Long Non-coding RNA LINC02085 Mediates Cell Growth and Inflammatory Response of Fibroblast-Like Synoviocytes by Activating PI3K/AKT Signals in Rheumatoid Arthritisis

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Abstract

Background: The present study explored the possible functions and the underlying mechanism of Long Non-coding RNA LINC02085 in rheumatoid arthritis (RA).

Methods: Peripheral blood mononuclear cells (PBMCs) were collected from patients with RA. Primary fibroblast-like synoviocytes (FLS) were separated from synovial tissues and was established cell lines, then cultured for subsequent cell experiments by transfecting different vectors. The RT-qPCR analysis was employed for evaluating the levels of LINC02085 in the PBMCs and RA-FLS. ELISA analysis was employed to detect the levels of inflammatory cytokines. CCK8 assay, migration and invasion assays were used to evaluate the proliferation, migration and invasion abilities of cells, respectively. Besides, the levels of the the PI3K/AKT pathway-related proteins were measured by WB and IF. Spearman correlation analysis, association rule analysis, logistic regression analysis were used to assess the correlation between LINC02085 and clinical parameters.

Results: The expression level of LINC02085 was significant high in patients with RA, and positively associated with age, ESR, CRP, RF, CCP and DAS28. Moreover, logistic regression analysis indicated that ESR, CRP, RF and DAS28 were risk factors for LINC02085. We found that LINC02085 was upregulated in RA -FLS and TNF-αstimulated. And overexpression of LINC02085 could promote proliferation, migration and invasion induced by TNF-α, through upregulating the levels of TNF-αand TNFAIP2 and promoting the activation of PI3K/AKT pathway. Whereas knockdown of LINC02085 received the opposite results.

Conclusion: In conclusion, the present study revealed that LINC02085 could regulate cell growth and inflammatory response of RA-FLS by activating the PI3K/ AKT signaling pathway, subsequently playing important roles in promoting the occurrence and development of RA.

Background

Rheumatoid arthritis (RA), a chronic systemic disease, is featured with inflammatory synovitis, which can lead to destruction on bone and cartilage and even cause disability[1; 2]. The chronic and recurrent pain imposes a substantial burden on patients'quality of life, and brings great mental impact and economic pressure to patients and their families[3; 4]. Fibroblast-like synoviocytes (FLSs) have been proven to be the main effector cells, which play a critical role in the initiation and regulation of immune responses[5; 6]. Tumor necrosis factor-α (TNF-α), the pro-inflammatory cytokine, critically promotes the progress of RA[7; 8]. Despite the development of various treatment strategies for RA, the management of RA is still a challenge, which suggesting the need for a deeper understanding of the mechanism of RA, especially at molecular level[9; 10].

A substantial portion of this "genomic dark matter" is long non-coding RNA (lncRNA), defined as ncRNA greater than ~ 200 nucleotides in length, accounting for 68% of RNA molecules[11; 12]. Recently, the functions of lncRNAs and their associations with human diseases have attracted much attention from researchers because increasing evidences indicated that lncRNAs play critical roles in the development of

various autoimmune diseases, such as ankylosing spondylitis (AS)[13], osteoarthritis (OA) [14]and systemic lupus erythematosus (SLE) [15]with the complete mechanism. LncRNAs can crosstalk with immune cells and mediate immunological and inflammatory response through phosphoinositide-3-kinase (PI3K) / protein kinase B (AKT) signaling pathway[16]. Recently, a number of studies indicated that a number of dysregulated lncRNAs contribute to the inflammatory response in RA[17]. Certain differentially expressed lncRNAs in RA have been reported to affect the disease activity[18]. In our previous study, many differentially expressed lncRNAs were screened out from a high-throughput sequencing analysis, including LINC02085[19]. Nevertheless, the precise role and mechanisms of LINC02085 in RA pathogenesis remain unclear, particularly regarding its role in regulating inflammation.

In the present research, we selected LINC02085 as the subject and investigated its levels in the PBMCs of patients with RA. Next, we performed cellular experiments to verify the possible mechanism and assess the effects of the abnormally expressed LINC02085 in inflammatory responses and cell biological processes of RA-FLS. In addition, PI3K/AKT signal pathway aspect was found to be active under the LINC02085 condition. Based on these encouraging results, we believe that the present study could further elucidate a theoretical basis for the underlying mechanism of LINC02085 in RA.

Materials And Methods

Ethics approval

This study was approved by the Ethics Committee of the First Affiliated hospital of Anhui University of Traditional Chinese Medicine, and all patients signed a statement of informed consent.

Subjects and Samples

The present study recruited 30 patients with RA from May 2015 to July 2015. The inclusion criteria were (1) all the subjects fulfilled the 2010 American College of Rheumatology (ACR) criteria for the diagnosis of RA[20]; (2) complete clinical data of all patients were available. The exclusion criteria were (1) patients below the age of 18 years and older than 75 years were also excluded; (2) pregnant women, people with severe mental illness, people with liver and kidney function injury were excluded; (3) people for biologic agents treatment were excluded. In addition, 30 healthy participants served as healthy controls (HC).

Observation Indexes

Clinical indicators: sex, age, duration of disease, erythrocyte sedimentation rate (ESR), high-sensitivity C-reactive protein (CRP), rheumatoid factor (RF), anti-cyclic citrullinated peptide antibody (CCP), immunoglobulins A (IGA), immunoglobulin G (IGG), immunoglobulin M (IGM), complement 3 (C3), and complement 4 (C4).
Self-perception of patients (SPP): Disease activity score (DAS28), Visual analog scale (VAS), Anxiety self-assessment scale (SAS), Depression self-assessment scale (SDS) scores.

PBMC collection and total RNA extraction

Peripheral blood (5 ml) was collected from all subjects in vacuum blood tubes containing EDTA-K2. PBMCs were isolated by density gradient centrifugation (Histopaque-1077, Sigma). Total RNA was extracted from PBMCs using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturers protocol and and stored at -80˚C. The RNA concentration was determined with a NanoDrop (Thermo Scientific), and RNA integrity was assessed by agarose gel electrophoresis.

Cell culture

Primary FLS were separated from synovial tissues. Briefly, synovial tissue samples were obtained from RA patients who had undergone joint replacement surgery, which was cut into small pieces and then minced and digested with 4 mg/mL collagenase type I (Sigma) for 1 h in Dulbecco's modified Eagle's medium (DMEM; Sangon Biotech, Shanghai, China) at 37 °C for 1 h. The cells were then digested using 0.25% trypsin. After centrifugation at 800 g and further at 1000 g, the RA-FLS was maintained in RPMI-1640 (HyClone, Logan, UT, USA) supplemented with 10% fetal bovine serum (FBS), 100 mg/L streptomycin and 1 × 105 U/L penicillin. The separated RA-FLS were cultured to the third to sixth generations and were used for further study.

Cell Transfection

The coding sequence of LINC02085 was amplified and inserted into pcDNA3.1 (+) to produce the overexpressed vector of LINC02085 (pcDNA3.1-LINC02085). The silenced vectors si-LINC02085 and si-NC, and scrambled siRNA (si-NC) were obtained from GenePharma Co., Ltd. (Shanghai, China). The RA-FLS were transiently transfected with the above vectors using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA). Next, the transfected RA-FLS were incubated for 24 h and the cells were treated with TNF-α (10 ng/mL) for 24 h.

