Association of the X-chromosomal hsa_circ_0140271 with Female Rheumatoid Arthritis

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Abstract

Background: Rheumatoid arthritis (RA) commonly affects women. Circular RNAs (circRNAs) have been reported to be related RA progress. However, it is still unknown the role of circRNA in female RA.

Methods: In this study, we extracted total RNA of peripheral blood mononuclear cells (PBMC) from four RA patients and four healthy control donors, then performed RNA sequencing analysis. To validation, we recruited PBMC samples from 47 RA patients and 47 healthy control donors. The diagnosis ability of candidate circRNA in female RA was explored by Receiver Operating Characteristic (ROC) curve analysis. Levels of inflammation factors in serum, such as IL-1α, IL-1β, IL-6, IL-8,TNF-α and INF-γ, were detected in female RA patients and female healthy donors to analysis the correlation between those factors and candidate circRNA. KEGG pathway enrichment analyses were also performed to predict function of candidate circRNA.

Resuluts: By analyzing circRNA expression profiles, we identified hsa_circ_0140271, generated from X chromosome gene-MED14, was specifically expressed in female RA patients. We verified hsa_circ_0140271 expression in PBMC samples from a cohort of 47 RA patients and 47 healthy control subjects via RT-qPCR and found hsa_circ_0140271 was also specifically highly expressed in female RA. In addition, ROC curve analysis indicated that the specificity of hsa_circ_0140271 in female RA patients was 1 comparing to female healthy subjects, osteoarthritis (OA) patients or Ankylosing spondylitis (AS) patients. Levels of IL-6, IL-8 and TNF-α was higher in serum from female RA patients than that from female healthy subjects. Furthermore, KEGG analysis indicated that hsa_circ_0140271 might regulate fatty acid metabolism in female RA patients.

Conclusion: Our results suggested that hsa_circ_0140271 has a potential role in detect female RA and may contribute RA progress by fatty acid metabolism.

Introduction

Rheumatoid arthritis is a systemic autoimmune disease characterized by chronic inflammation and erosive arthrosynovitis, with a prevalence of 0.5-1% in the worldwide[1] [2]. Like other autoimmune diseases, RA is more prevalent among women, with some studies suggesting as high as 3:1 female to male ratio. The etiology of RA is not fully elucidated, however, hormonal change and genetic factors are related to high incidence of female RA[3]. Women with early menopause are more susceptible to RA[4]. Besides hormone, genes from X chromosome are also closely associated with RA. A higher rate of a skewed X chromosome inactivation has been observed in female RA patients[5]. And interleukin 1 receptor associated kinase (IRAK1) from X chromosome has been reported to be related to female predominant in RA[6]. Thus, researches on X-chromosomal genes might be a good way to elucidate the mechanism of female RA etiology.

Circular RNA is a class of endogenous RNA, which is characterized by a covalently closed loop structure without a 5’ end cap structure and 3’ end poly (A) tail[7]. Circular RNA is widely presented in eukaryotic

cells and showed cell or tissue specific expression pattern[8]. Due to loop structure, circular RNA is resistant to degradation, and this feature has been applied to clarify the potential pathogenesis of diseases[9]. Recently, several studies have been demonstrated that circular RNAs are highly relevant to RA[10]. Those studies have explored various roles of circRNAs in RA, however, there is few concerned about association between X chromosome generated circRNAs and female predominance in RA. In this study, we aimed to explore X-chromosomal circRNA and its role in female RA. Furthermore, we also evaluated the clinical potential of the candidate circRNA as a biomarker of female RA.

Material And Methods

Patient variables

Peripheral blood was collected from a total of 127 participants who were recruited consecutively for this study: 47 patients with RA, 47 healthy controls, 23 female osteoarthritis (OA) and 7 female Ankylosing spondylitis (AS) patients. All RA, OA and AS patients were diagnosed at the Department of Rheumatology and Immunology, and Department of Orthopedics at The Fifth Affiliated Hospital of Sun-Yat-Sen University in 2020. Age and sex-matched patients without RA, OA or AS from the same hospital were recruited as healthy controls. All RA patients fulfilled the American College of Rheumatology criteria for the classification of RA. Among these, 5 patients were new onset rheumatoid arthritis (< 6 months of disease duration) .The disease activity was evaluated by Disease Activity Score 28 (DAS28) using C-reactive protein (CRP). The characteristics of RA patients and healthy controls and controls are shown in Table 1. All study protocols were approved by the ethics committee of The Fifth Affiliated Hospital of Sun-Yat-Sen University. All participants in this study were informed and signed written consent.

Table 1
Characteristics of healthy controls and patients with rheumatoid arthritis.

Characteristics	RA	HC
Number	47	47
Age(years)[a]	57.13 ± 12.27	60.70 ± 10.89
Sex (M/F)	16/31	16/31
Disease duration (Month)[b]	1008.00(158.40,1872.00)	NA
Anti-CCP(U/ml)[a]	40.28 ± 15.20	32.34 ± 10.86
DAS28[a]	4.06 ± 1.14	NA
CRP(mg/L)[b]	25.14(5.10,73.40)	3.1(1.00,42.65)
ESR(mm/h)[a]	61.17 ± 34.13	33.12 ± 27.18
Swollen joints[b]	6.00(2.00,10.00)	NA
Tender joints[b]	6.00(3.00,10.00)	NA
ILD positive(%)	17.02%	NA
In the groups providing PBMCs, there was no significant difference in age (p = 0.139 ,Student's t-test) and sex (p = 1.000, Chi-square) between RA patients and HC. Abbreviations: RA: rheumatoid arthritis;HC:healthy controls;M/F: male/female;Anti-CCP:anti-cyclic citrullinated peptide antibodies;DAS28:disease activity score in 28 joints;CRP:C-reactive protein;ESR: erythrocyte sedimentation rate;ANA: antinuclear antibody;ILD:interstitial lung disease;NA: not available.		
[a]Expressed as mean ± standard deviation.		
[b]Expressed as the median (25th to 75th percentile).		

