatoid arthritis. Clin Exp Rheumatol 34 (5 Suppl 101): S40-S44, 2016.