

RESEARCH

Open Access



# Potential role of RhoA GTPase regulation in type I interferon signaling in systemic lupus erythematosus

Wei Fan<sup>1\*</sup>, Bo Wei<sup>2</sup>, Xuyan Chen<sup>1</sup>, Yi Zhang<sup>1</sup>, Pingping Xiao<sup>1</sup>, Kaiyan Li<sup>1</sup>, Yi qin Zhang<sup>3</sup>, Jinmei Huang<sup>1</sup>, Lin Leng<sup>4</sup> and Richard Bucala<sup>4\*</sup>

## Abstract

**Objective** Systemic lupus erythematosus (SLE) is an autoimmune disorder characterized by abnormal activation of the type I interferon (IFN) pathway, which results in tissue inflammation and organ damage. We explored the role of the RhoA GTPase in the type I IFN activation pathway to provide a potential basis for targeting GTPase signaling for the treatment of SLE.

**Methods** Total RNA was extracted from peripheral blood mononuclear cells (PBMCs) of SLE patients and healthy controls, and the mRNA expression levels of RhoA and IFN-stimulated genes were measured by SYBR Green quantitative reverse transcriptase-polymerase chain reaction. IFN- $\alpha$ -stimulated response element (ISRE)-luciferase reporter gene assays and Western blotting were conducted to assess the biologic function of RhoA. An enzyme-linked immunoassay (ELISA) measured C-X-C motif chemokine ligand 10 (CXCL10) protein expression.

**Results** Our studies demonstrate that the expression of RhoA in the PBMCs of SLE subjects was significantly higher than in healthy controls and positively correlated with type I IFN scores and type I IFN-stimulated gene (ISGs) expression levels. siRNA-mediated knockdown of RhoA and the RhoA/ROCK inhibitor Y27632 reduced the activity of the type I IFN-induced ISRE, the signal transducer and activator of transcription 1 (STAT-1) phosphorylation, and the expression of CXCL10 and 2'-5'-oligoadenylate synthetase 1 (OAS1). Finally, we verified that Y27632 could significantly down-regulate the OAS1 and CXCL10 expression levels in the PBMCs of SLE patients.

**Conclusion** Our study shows that RhoA positively regulates the activation of the type I IFN response pathway. Reducing the expression level of RhoA inhibits the abnormal activation of the type I IFN system, and the RhoA/ROCK inhibitor Y27632 decreases aberrant type I IFN signaling in SLE PBMCs, suggesting the possibility of targeting the RhoA GTPase for the treatment of SLE.

**Keywords** Autoimmunity, Systemic lupus erythematosus, RhoA, Type I IFN, RhoA/ROCK inhibitor

\*Correspondence:

Wei Fan

fanwei1202003@163.com

Richard Bucala

Bucala.Richard@Yale.edu

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

## Background

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease that causes immune dysregulation and chronic inflammation, leading to progressive end-organ damage [1]. Studies over several decades have underscored the pathogenic complexity of SLE, but type I IFN produced by innate immune cells and a dysregulated activation of the adaptive immune system are considered important for the initiation and maintenance of the disease [2, 3]. Sustained activation of the type I IFN pathway leads to excessive production of numerous tissue-damaging inflammatory cytokines, which mediate many of the long-term pathologic sequelae in the skin, kidneys, and other organ systems [4–7].

The majority of patients with SLE exhibit abnormal expression of multiple type I IFN-inducible genes, known as the type I IFN signature [2, 3, 8]. The type I IFN expression signature determined in the tissues or serum of lupus patients is associated with pathogenesis, clinical manifestations, and disease activity [9–12]. Previous reports have shown that the therapeutic use of IFN- $\alpha$ , a major type I IFN family member, can induce an SLE-like syndrome, while blocking the action of type I IFNs or their common receptor reduces immunological dysfunction and decreases tissue damage in SLE [13–16].