Preparation of Peripheral Blood Samples and Isolation of RNA and plasma

Peripheral blood samples (6 ml) were collected from each patient and control subjuect into EDTA-2K-containing tubes. Plasma and peripheral blood mononuclear cells (PBMCs) were extracted as soon as possible by using the Histopaque-1077 (Sigma-Aldrich, UK) according to the manufacturer's protocol. Then the plasma was immediately separated and transferred to a fresh RNase free tube and stored at −80 °C for Elisa. Total RNA was isolated from freshly obtained PBMCs using the Total RNA Kit I (Omega Bio-tek, USA). The concentration and quality of the RNA were assessed by absorbance spectrometry measuring absorbance ratios of A260/A280 and a 260/A230 using a NanoDrop ND-1000 spectrophotometer (ThermoFisher Scientifc, USA). Total RNA were kept at -80 °C or immediately used for reverse transcription.

RNA-seq analysis

Before RNA-sequencing, the quality of RNA was tested by Agilent 2100 Bioanalyzer (Agilent Technology). 2 µg of RNA sample was taken for RNA-sequencing. RNase R digested and rRNA depleted RNAs were taken to generated the sequencing libraries by using Total RNA-seq (H/M/R) Library Prep Kit for Illumina (Vazyme Biotech) following manufacturer's recommendations. The library preparations were sequenced on Hiseq X Ten (Illumina).

Differential expression analysis

The reads were first mapped to the latest UCSC transcript set using Bowtie2 version 2.1.0[11] and the gene expression level was estimated using RSEM v1.2.15[12]. For circRNA expression analysis, the reads were to mapped genome using the STAR[13] and DCC[14] was used to identify the circRNA and to estimate the circRNA expression.TMM (trimmed mean of M-values) was used to normalize the gene expression. Differentially expressed genes were identified using the edgeR program[15]. Genes showing altered expression with $p < 0.05$ and more than 1.5 fold changes were considered differentially expressed.

Real time- quantitative PCR (RT-qPCR) analysis.

A total of 500 ng of each RNA sample was used for reverse transcription using a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientifc, Lithuana). The relative expression of circRNA (Forward Primer:5'-atcggcttgtaaccacagat-3', Reverse Primer: 5'-ctcataaagttgttctctcctg-3') and MED14 (Forward Primer:5'-cgccaactcttcgttcgatta-3', Reverse Primer: 5'-gtccacaaacaggatggcttg-3') was determined on an CFX96TM Touch Real-Time PCR Detection System (BIO-RAD,USA) using EverGreen® Forget-Me-Not qPCR Master Mix (Biotium, USA), as the following PCR thermocycler protocol: Initial Enzyme activation step at 95˚C for 2 min, followed by 40 cycles of 95˚C for 5 sec (denaturation), 60˚C for 10 sec (annealing) and 72˚C for 10 sec (extension). GAPDH (Forward Primer:5'-agccacatcgctcagca-3', Reverse Primer: 5'-gcccaatacgaccaaatcc-3') was used as an internal control.

ELISA

Plasma concentrations of IL-1α, IL-1β,IL-6,IL-8,TNF-α and IFN-γ were measured by commercially available enzyme linked immunosorbent assay (ELISA) kits (Saicheng Bio-tek, China) according to the manufacturer's instruction.

miRNA prediction and KEGG pathway analysis

The miRNAs, potentially related to candidate circRNA, were predicted by circBank database (http://www.circbank.cn/) and circular RNA Interactome database (https://circinteractome.nia.nih.gov/). Based on predicted miRNAs, KEGG pathway enrichment analyses were performed by DIANA-mirPath (http://www.microrna.gr/miRPathv2). The KEGG pathway analysis was also performed to represent the

knowledge on the molecular interaction and reaction networks of the target gene. The KEGG pathway with a $p < 0.05$ was considered the significance of the pathway correlations.

Statistical Analysis.

Data were statistically described in terms of mean ± standard deviation, medians (quartiles), or proportions when appropriate. All experimental data were analyzed using SPSS software 22.0 (IBM,USA) and GraphPad Prism 8.0 (GraPhpad Software, CA). Student's t-test and Mann-Whitney's U test were employed to compare normally distributed parameters and those with skewed distribution, the Pearson method or the nonparametric Spearman method was used for correlation analysis, and logistic regression analysis were used, as appropriate. Receiver operating characteristic (ROC) curves were performed to evaluate the diagnostic value of circRNA. The area under curve (AUC) was calculated with SPSS software22.0. $p < 0.05$ was considered to be statistically significant.

Results

Screening of female rheumatoid arthritis associated circRNA

To explore female RA associated circRNAs, we firstly collected total RNA of PBMC samples from four RA patients and control healthy subjects with gender and age matched. Then, we performed RNA sequencing analysis and compared circRNAs expression between rheumatoid arthritis patients and healthy control group. We identified 162 significantly differentially expressed circRNAs with a fold change ≥ 1.5 and a p-value ≤ 0.05 (Fig. 1A, Supplemental Table 1). Next, we used cluster screening to analyze the effect of gender against the background of female healthy samples and male rheumatoid arthritis samples (Fig. 1B,C, Supplemental Table 2,3). This enabled us to further identify female RA associated circRNAs. As shown in the Venn diagram, we identified three circRNAs (hsa_circ_0140271, hsa_circ_0105101 and hsa_circ_0010474) (Fig. 1D). Among those circRNAs, we were interested in hsa_circ_0140271, which were generated from MED14 gene located on X chromosome and highly expressed in three paired comparisons (Supplemental Table 4). To further explore the role of hsa_circ_0140271 in female RA, we designed specific primer to target hsa_circ_0140271, and then performed Sanger sequencing analysis and RT-qPCR. As the results shown, we identified the back-splice junction of hsa_circ_0140271 through Sanger sequencing analysis, and also found hsa_circ_0140271 was resistant to RNase R. While the liner RNA of MED14 was significantly decreased (Fig. 1E,F). These results confirmed the primer correctly amplified hsa_circ_0140271.