Cell Counting Kit 8 (CCK-8) assay

CCK-8 was utilized to determine the viability of both the treated and untreated RA-FLS. Transfected cells were placed in three replicates at a density of 3×10^4 cells per well in a 96-well plate with 100 μl of medium and incubated for 0, 24, 48, and 72 h after treatment with 10 ng/ml TNF-α. Then, the cells were incubated for 2 h in 10 μl of CCK-8 solution. Absorbance (optical density, OD) was assessed at 490 nm using an absorbance reader. The experiment was performed in triplicate.

Quantitative real-time PCR (RT-qPCR)

Total cellular RNA was extracted using TRIzol reagent (Invitrogen, NY, USA). To measure the expression of LINC02085, cDNA was reversely transcribed from total RNA by Prime Script TM RT reagent Kit with gDNA Eraser (TaKaRa, Dalian, Liaoning, China). And then qPCR was performed using TB Green™ Premix Ex Taq ™ (Tli RNaseH Plus) (TaKaRa, Shiga, Japan) according to the manufacturer's protocol. β-actin was used as an internal control. The primers were synthesized by Sangon Biotech. The primer sequences are listed in Table 1. The data were analyzed using the $2 - ^{\Delta\Delta ct}$ method.

Table 1
Primer sequences for LINC02085

Gene	Amplicon Size (bp)	Forward primer (5'→3')	Reverse primer (5'→3')
β-actin	96	CCCTGGAGAAGAGCTACGAG	GGAAGGAAGGCTGGAAGAGT
LINC02085	82	GAGTCTGATTCTTTCAAGGTGG	TTTCATTCTTCTGTCTGTGGC

Enzyme-linked immunosorbent assay (ELISA)

The supernatants of treated RA-FLS were collected and centrifuged at 1500 × g for 10 min at 4 °C. The concentrations of TNF-α (Cat No. JYM0110Hu) and TNFAIP2 (Cat No. JYM2468Hu) in supernatants were determined using the corresponding enzyme-linked immunosorbent assay (ELISA) Kits (Sangon Biotech) according to the manufacturers instructions. The absorbance at 450 nm was read using a Microplate Reader (BioTek, USA). The concentrations of TNF-αand TNFAIP2 were calculated based on the standard curve.

Cell migration assay

After 48 h, RA-FLS were digested using 0.25% trypsin (Gibco; Thermo Fisher Scientific, Inc.). 3 × 104 RA-FLS were plated to the top chamber of transwell (24-well insert, pore size, 8 μm, Coring, USA) precoated with Matrigel (BD, Bioscience). Then, 600 μL of DMEM containing 10% FBS was added to the lower chamber. After 24 h of incubation at 37 °C, the RA-FLS in the upper chamber were carefully removed with cotton swabs, and cells in the lower chamber were washed with PBS. Afterwards, cells were fixed with 4% paraformaldehyde for 20 min and stained with 0.1% crystal violet for 30 min. Positive stained cells were observed under a microscope (Olympus) and counted at four randomly selected fields.

Cell invasion assay

Transwell invasion assay was carried out following a similar procedure as the Transwell migration assay. In brief, wells in a 96-well plate were pre-coated with 5 μg of Matrigel (BD Matrigel matrix, Matrigel basement membrane matrix, Biosciences). Matrigel was diluted 1:5 with DMEM. RA-FLS were transfected for 48 h and suspended in 100 μL serum-free medium at a final concentration of 3×10^4 cells/ml were seeded in the upper well. Similarly, in each lower chamber, 600 ml of DMEM medium with 10% FBS was added. Microscopic visualization and cells counting were conducted as described with the migration assays.

Western blotting (WB)

The RA-FLS were lysed by RIPA lysis buffer (Sangon Biotech), following which cytoplasmic and nuclear proteins were extracted from RA-FLS using a commercial kit (Pierce, Rockford, IL, USA). Each sample (25 mg protein) was prepared for electrophoresis running on 10% SDS/PAGE gel and then transferred onto PVDF membranes (Millipore). The membrane was then blocked in a 5% nonfat dry milk/Tris-buffered solution and incubated at 4 °C with primary antibody overnight: rabbit anti-human anti-*p*-PI3K

(dilution, 1:1000; cat. No. ab182651), rabbit anti-human anti-*p*-AKT (dilution, 1:2000; cat. No. 4060s). The membrane was then washed in PBST for three times, and incubated with the horseradish peroxidase (HRP)-labeled goat anti-rabbit immunoglobulin G (IgG) secondary antibody (dilution, 1:20000, ab6721) at 37 °C for 1.5 h. Lastly, the proteins were detected by enhanced chemiluminescence (ECL, Millipore, USA). The densities of the bands were quantified by Image J Software (version 1.45, NIH, USA).

Immunofluorescence assay (IF)

The RA-FLS were fixed with 4% paraformaldehyde at 48 h post transfection, washed three times with PBS and blocked with 2% BSA in PBS for 15 min. After blocking, cells were incubated with primary antibodies diluted in blocking buffer overnight at 4 °C. The RA-FLS were washed three times with PBS and incubated for 1 h with fluorophore-conjugated secondary antibodies (1:1000; Invitrogen) in blocking buffer. Cell nuclei were stained with DAPI (Invitrogen). Images were acquired using a Zeiss LSM710 confocal microscope.

Association rule mining (ARM)

The clincial indexes rise was set to “T”, while the clincial indexes decline was set to “F”. The Aprior module of SPSS Clementine 11.1 software was used to analyze the correlation between observation clincial indexes.The most famous association rule is the Apriori algorithm, which aims to find out the relationship between items in a data set, also known as shopping blue analysis. In our data, each drug was treated as a variable[21]. The formulae were as follows:

$$\text{support}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{N},$$

$$\text{counfidence}(X \rightarrow Y) = \sigma \frac{(X \cup Y)}{\sigma(X)},$$

$$\text{lift}\ (X \rightarrow Y) = \text{counfidence}\ \frac{(X \rightarrow Y)}{\sigma(Y)},$$

where $X \rightarrow Y$ is an association rule, X (left-hand side [LHS]) and Y (right-hand side [RHS]) represent the set of LINC02085, $\sigma(X)$ is the frequency of itemset X, $X \cup Y$ is the union of itemset X and Y, $\sigma(X \cup Y)$ is the frequency with which itemset X and itemset Y appear together, support($X \rightarrow Y$) is the frequency with which X and Y appear together, and confifidence($X \rightarrow Y$) is the probability that itemset Y appears in the presence of X. The lift is the ratio of the probability of itemset Y appearing in the presence of X to the frequency of Y. Support and confifidence are often used to eliminate meaningless combinations; lift is the validity of the rules.

Statistical analysis

Statistical analyses were performed with GraphPad Prism software 8.0 (GraphPad). Data are represented as the mean ± SD or median (interquartile ranges) and analyzed using Students t-test or one-way ANOVA. A Chi square test was used to compare categorical variables. Spearman correlation analysis was introduced to evaluate the correlations between the LINC02085 with the items of ESR, RF, CCP, DAS28, et al. Logistic regression analysis was used to identify the independent risk factors of LINC02085. A statistically significant difference was defined as $p < 0.05$.

Results

Characteristics of the study subjects

An independent cohort consisting of 30 RA patients and 30 HC were enrolled in the validation set for evaluation of abnormal LINC02085. The characteristics of the study subjects are summarized in Table 2. There were no significant differences between RA patients and HC regarding age or sex. However, patients with RA had higher ESR, CRP, RF, CCP, IGA, IGG, IGM, C4, C4, DAS28, VAS, SAS and SDS than those in HC ($p < 0.05$; Table 2).

Table 2
The general characteristics of RA patients

Variables	RA(n = 30)	HC(n = 30)	Normal range
Sex(M/F)[a]	3/27	3/27	NA
Age(year)[a]	45.73 ± 10.88	45.63 ± 10.96	NA
Duration of disease (year)	7.03 ± 4.61	NA	NA
ESR (mm/h)[b]	61.00(32.50, 82.25)	3.91(2.96, 4.69)	2.0–6.0
Hs-CRP (mg/L)[b]	41.02(18.79, 89.54)	2.15(1.35, 2.90)	0.00–3.00
RF (U/ml)[b]	117.71(44.90, 271.35)	8.26(6.92, 10.45)	0.0–14.0
CCP (U/ml)[b]	97.65(32.15, 210.50)	2.12(1.54, 2.89)	< 4.00
IGA (g/L)[b]	2.77(2.31, 3.12)	2.48(1.82, 3.28)	0.70–4.06
IGG (g/L)[b]	15.84(13.99, 17.02)	12.06(9.19, 16.31)	6.80–14.50
IGM (g/L)[b]	1.78(1.51, 2.42)	1.81(1.14, 2.63)	0.34–2.14
C3 (g/L)[b]	1.56(1.35, 1.76)	1.09(0.62, 1.32)	0.9–1.8
C4 (g/L)[b]	0.38(0.27, 0.46)	0.38(0.27, 0.43)	0.1–0.4
DAS28 score	6.54(5.77, 7.47)	NA	< 2.6
VAS score[b]	7.00(6.00, 8.00)	3.78(3.06, 4.27)	NA
SAS score[b]	55.25(54.38, 62.50)	82.86(77.75, 87.24)	NA
SDS score[b]	55.00(51.25, 65.00)	76.60(71.95, 80.48)	NA
ESR: Erythrocyte sedimentation rate, hs-CRP: high-sensitivity C-reactive protein, RF: rheumatoid factor, CCP: anti-cyclic citrullinated peptide antibody, IGA: immun-oglobulin A, IGG: immunoglobulin G, IGM: immunoglobulin M, C3: complement 3, C4: complement 4, DAS: Disease Activity Score, VAS: Visual Analogy Scale, SAS: Anxiety Self-Assessment Scale, SDS: Depression Self-Assessment Scale.			
[a] $p > 0.05$, [b] $p < 0.01$			

The expression of LINC02085 in PBMCs of RA patients

To explore the levels of LINC02085 in patients with RA, RT-qPCR was performed and the results showed significantly higher levels of LINC02085 in patients with RA compared with HC ($p < 0.01$; Fig. 1A). To evaluate the diagnostic value of LINC02085, receiver operating characteristic (ROC) curve analysis was

performed. The AUC of LINC02085 was 0.8861 (95% CI 0.7958—0.9765), which suggested that LINC02085 had potential diagnostic value for RA patients (Fig. 1B).

Spearman correlation analysis of LINC02085 with clinical characteristics of RA patients

In addition, Spearman correlation analysis showed that the expression level of LINC02085 levels in RA PBMCs positively correlated with age (r = 0.6241, p = 0.0002; Fig. 1A), ESR (r = 0.6109, p = 0.0003; Fig. 1B), CRP (r = 0.5254, p = 0.0029; Fig. 1C), RF (r = 0.6521, p < 0.0001; Fig. 1D), CCP (r = 0.7882, p < 0.0001; Fig. 1E) and DAS28 (r = 0.8456, p < 0.0001; Fig. 1F). All these findings revealed that the abnormal expression of LINC02085 could be involved in the biology of RA.

Association rule analysis of LINC02085 with clinical characteristics of RA patients

Then we conducted an association rule analysis to determine the confidence, support, and lift value of LINC02085 and clinical characteristics of RA patients. The results are shown that the confidence and support value of LINC02085 and clinical characteristics both higher than 80%, and the degree of lift was more than 1 and P□0.05 through Aprior module analysis (Table 3).

Table 3
Association rules analysis of LINC02085 and clincial characteristics

Items(LHSRHS)	Support	Confidence	Lift	*P* value
{ LINC02085 ↑ }{ ESR ↑ }	83.78%	92.72%	1.15	□0.01
{ LINC02085 ↑ }{ CRP ↑ }	83.85%	91.43%	1.04	□0.01
{ LINC02085 ↑ }{ RF ↑ }	81.35%	91.83%	1.04	□0.01
{ LINC02085 ↑ }{ IGA ↑ }	83.95%	87.91%	1.15	□0.01
{ LINC02085 ↑ }{ IGG ↑ }	87.07%	87.55%	1.15	□0.01
{ LINC02085 ↑ }{ C3 ↑ }	81.15%	87.55%	1.05	□0.01

Logistic regression analysis of LINC02085 with clinical characteristics of RA patients

To assess risk factors for LINC02085, logistic regression analysis was carried out. Significant differences in LINC02085 was found between RA patients with ESR (p = 0.023), RF (p = 0.000), CCP (p = 0.013) and DAS28 (p = 0.002), indicating that ESR, CRP, RF and DAS28 were risk factors for LINC02085, the higher expression of ESR, CRP, RF and DAS28, the higher expression of LINC02085 (Fig. 3).

The expression of LINC02085 in RA-FLS

To evaluate the expression level of LINC02085 in RA-FLS, we detected LINC02085 by RT-qPCR analysis in RA-FLS. A significant upregulation in the expression level of LINC02085 was observed in the RA-FLS were stimulated with TNF-αcompared with RA-FLS (Fig. 4). Also, the efficiency of overexpression and knockdown was assessed by RT-qPCR. The results suggested that LINC02085 was significantly elevated

by the transfection of LINC02085 overexpression vector, and the knockdown of LINC02085 resulted in significantly reduced LINC02085 expression (Fig. 4).

Effects of LINC02085 aberrant expression on cell proliferation

Furthermore, the cell viability of RA-FLS was remarkably increased by the TNF-α level relative to that in RA-FLS (Fig. 5). Consistently, TNF-αinduced cells with pc-DNA3.1 LINC02085 showed higher cell viability relative to the TNF-αinduced cells (Fig. 5). In addition, the cell viability was dramatically decreased in TNF-αinduced cells with si-LINC02085 relative to TNF-αinduced cells (Fig. 5).

Effects of LINC02085 aberrant expression on cell migration and invasion

To explore the effects of LINC02085 on RA-FLS migration and invasion ability, we performed a migration assay and an invasion assay using Boyden chamber. Transwell migration and invasion assays showed that TNF-α increased the ability of the migration and invasion of RA -FLS significantly, overexpression of LINC02085 effectively increased the migration and invasion ability of the RA-FLS, while knockdown of LINC02085 inhibited the migration and invasion of the RA-FLS (Fig. 6A-B).

Effects of LINC02085 aberrant expression on inflammatory cytokines

The ELISA results revealed that TNF-αtreatment enhanced the levels of TNF-αand TNFAIP2. Furthermore, TNF-αand TNFAIP2 levels were remarkably reduced when TNF-αinduced cells were transfected with si-LINC02085 compared with those in TNF-αinduced cells (Fig. 7A-B). Furthermore, TNF-αinduced cells with pcDNA3.1-LINC02085 showed higher TNF-αand TNFAIP2 levels than TNF-αinduced cells (Fig. 7A-B).