hsa_circ_0140271 was highly expressed in female rheumatoid arthritis patients' PBMC

In order to further analyze hsa_circ_0140271 expression in female rheumatoid arthritis patients' PBMC, we recruited PBMC totally from 47 RA patients and 47 healthy control donors. These two cohorts were also gender and age matched. The total RNA was also extracted and quantified hsa_circ_0140271 expression by RT-qPCR. As expected, hsa_circ_0140271 was significantly highly expressed in RA samples (Fig. 2A). To assess whether hsa_circ_0140271 would be specifically expressed in female RA samples, we stratified RA and healthy control samples according to gender. Consistent with previous results, hsa_circ_0140271 was also significantly highly expressed in female RA samples comparing to that in female healthy samples or male RA samples (Fig. 2B). However, it was not observed any difference in expression of mRNA of MED14 between RA and control group (Fig. 2C).

Next, we analyzed whether hsa_circ_0140271 expression was correlated with clinical status of female RA patients. According to DAS28 scores, we divided female RA patients into Remission group (DAS28 < 2.6) and Active group (DAS28 > 2.6). RT-qPCR results showed hsa_circ_0140271 expression was significantly higher in the Remission group and Active group comparing to that in female healthy group, while there was no difference between Remission group and Active group (Fig. 2C). We also analyzed effect of RA duration on hsa_circ_0140271 expression. We found that hsa_circ_0140271 expression was significantly higher in the female early RA (ERA) group (< 6 months) and RA group (> 6 months), but there was also no difference between ERA and RA group (Fig. 2D). Finally, we examined hsa_circ_0140271 expression in female RA-associated interstitial lung disease (ILD). Among female RA patients, there were four patients who suffered ILD. And hsa_circ_0140271 expression from those patients was not different to that from non-ILD patients (Fig. 2E). These data confirmed that hsa_circ_0140271 was specifically expressed in PBMC from female RA patients and its expression was independent with disease activity, duration and ILD.

hsa_circ_0140271 serves as a potential diagnostic biomarker for female RA

Based on the results showing that hsa_circ_0140271 was specifically highly expressed in PBMC of female RA patients, we established ROC curve analysis to explore the potential utility of hsa_circ_0140271 as a diagnostic biomarker of female RA. According to ROC analysis, the area under curve (AUC) for hsa_circ_0140271 was up to 0.704 (sensitivity = 0.419, specificity = 1), respectively (Fig. 3A, Table 2). We also performed ROC analysis by combining hsa_circ_0140271 and anti-cyclic citrullinated peptide (anti-CCP). The data suggested that the AUC and sensitivity were increased to 0.818 and 0.806, while the specificity was decreased to 0.742 (Fig. 3B, Table 2). These results implied that hsa_circ_0140271 was highly specificity for female RA and combination with anti-CCP improved its predictive value.

Table 2
ROC curve Validates the diagnostic value of differentially expressed hsa_circ_0140271 from female patients with RA.

Variables	AUC	SEM	P value	95%CI	Sensitivity	Specificity	Cutoff value
hsa_circ_0140271	0.704	0.067	0.006	0.572–0.837	0.419	1.000	0.110
Anti-CCP	0.738	0.738	0.001	0.609–0.867	0.581	0.903	40.719
Anti-CCP + hsa_circ_0140271	0.818	0.054	< 0.001	0.699–0.904	0.806	0.742	NA

Since some cytokines, like IL-1α, IL-1β, IL-6, IL-8, TNF-α and INF-γ and so on, were contributed to pathology of RA[16], we analyzed the relation between hsa_circ_0140271 and those cytokines. Based on Cutoff values of hsa_circ_0140271 ROC analysis, we divided female RA patients into hsa_circ_0140271 positive group (> 0.110) and negative group (< 0.110). Then we detected IL-1α, IL-1β, IL-6, IL-8, TNF-α and INF-γ expression in serum from both groups. Unfortunately, there was no difference in the level of those factors between two groups (Supplemental Fig. 1). However, when dividing female RA and female healthy donors according to Cutoff values, the results showed that IL-6, IL-8 and TNF-α expression were higher in hsa_circ_0140271 positive group (Fig. 4).

hsa_circ_0140271 possibly regulates fatty acid metabolism in female RA patients

Accumulating evidences have been shown that circRNAs play a role in pathogenesis of diseases through function of miRNA sponge[17, 18]. To elucidate the role of hsa_circ_0140271 in RA, we predicted hsa_circ_0140271 related miRNA using circBank database (http://www.circbank.cn/) and circular RNA Interactome database (https://circinteractome.nia.nih.gov/) (Fig. 5A). By analyzing those two databases, it was shown eight miRNAs might be closely related to hsa_circ_0140271, which were has-miR-600, has-miR-1244, has-miR-576-5p, hsa-miR-941, has-miR-657, has-miR-635, has-miR-574-5p, and has-miR-1305. Then, to further predict function of hsa_circ_0140271, we performed KEGG analysis based on predicted miRNAs using DIANA-mirPath (http://www.microrna.gr/miRPathv2) (Fig. 5B). According to analysis, we found 9 enriched KEGG terms. Among those terms, we found 3 terms were related to lipid metabolism, which were “Fatty acid biosynthesis”, “Pantothenate and CoA biosynthesis” and “Fatty acid metabolism”. This analysis indicated function of hsa_circ_0140271 might be associated with lipid metabolism. To verify our hypothesis, we checked laboratory characteristics of female RA patients. Although there was no correlation between hsa_circ_0140271 and lipid associated categories, such as TG, TC, HDL-C and LDL-C, it was found that expression of TG from female RA patients was lower than that from female control groups (Fig. 5C-F, Supplemental Table 5). Taken together, hsa_circ_0140271 might play a role in lipid metabolism in female RA patients through function of miRNA sponge.

hsa_circ_0140271 was a potential biomarkers in discriminating female RA from OA or AS

Since female is predominant in autoimmune diseases[3], we also analyzed hsa_circ_0140271 expression in PBMC from female osteoarthritis (OA) and Ankylosing spondylitis (AS) patients. We recruited 24 female OA patients and 7 female As patients and PBMC of those patients was also extracted to perform RT-qPCR. RT-qPCR analysis demonstrated that hsa_circ_0140271 expression from OA and AS was significantly lower than that from RA, even lower than that from healthy control (Fig. 6A). Then, we performed ROC curve analysis to evaluate the potential value of hsa_circ_0140271 discriminating RA from OA or AS. ROC curve from patients with RA and AS showed AUC area was 0.922 (sensitivity = 0.806, specificity = 1). When distinguish RA patients from OA patients, AUC area was 0.868 (sensitivity = 0.645, specificity = 1). It was also analyzed by combining OA and AS. By combination, AUC area was 0.881 (sensitivity = 0.645, specificity = 1) (Fig. 6B,C, Table 3). Those analyses implied that hsa_circ_0140271 was specifically expressed in female RA patients and might discriminate RA from OA or AS.

Table 3
ROC curve analysis of the confirmed hsa_circ_0140271 as RA female specific diagnosis.

Variables	AUC	SEM	P value	95%CI	Sensitivity	Specificity	Cutoff value
hsa_circ_0140271(RA vs AS)	0.922	0.045	< 0.001	0.787–0.984	1	0.806	0.034
hsa_circ_0140271(RA vs OA)	0.868	0.048	< 0.001	0.748–0.945	1	0.645	0.072
hsa_circ_0140271(RA vs AS + OA)	0.881	0.045	< 0.001	0.772–0.950	1	0.645	0.072

Discussion

RA is a chronic autoimmune disease characterized by rheumatoid nodules, pulmonary involvement or vasculitis, and systemic comorbidities[19]. Currently, increasing studies have been demonstrated that circRNAs are involved in or related to RA progress[10]. In this study, we found hsa_circ_0140271 was highly expressed in PBMC from female RA patients comparing to that from female healthy control or male RA patients.

Like other autoimmune disease, women are more frequently to be affected by RA than men, with a female to male ratio of about 3:1[3]. Genes from X chromosome is one factors contribute to predominance of RA in women[20]. Normally, a female karyotype is comprised of two X chromosomes, which are originated from each parent. During early stage of embryogenesis, one of X chromosomes is randomly silenced. However, 15% of gene would be escaped from silencing and X chromosomes inactivation (XCI) is associated with female RA[5, 20, 21]. One of mechanism of XCI is DNA methylation, which is potential related to circular RNA biogenesis and RA[21–23]. And generation of circRNAs was also regulated by

DNA methylation[22]. In this study, we performed RNA sequencing analysis and found hsa_circ_0140271 expression might be related to female RA. We also detected X chromosomal gene-MED14 expression, from which hsa_circ_0140271 was generated. However, there was no difference in MED14 mRNA expression between female RA group and female healthy group. These results indicated that the difference of hsa_circ_0140271 expression was not caused by gene of MED14 itself, while it may be closely associated with female RA or other things, like DNA methylation. And it is needed to further explore the mechanism of generation of hsa_circ_0140271 in female RA.

It is well accepted that early diagnosis and assessment of disease activity are favorable outcome in RA[24–26]. In this study, we observed that there was no difference in hsa_circ_0140271 expression between Remission and Activity group in female RA patients. Furthermore, there was also no difference in hsa_circ_0140271 expression between early stage RA and RA group in female RA patients, which meant hsa_circ_0140271 expression might be independent of duration or activity in female RA. Comparing to female healthy control, AS or OA group, ROC curve analysis also indicated that the specificity of hsa_circ_0140271 was all 1, which implied that it might had a good diagnosis value for female RA. We also detected hsa_circ_0140271 expression in one of RA complications- interstitial lung disease (ILD). In this analysis, we also did not found any difference in hsa_circ_0140271 expression between ILD and non-ILD group. These analyses indicated that hsa_circ_0140271 could not serve as a biomarker for assessing disease activity in RA or identifying complications of RA like ILD, however, it might be a potential biomarker in female RA.

RA is a chronic inflammation disease and proinflammation factors play an important role in RA progress[16]. In this study, we detected IL-1α, IL-1β, IL-6, IL-8, TNF-α and INF-γ levels in serum among female RA patients and female healthy groups. Although there were no difference in those factors level between hsa_circ_0140271 positive and negative group among female RA patients, it was observed IL-6, IL-8 and TNF-α levels were higher in the hsa_circ_0140271 positive group when calculating female RA patients and female healthy subjects together. Regarding to this results, we estimated that the limited sample size affected the results in female patients, and with increasing sample size there would be also difference in those factors level between hsa_circ_0140271 positive and negative group among female RA patients. Interestingly, it have been found that level of IL-6, IL-8 and TNF-α in serum were associated with early stage of RA patients[27, 28].These results implied that hsa_circ_0140271 might be related to onset or pathology of early stage of RA. And combing those analysis and previous analysis of hsa_circ_0140271 expression in early stage RA and RA group, we estimated that hsa_circ_0140271 might be also a biomarker of early stage of female RA.

To further explore function of hsa_circ_0140271, we predicted some miRNAs related hsa_circ_0140271 based on miRNA sponge theory, and then performed KEGG pathway analysis to found some signaling pathways potentially regulated by hsa_circ_0140271. Among those pathways, we detected three fatty acid related pathways, such as Fatty acid biosynthesis, Pantothenate and CoA biosynthesis and Fatty acid metabolism. It has been demonstrated that fatty acids play a role in various of RA processes, such as inflammation and pain, by interacting with immune cells like Th17 cells[29, 30]. It has been

demonstrated that fatty acid contributed synthesis of Th17 cells[31]. As a source of fatty acid, level of TG was decreased in female RA group comparing to female healthy subjects. Taken together, we hypothesize that hsa_circ_0140271 might regulate fatty acid metabolism in female RA.