Effects of LINC02085 aberrant expression PI3K/AKT pathway

To further find the possible correlation of LINC02085 abnormal expression and the pathway involved in RA, we performed experiments to detect the expression of PI3K/AKT pathway–related proteins. In relation to the untreated cells, the proteins expression of *p*-PI3K and *p*-AKT were significantly upregulated in TNF-αinduced cells (Fig. 8A-C). The TNF-αinduced cells transfected with pcDNA31.-LINC02085 showed dramatically enhanced relative protein expression of *p*-PI3K and *p*-AKT compared with those in TNF-αinduced cells (Fig. 8A-C), whereas the protein levels were significantly reversed in the TNF-αinduced cells transfected with si-LINC02085 compared with those in the TNF-αinduced cells (Fig. A-C). Similar results were also illustrated in the hippocampus by immunofluorescence analysis (Fig. 8E-F). These findings indicated that the PI3K/ AKT signal pathway could be activated by the highly expressed LINC02085 in RA.

Discussion

Expanding numbers of studies have documented that lncRNAs play critical roles in physiological and pathological responses in different human disease including RA[22; 23]. The current study firstly provided evidence that LINC02085 was overexpressed in PBMCs of patients with RA compared with the healthy participants. The LINC02085 levels in RA blood samples positively correlated with those of age, ESR, CRP, RF, CCP and DAS28. Additionally, there are high confidence, support and lift value between LINC02085 and clinical characteristics. Meanwhile, ESR, RF, CCP and DAS28 are independent risk factors for

LINC02085. Further analysis showed that overexpression of LINC02085 promoted RA-FLS proliferation, migration and invasion ability, enhanced the inflammatory response by increasing the levels of TNF-αand TNFAIP2. Furthermore, upregulated LINC02085 remarkably increased the expression of *p*-PI3K and *p*-AKT. However, the opposite results results were observed for LINC02085 knockdown. LINC02085 offers promising therapeutic strategy for RA patients.

An increasing number of studies have indicated that abnormal lncRNAs expression is involved in various immune-mediated diseases and serves an important role in regulating the inflammatory response and cell growth[24]. A study revealed that lncRNA PVT1 can regulate the proliferation and inflammatory responses of RA-FLS by targeting microRNA-145-5p[25]. Another additional study demonstrated that inhibiting role of LINC01197 in inflammation in RA through the microRNA-150/THBS2 axis[26]. The present study found the upregulation of LINC02085 in RA. A recent study further demonstrated that abnormal expression levels of lncRNA in RA closely related to the severity of symptoms[27; 28]. Similarly, our study revealed a positive correlation of LINC02085 levels with those of ESR, CRP, RF and DAS28, indicating LINC02085 levels were associated with the severity of RA. In addition, the current study found that upregulated LINC02085 enhanced proliferation, migration, and invasion ability of cells. RA-FLS proliferation, migration and invasion are the most important pathologic features of RA, which together with inflammatory responses affect and promote each other, and involved in the pathogenesis of RA[29]. Our results suggested that overexpression of LINC02085 could promote RA-FLS cell growth. The previous studies have confirmed that RA-FLS could secrete various kinds of inflammatory cytokines, thereby directly aggravating the inflammatory response[30; 31]. Our findings showed that inflammatory cytokines of TNF-αand TNFAIP2 were dramatically increased in TNF-α-treated RA-FLS transfected with pcDNA3.1-LINC02085; however, the levels were reversed when LINC02085 was suppressed, implying that the reduction in LINC02085 expression could suppress cell inflammatory response. Thus, we speculated that LINC02085 might participate in the occurrence and development of RA by involving in cell growth and inflammatory response of FLS.

To further find out the potential correlation and mechanism of LINC02085 at the level of the signal pathway in RA-FLS, we selected the PI3K/AKT signaling pathway based on the following aspects. First, the PI3K/AKT signaling pathway has been showed to articipate in the pathogenesis of RA and may serve as an important target in RA therapies. For example, Huang et al. demonstrated that miR-26a-5p enhances cells proliferation, invasion, and apoptosis resistance of FLS in RA by regulating PTEN/PI3K/AKT pathway[32]. Similarly, Li reported that cinnamaldehyde attenuates the progression of RA through down-regulation of PI3K/AKT signaling pathway[33]. Second, lncRNA THRIL mediates cell growth and inflammatory response of FLS by activating PI3K/AKT signals in RA[16]. Based on these findings, we speculated the existence of a possible direct or indirect regulatory correlation between LINC02085 and the PI3K/ AKT pathway. Our findings showed that overexpression and knockdown of LINC02085 retrained the protein expression of *p*-PI3K and *p*-AKT, which verified our speculation. It can be said that PI3K/AKT not merely involved in immune-mediated responses, but also mediates important signaling transduction in cell biological progression. The activation of *p*-PI3K could first induce the downstream *p*-AKT, followed by inducing high expression levels of inflammatory factors TNF-αand

TNFAIP2, subsequently increased proliferation, migration and invasion ability. This study concluded that LINC02085 could induce the PI3K/ AKT signaling pathway activation. Therefore, we speculated that LINC02085 mediates cell growth and inflammatory response of RA-FLS by activating PI3K/AKT signals.

In summary, the present study revealed that LINC02085 was highly expressed in RA and its abnormal expression could regulate the cell growth and inflammatory response of RA-FLS by activating the PI3K/AKT signaling pathway through affecting proliferation, migration and invasion ability of RA-FLS, thereby promoting the occurrence and development of RA. Additionally, the model of sponge which lncRNA interacts with other miRNA may be one of the important regulatory mechanisms in cells. Based on the the current findings, we have great interests in this research, our research team is attempting to explore the possible ceRNA molecular mechanism of LINC02085 in RA in our future work.

Abbreviations

PBMCs: Peripheral blood mononuclear cells; FLS: Fibroblast-like synoviocytes; RA: Rheumatoid arthritis; TNF-α: Tumor necrosis factor-α; LncRNA: Long non-coding RNA; PI3K: phosphoinositide-3-kinase; AKT: protein kinase B; AS: Ankylosing spondylitis; OA: Osteoarthritis; SLE: Systemic lupus erythematosus; ESR: erythrocyte sedimentation rate; Hs-CRP: high-sensitivity C-reactive protein; RF: rheumatoid factor; CCP: anti-cyclic citrullinated peptide antibody; IGA: Immunoglobulins A; IGG: Immunoglobulin G; IGM: Immunoglobulin M; C3: Complement 3; C4: Complement 4; SPP: Self-perception of patients; DAS28: Disease activity score; VAS: Visual analog scale; SAS: Anxiety self-assessment Scale; SDS: Depression self-assessment scale; CCK-8: Cell Counting Kit 8; RT-qPCR: Quantitative real-time PCR; ELISA: Enzyme-linked immunosorbent assay; WB: Western blotting; IF: Immunofluorescence assay; ARM: Association rule mining.

Declarations

Acknowledgements

Not applicable.

Authors’contributions

WJT and LJ contributed to the study design. WJT contributed to data analysis, wrote the first draft, and revised the manuscript. WX and WJ contributed to the specimen and data collection. LJ supervised the project and contributed to the manuscript revision. All authors reviewed and accepted the content of the final manuscript.

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Availability of data and materials

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

Consent for publication

All authors read and approved the paper.

Competing interests

The authors declare that they have no competing interests.