In general, we suggested hsa_circ_0140271 as a potential candidate of female RA. However, there were some limitations should be considered in this study. The small sample size was the main and significant limitation. In this study, we recruited 31 female RA patients and 31 female healthy control subjects. As mentioned above, the level of inflammation factors, such as IL-6,IL-8 and TNF-α, were shown to be different when calculating female RA patients and female healthy subjects together, however, it was not seen any difference in female RA patients alone. Therefore, a large number cohort study would be needed to be performed in future study. Besides that, it is also needed more research on function of hsa_circ_0140271 in further study. In this study, we predicted function of hsa_circ_0140271 based on theory of miRNA sponge. Except function of miRNA sponge,circular RNA also interacts with functional protein to regulate transcription activity in cells[32]. Thus it should carefully explore the function of hsa_circ_0140271 via more relative experiments in future.

Conclusions

In this study, we aimed to explore X chromosomal circRNAs to elucidate its potential role in diagnosis and treatment of female RA. Our results have provided evidences that hsa_circ_0140271, generated from gene of X chromosome, was specifically highly expressed in female RA patients. Furthermore, hsa_circ_0140271 would present great potential to be a biomarker for female RA detection. In addition, we predicted hsa_circ_0140271 would play a role in regulating inflammation in RA via fatty acid metabolism.

Abbreviations

circRNAs: circular RNAs; RA: Rheumatoid arthritis; PBMC: peripheral blood mononuclear cells; ROC: Receiver Operating Characteristic; OA:osteoarthritis; AS: Ankylosing spondylitis; IRAK1: interleukin 1 receptor associated kinase; DAS28: Disease Activity Score 28; CRP: C-reactive protein; ERA: early RA; ILD: interstitial lung disease; AUC: areas under ROC curve; anti-CCP: anti-cyclic citrullinated peptide; XCI: X chromosomes inactivation;

Declarations

Ethics approval and consent to participate

The experimental protocol was established, according to the ethical guidelines of the Helsinki Declaration and was approved by the Human Ethics Committee of The Fifth Affiliated Hospital of Sun-Yat-Sen University. Written informed consent was obtained from individual or guardian participants.

Consent for publication

Not applicable

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

Y.C and X.X designed experiments and analyzed data; Y.C and X.L performed experiments; J.S and N.J performed parts of Elisa experiments; W.B, W.B and J.Z collected clinical samples; S.Z and H.L provided techniques and discussion; Y.C and X.X wrote the manuscript.