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Figures

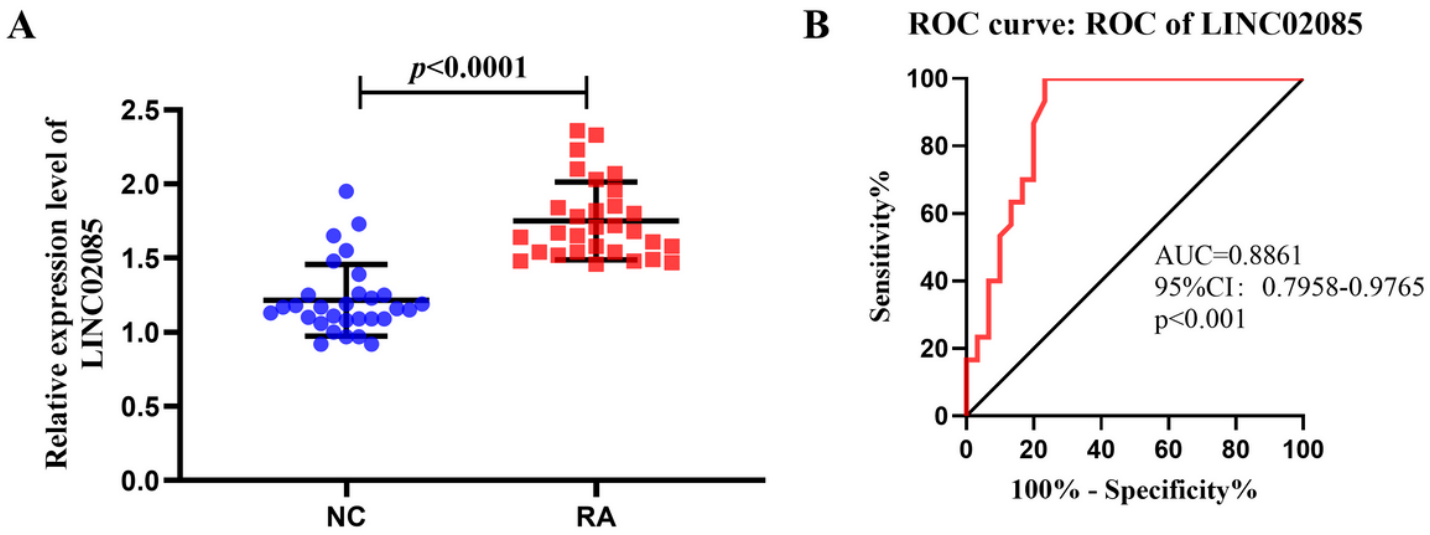

Figure 1

Validation and ROC curve analysis of abnormally expressed LINC02085 in PBMCs of RA patients. (A) The expression of LINC02085 in patients RA was significantly higher compared with that in HC. (B) ROC curve analysis of PBMC LINC02085 in RA patients vs. HC.

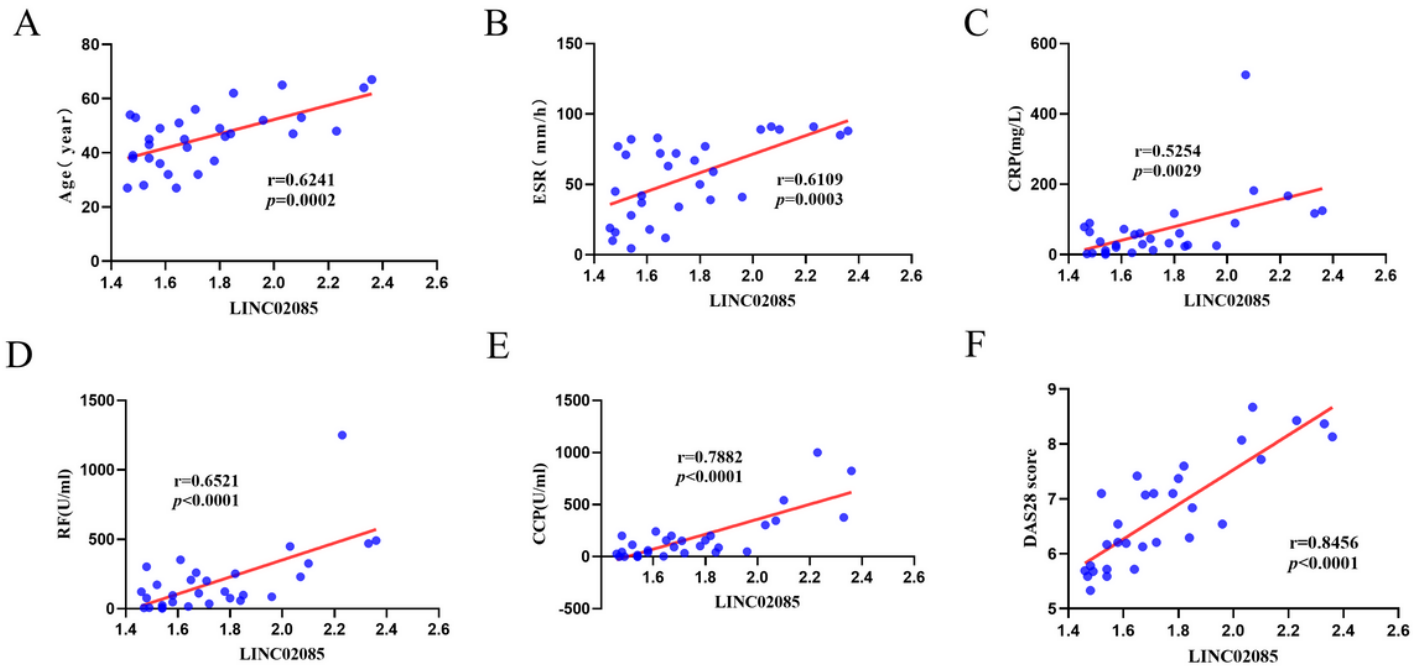

Figure 2

Correlation of PBMC LINC02085 expression with clinical characteristics of RA. (A, B, C, D, E, F) The expression of PBMC LINC02085 in RA patients was positively correlated with age, ESR, CRP, RF, CCP, DAS28.