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Figures

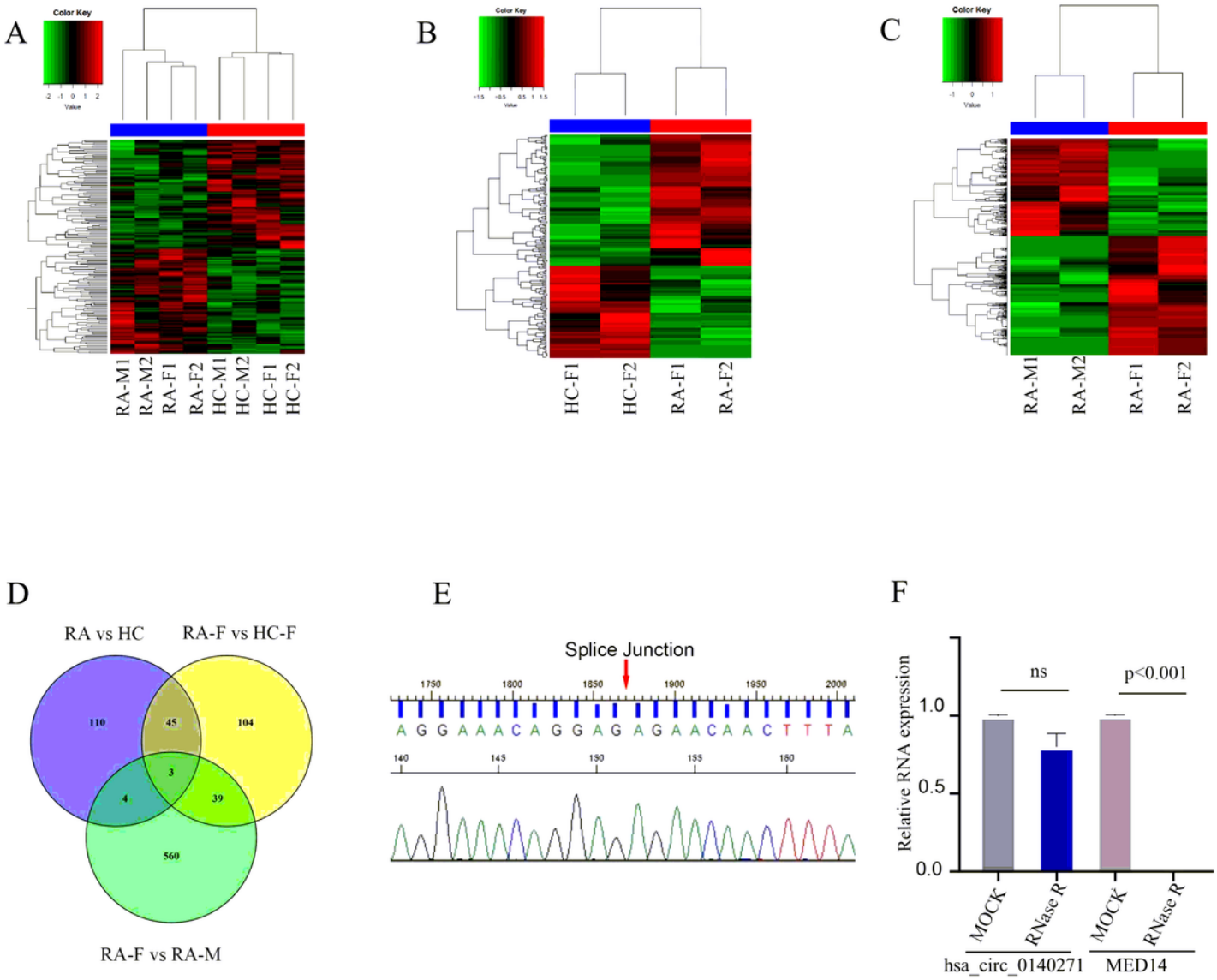

Figure 1

RNA sequencing analysis determined the circRNAs expression profiles and Identification and characterization of circRNAs. (A-C) Heat map of differentially expressed circRNAs in four RA patients and four healthy control donors with age and gender matched(A), two RA female patients and two healthy female controls (B), two RA female patients and two RA male patients (C) ;'Red'indicates high relative expression, and'green'indicates low relative expression. (D) Venn diagram analysis of three RNA-seq cohort (RA vs HC; RA female vs HC female; RA female vs RA male); (E) Sanger sequencing for hsa_circ_0140271; (F) relative expression of hsa_circ_0140271 and mRNA of MED14 in PBMC from RA patients treated with or without RNase R.

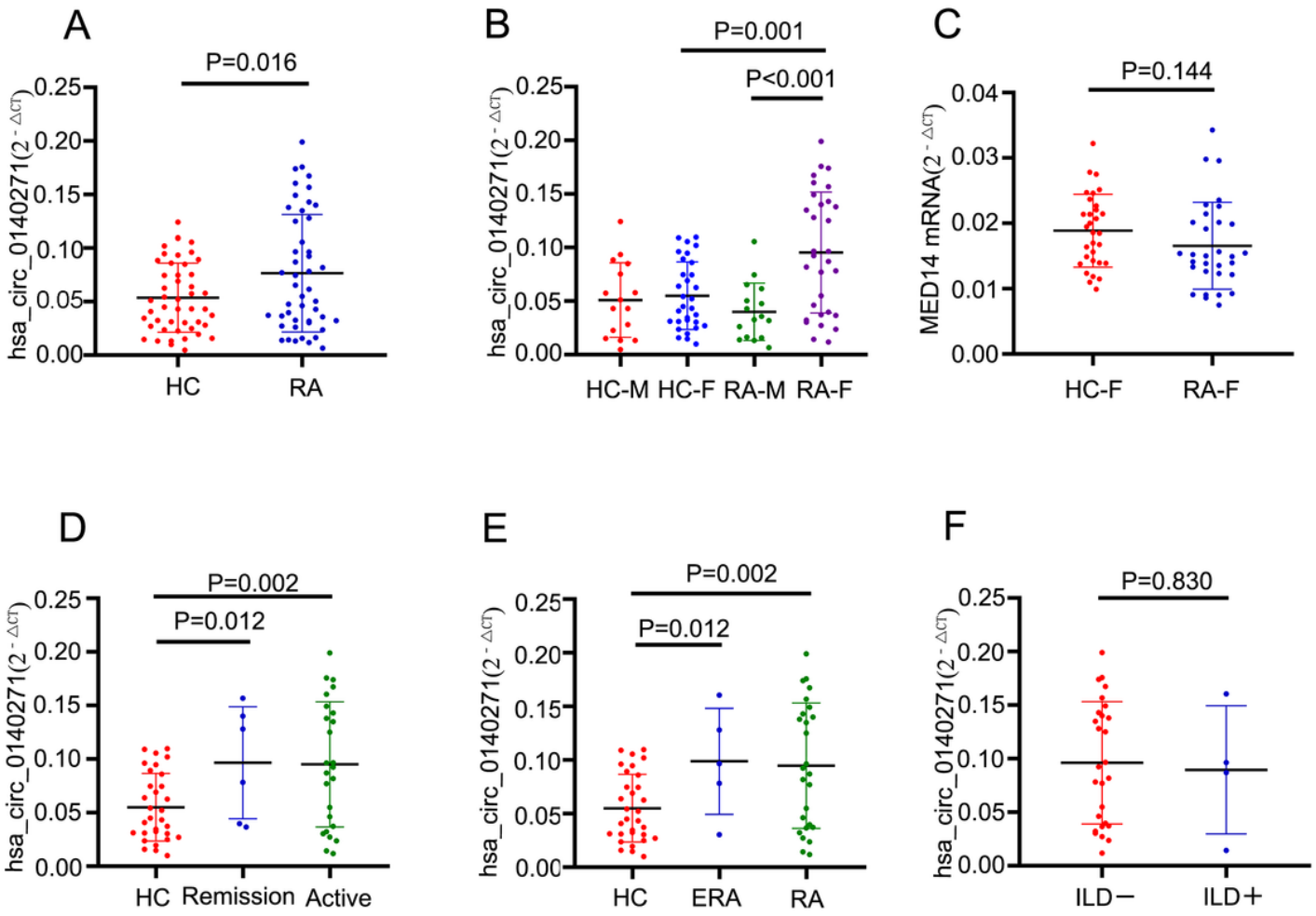

Figure 2

RT-qPCR determined the relative expression level of hsa_circ_0140271 and mRNA of MED14 in PBMCs from RA patients and healthy donors. (A) Detected expression level of hsa_circ_0140271 in PBMC of RA patients and healthy control (HC) donors. (B) RA patients and HC donors were divided according to gender in each group and then detected expression level of hsa_circ_0140271. (C) Detected MED14 mRNA in PBMC of female RA (RA-F) patients and female HC donors. (D) Female RA patients were divided according to activity of RA and detected expression level of hsa_circ_0140271 in Remission and Active with comparing to HC. (E) Female RA patients were divided according to stage of RA and detected expression level of hsa_circ_0140271 in early RA (ERA) and RA with comparing to HC. (F) Detected expression level of hsa_circ_0140271 in ILD positive group (ILD+) and ILD negative group (ILD-).