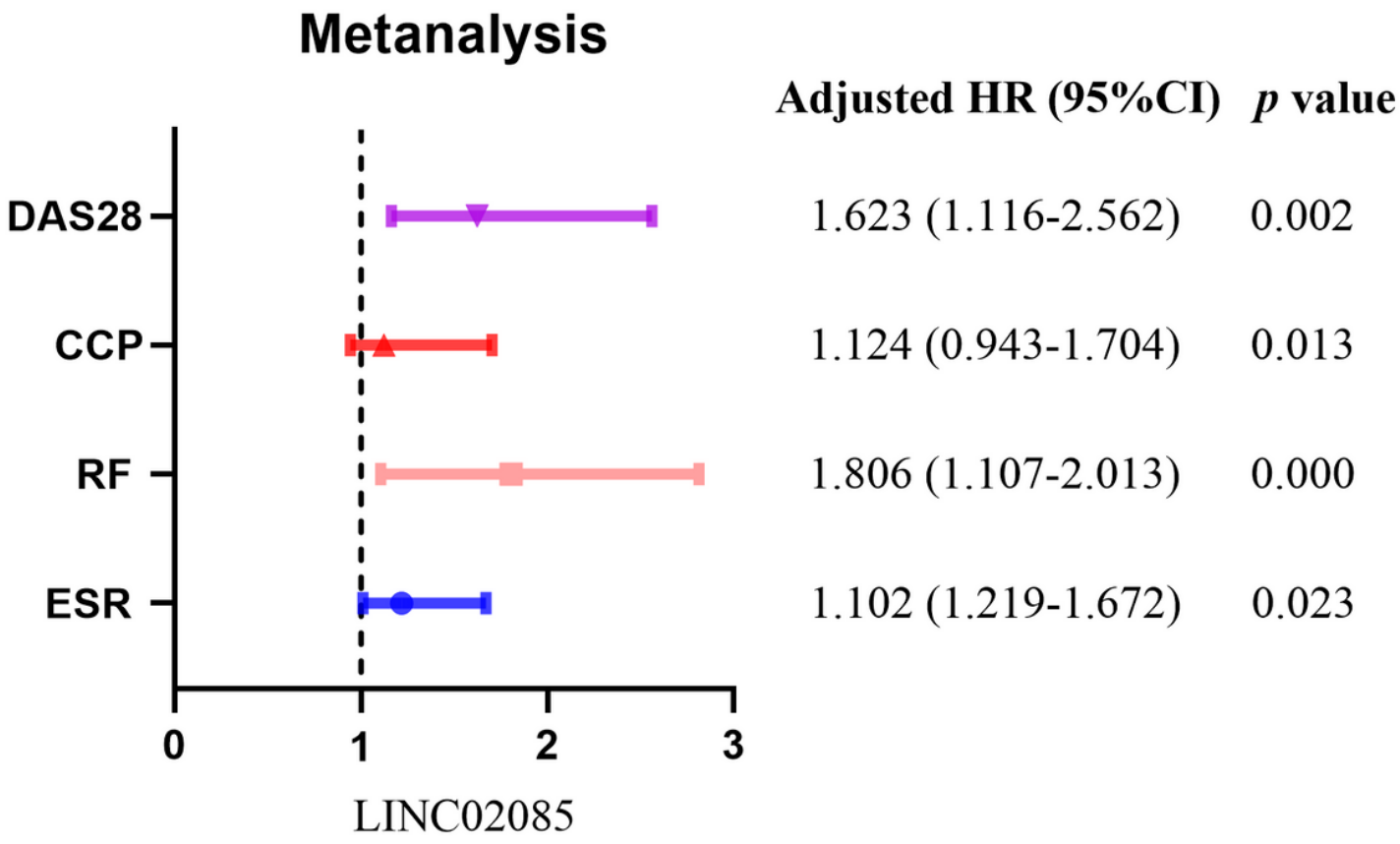

Figure 3

Logistic regression analysis of LINC02085 with clinical characteristics.

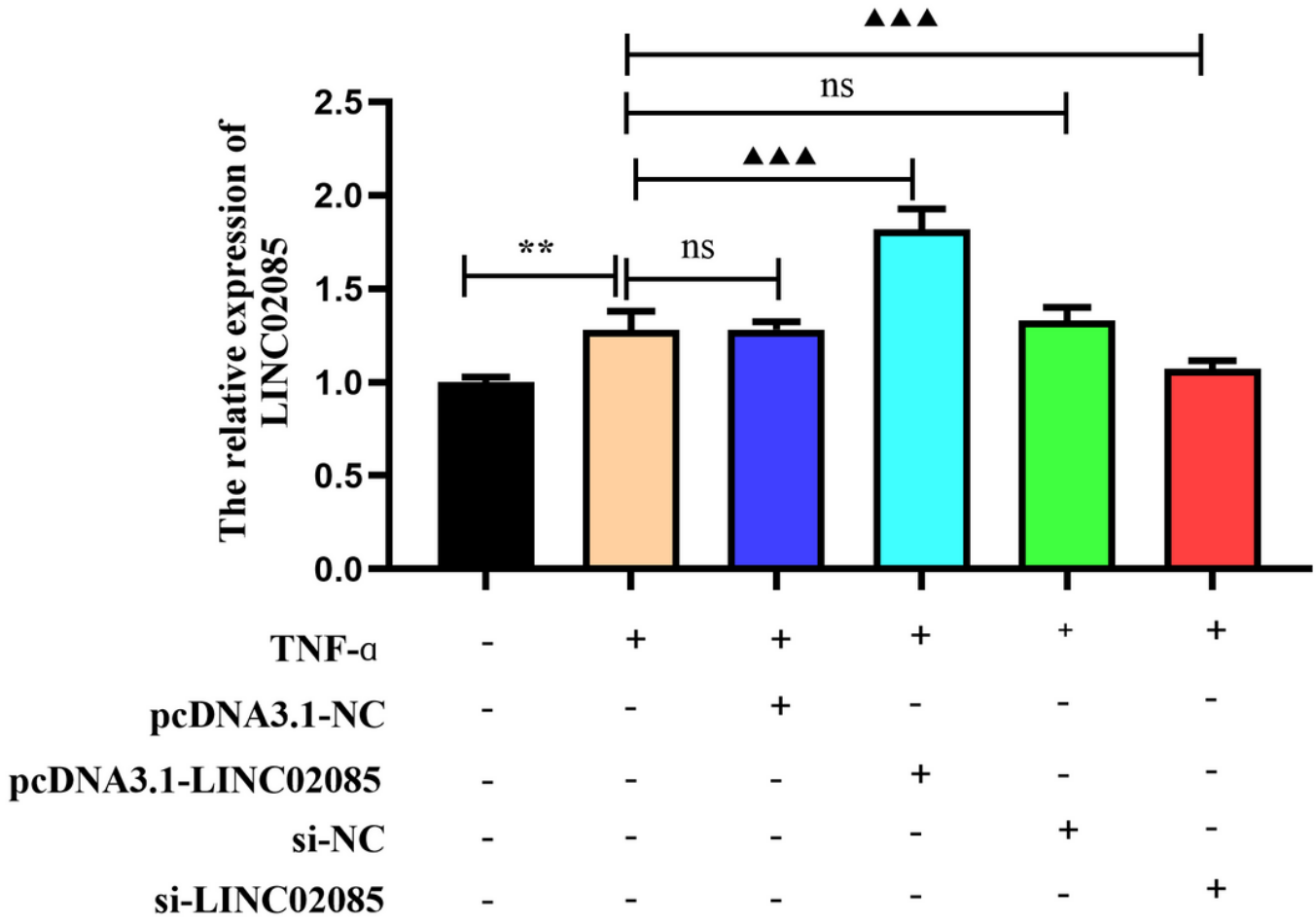

Figure 4

The expression of LINC02085 in RA-FLS. The LINC02085 level was detected in control RA-FLS, the cells were stimulated with TNF-α(10 ng/ml), the cells with pcDNA3.1-NC and TNF-α, pcDNA3.1-LINC02085 and TNF-α, si-NC and TNF-α, and si-LINC02085 and TNF-αby qRT-PCR. All experiments were repeated three times and data are expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with RA-FLS, ▲p < 0.05, ▲▲p < 0.01, ▲▲▲p < 0.001 compared with TNF-α+RA-FLS.

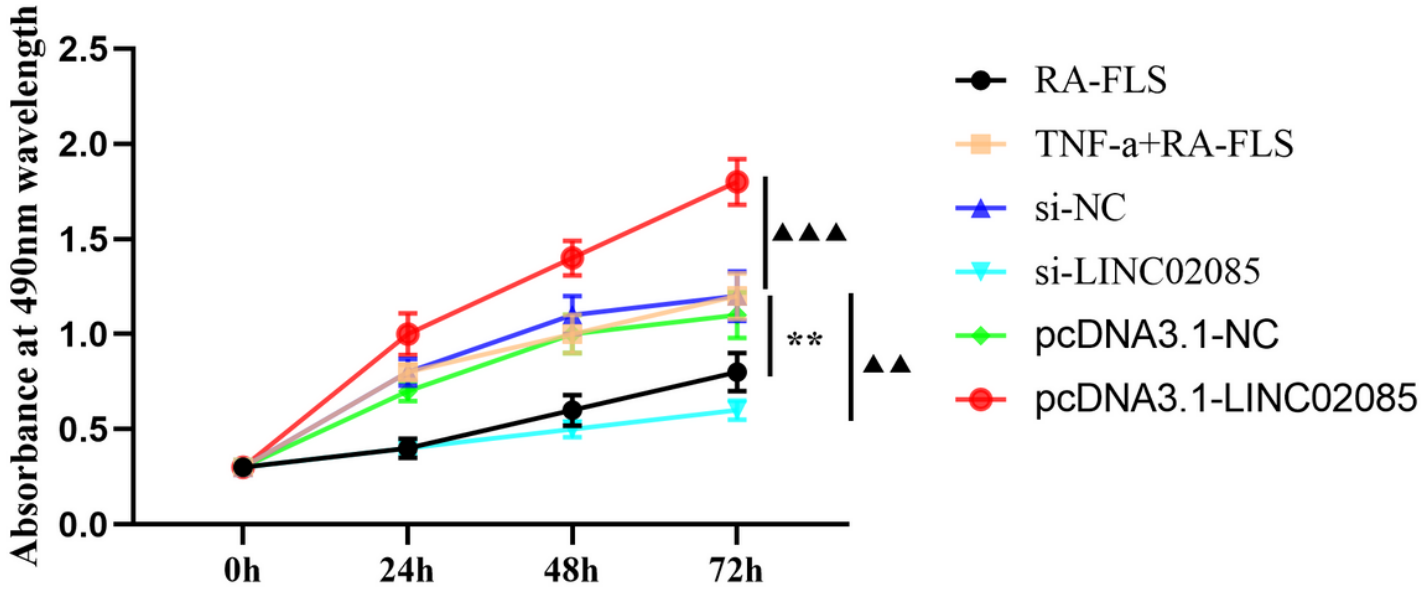

Figure 5

Effects of LINC02085 aberrant expression on cell proliferation. Cell viability was measured in control RA-FLS, the cells transfected with TNF-α, pcDNA3.1-NC and TNF-α, pcDNA3.1-LINC02085 and TNF-α, si-NC and TNF-α, and si-LINC02085 and TNF-αby CCK8 assay. All experiments were repeated three times and data are expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with RA-FLS, ▲p < 0.05, ▲▲p < 0.01, ▲▲▲p < 0.001 compared with TNF-α+RA-FLS.

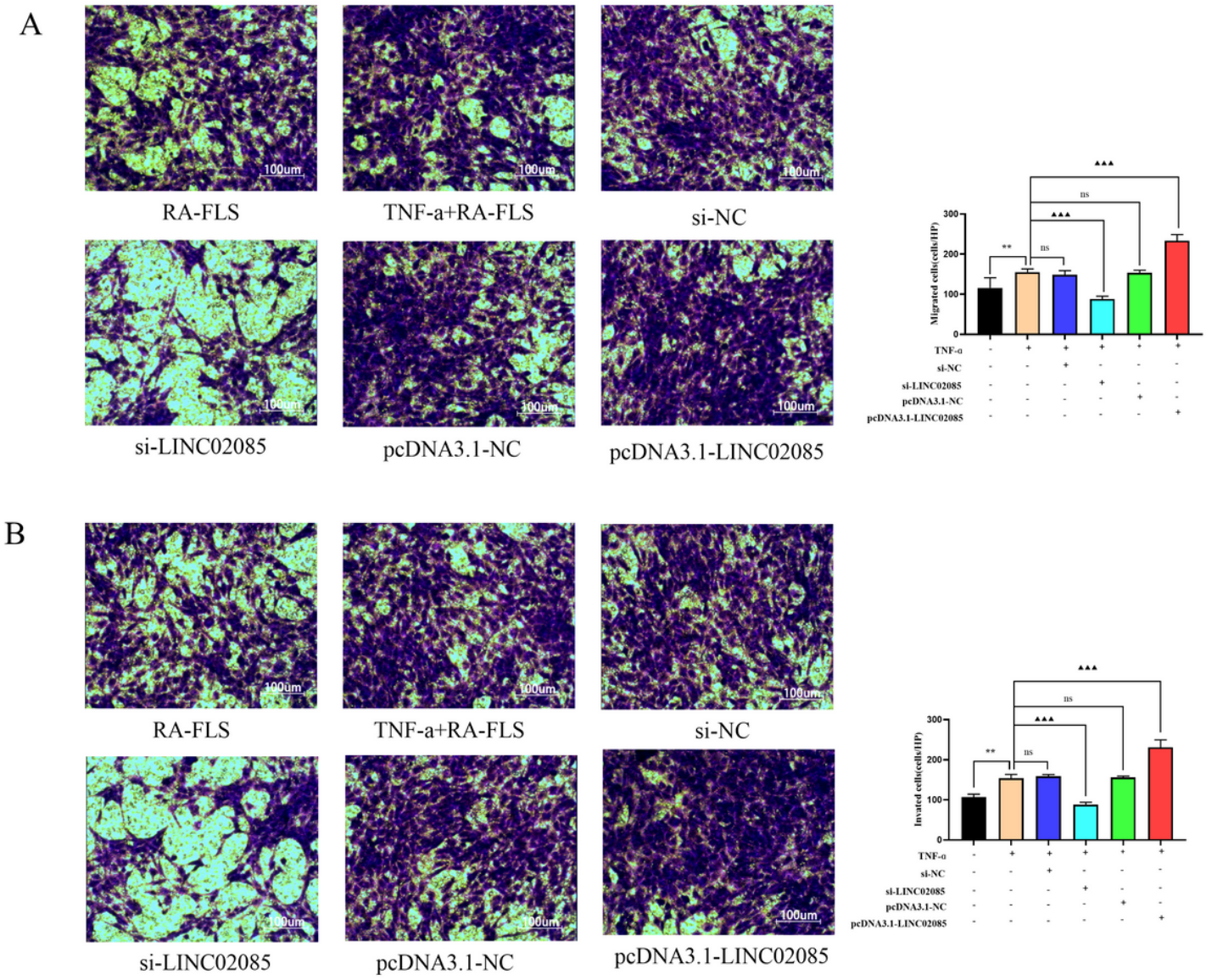

Figure 6

Effects of LINC02085 aberrant expression on cell migration and invasion. (A) The transwell assays revealed that LINC02085 overexpression could promote the migration of RA-FLS, and LINC02085 knockdown could inhibit the migration of RA-FLS. (B) The transwell assays revealed that LINC02085 overexpression could promote the invasion of RA-FLS, and LINC02085 knockdown could inhibit the invasion of RA-FLS. All experiments were repeated three times and data are expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with RA-FLS, ▲p < 0.05, ▲▲p < 0.01, ▲▲▲p < 0.001 compared with TNF-α+RA-FLS.