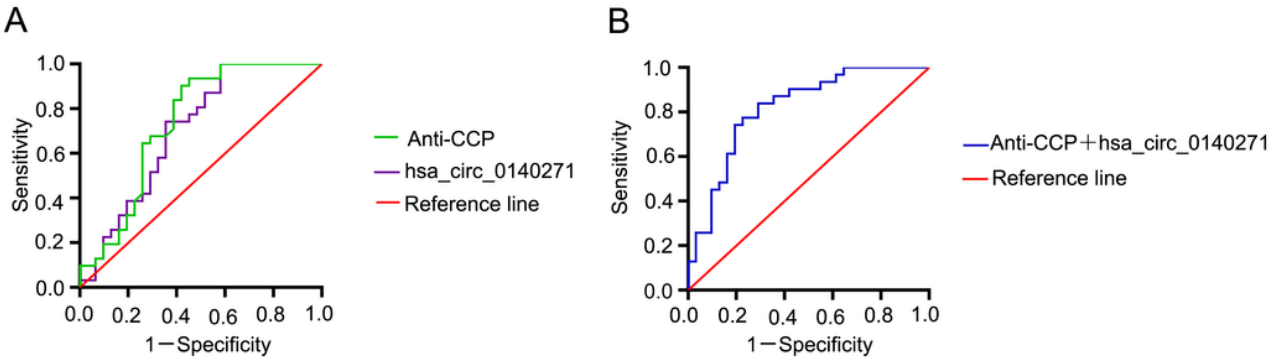

Figure 3

Validation of hsa_circ_0140271 as a potential biomarker in female RA patients. (A) The largest AUC was identified for Anti-CCP, followed by hsa_circ_0140271. (B) The AUC of combined Anti-CCP (Anti-CCP + hsa_circ_0140271) was 0.818, combination of Anti-CCP and hsa_circ_0140271 increased diagnostic accuracy.

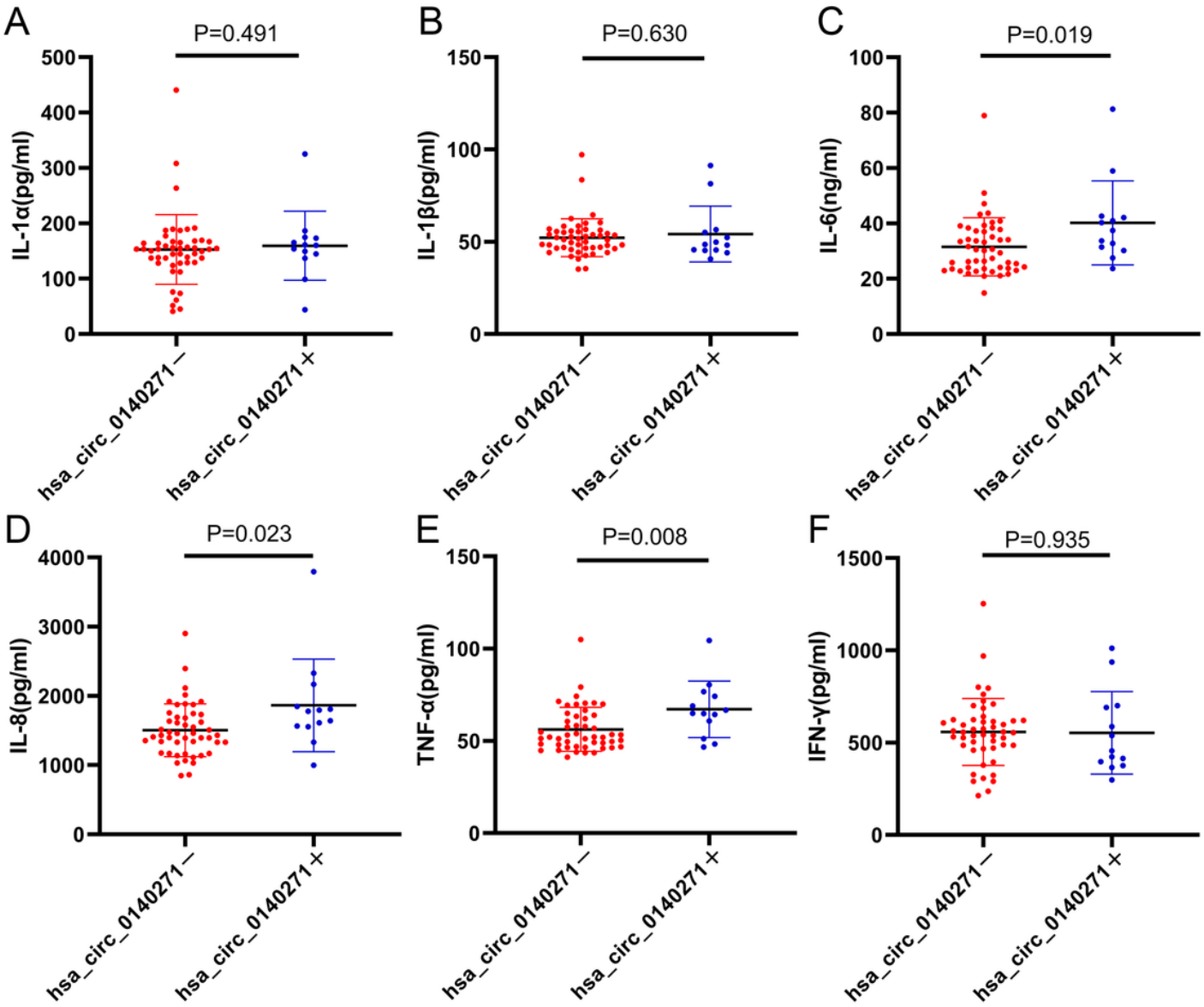

Figure 4

Determine levels of inflammatory factors in serum of female RA patients and female HC donors. 31 female RA patients and 31 female HC donors were divided according to Cutoff levels of hsa_circ_0140271 ROC analysis. Detected levels of IL-1α (A), IL-1β (B), IL-6(C), IL-8(D), TNF-α (E) and IFN-γ (F) in hsa_circ_0140271 positive group (hsa_circ_0140271+) and hsa_circ_0140271 negative group (hsa_circ_0140271-) .

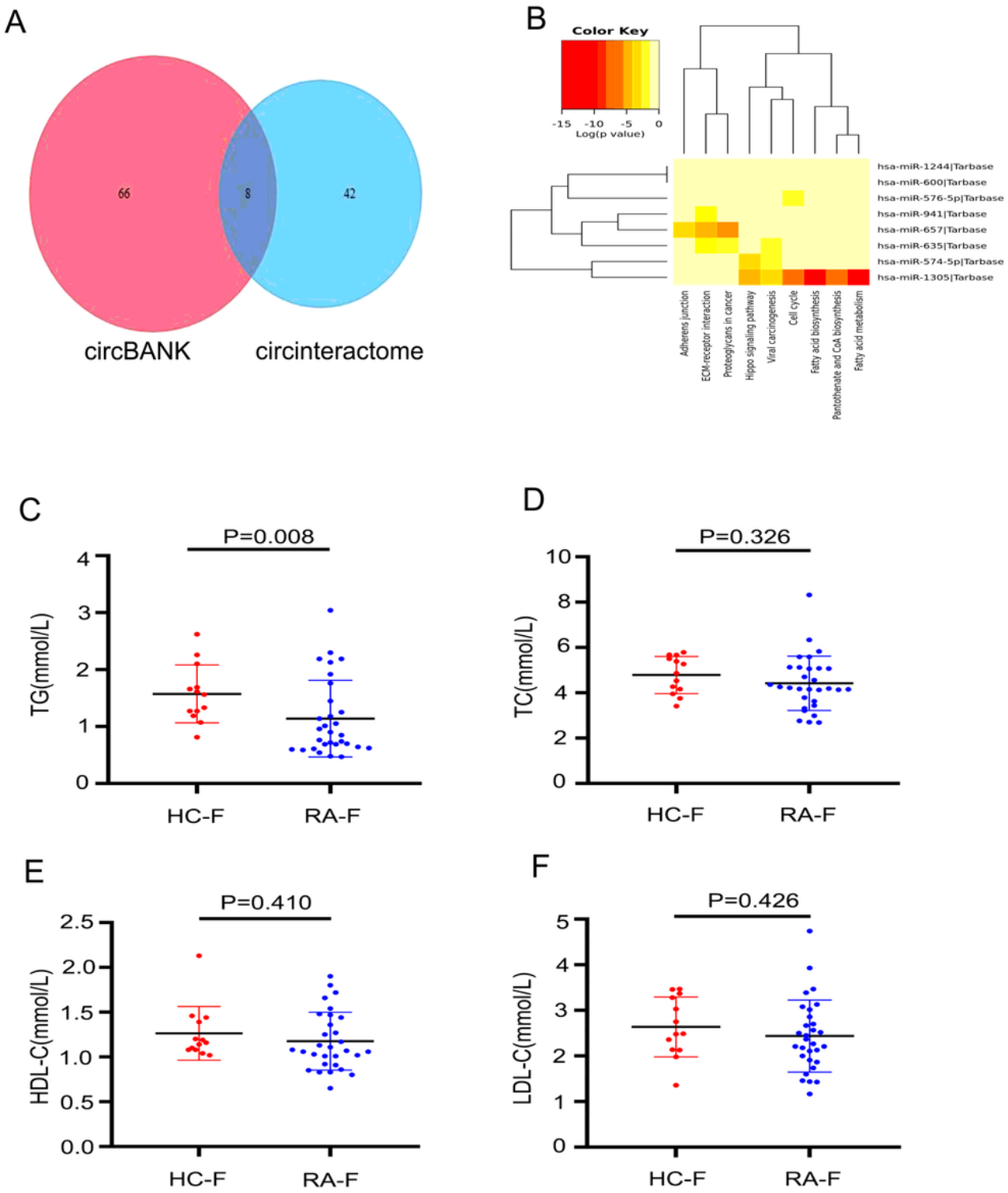

Figure 5

Prediction role of hsa_circ_0140271 in female RA patients. (A) Venn Diagram showed the number of potentially hsa_circ_0140271 associated miRNAs. (B) KEGG pathway analysis provided potential signaling pathway regulated by hsa_circ_0140271. (C-F) The levels of TG, TC, HDL-C and LDL-C of serum were detected in female RA patients and female HC donors.

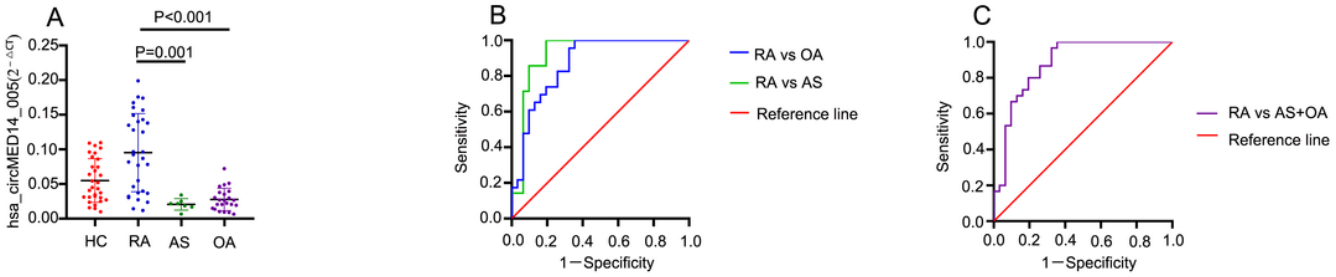

Figure 6

Validation the specific diagnostic values of hsa_circ_0140271 in female RA patients. (A) RT-qPCR confirmed hsa_circ_01402715 expression in female RA patients, female OA patients, female AS patients and female HC donors. (B, C) ROC curve analysis of hsa_circ_0140271 in female patients with RA compared with OA or AS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable1.xlsx
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