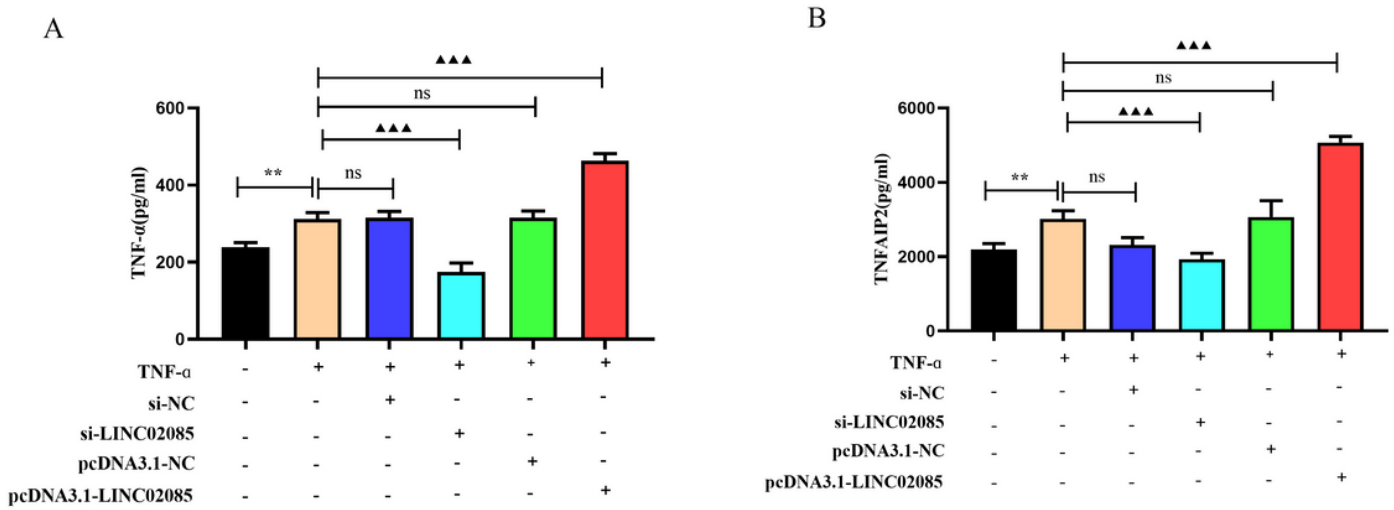

Figure 7

Effects of LINC02085 aberrant expression on inflammatory factors. (A, B) The contents of TNF-αand TNFAIP2 were detected in control RA-FLS, the cells transfected with TNF-α, pcDNA3.1-NC and TNF-α, pcDNA3.1-LINC02085 and TNF-α, si-NC and TNF-α, and si-LINC02085 and TNF-αby ELISA. All experiments were repeated three times and data are expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with RA-FLS, ▲p < 0.05, ▲▲p < 0.01, ▲▲▲p < 0.001 compared with TNF-α+RA-FLS.

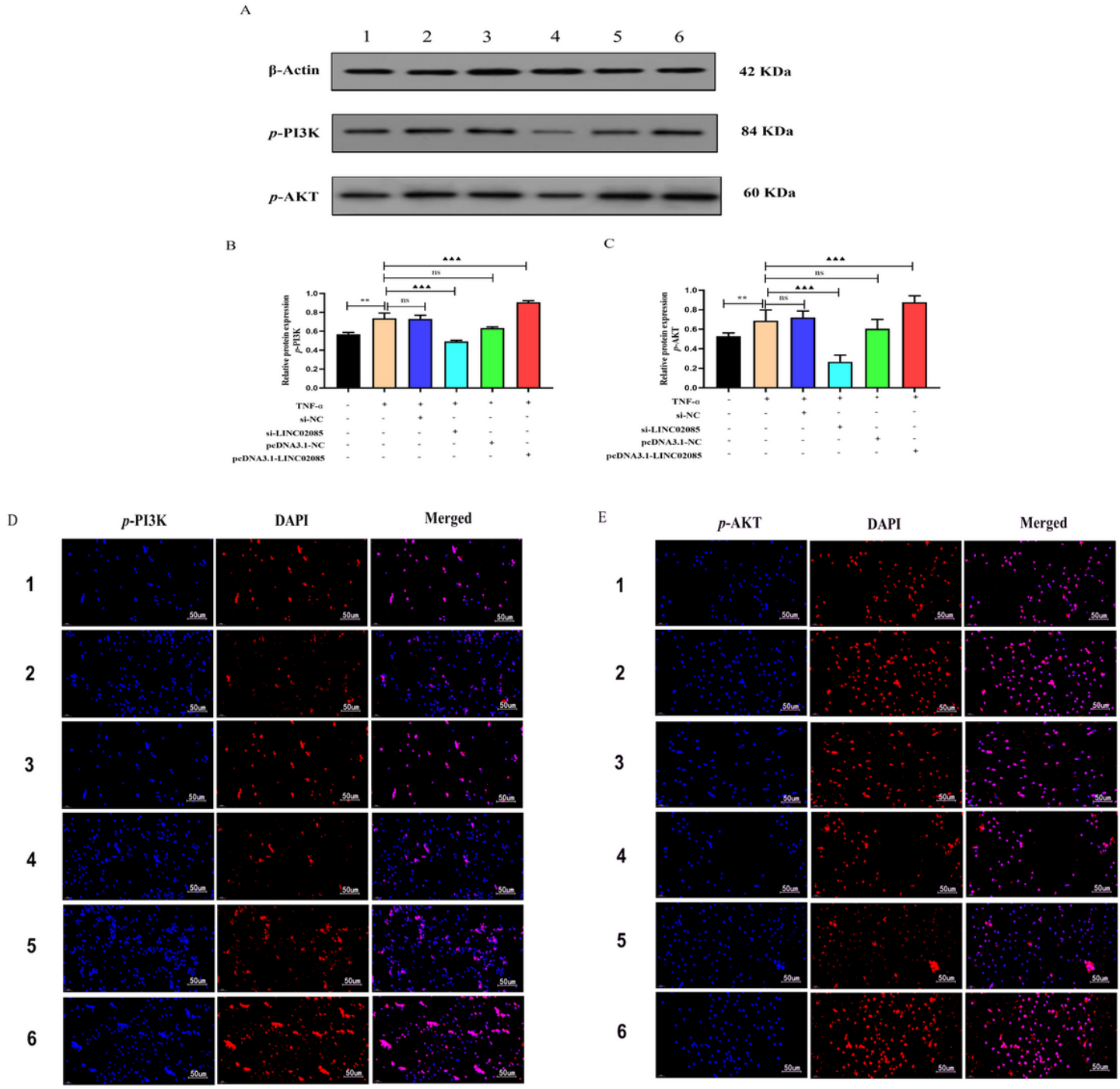

Figure 8

Effects of LINC02085 aberrant expression PI3K/AKT pathway. (A, B, C) The relative protein expressions of p-PI3K and p-AKT were detected in control RA-FLS, the cells transfected with TNF-α, pcDNA3.1-NC and TNF-α, pcDNA3.1-LINC02085 and TNF-α, si-NC and TNF-α, and si-LINC02085 and TNF-αby WB. (D, E) The relative protein expressions of p-PI3K and p-AKT were detected by IF. All experiments were repeated three times and data are expressed as mean ± SD. *p < 0.05, **p < 0.01, ***p < 0.001 compared with RA-FLS, ▲p < 0.05, ▲▲p < 0.01, ▲▲▲p < 0.001 compared with TNF-α+RA-FLS. 1 representes RA-FLS group, 2

representes TNF-α+ RA-FLS group, 3 representes si-NC group, 4 representes si-LINC02085 group, 5 representes pcDNA3.1-NC group, 6 representes pcDNA3.1-LINC02085 group.