RhoA is a member of the Rho-GTPase family that has a crucial role in a variety of biologic processes, including cell adhesion and migration, apoptosis, and in the regulation of immunologic functions [17–20]. RhoA exerts its major functions by activating Rho-associated serine/threonine protein kinases (ROCKs), which include the two isoforms: ROCK1 and ROCK [19]. Activation of the RhoA/ROCK pathway in autoimmune disease has been well described, although knowledge of its specific contribution to SLE is only emerging [17]. Previous studies have reported that inhibition of RhoA/ROCK signaling significantly reduced anti-dsDNA antibody levels in lupus-prone NZB/NZW F1 mice, effectively alleviating renal damage and reducing mortality [21]. Our previous study has demonstrated a significant increase in RhoA expression in lupus T cells [22]. In addition, abnormal activation of the RhoA-ROCK pathway has been observed in SLE patients, and targeting this pathway has the potential to rescue T cell dysfunction. Analyses of gene chip expression data have indicated that treatment with the RhoA/ROCK inhibitor Y27632 may affect elements of the type-I IFN signaling pathway [23, 24]. Furthermore, downstream of the IFN- $\alpha$ /b receptor (IFNAR), RhoA serves as an important signaling effector, promoting the survival of B cells by preventing IFN- $\alpha$ /b-induced apoptosis [25]. These prior observations support a mechanistic link between RhoA and IFN- $\alpha$ /b-mediated immune responses. The PBMCs of a majority of lupus patients

also exhibit up-regulated ROCK activity [24]. In the present report, RhoA mRNA expression further is shown to be higher in the PBMCs of lupus patients when compared to healthy controls, and expression levels correlate positively with type I IFN scores and interferon-induced genes, including CXCL10, OAS1, IFIT3, and MX1.

We hypothesize that the overexpression of RhoA leads to pathogenic activation of the type I IFN pathway, with consequences for immune activation in SLE. Accordingly, we examined the activation state of RhoA and its downstream actions in the PBMCs of lupus patients and assessed the consequences of RhoA pathway inhibition on selected type I IFN-induced genes relevant to immune dysregulation.

## Materials and methods

### Study subjects

We enrolled 36 SLE patients admitted to the Second Affiliated Hospital of Xiamen Medical College and defined according to the American College of Rheumatology 1997 revised criteria for SLE. Thirty-six age- and sex-matched healthy controls were drawn from volunteers with no personal history of autoimmune disease or immunosuppressive therapy. All participants signed written informed consent for this study. The SLEDAI-2000 assessment tool was used to quantify disease activity for the SLE patients [26]. The study was performed according to the current National Health and Family Planning Commission of China ethical standards and approved by the hospital ethics committee.

### Study samples

Approximately 15–25 ml of venous blood was drawn from each study subject into heparinized tubes and centrifuged at 2000 rpm for 10 min at 4°C to extract plasma, which was stored at –80°C. PBMCs were isolated from heparinized blood by density-gradient centrifugation using Ficoll-Paque Plus medium (GE Healthcare) in accordance with the manufacturer's instructions.

### Cells and reagents

Human THP1 monocytes or PBMCs were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal bovine serum (FBS/Gibco) and 1% penicillin–streptomycin (Hyclone, USA) in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. The pCMV6-RhoA plasmid overexpressing human-RhoA and pCMV6-Ctrl plasmid were purchased from ORIGENE. The two plasmids were confirmed by DNA sequencing and were prepared for transfection using the EndoFree Plasmid Maxi kit (Qiagen). The correct mRNA and protein expression of the clone plasmid were confirmed by reverse transcription-quantitative PCR (RT-qPCR) and Western blot analysis, respectively.

RhoA small-interfering RNA (siRNA) sequences and siRNA universal negative control were obtained from Sigma-Aldrich. The siRhoA sequences were as follows: sense: 5'-CAGAUACCGAUGUUAUACU-3' and anti-sense: 5'-AGUAUAACAUCGGUAUCUG-3'.

#### Cell transfection and stimulation

HEK-293 and THP1 cells were transfected with siRNAs (200 nM) or plasmid (4 mg/mL) controls using Lipofectamine RNAiMAX or Lipofectamine 2000 reagent following the manufacturer's recommendations (Invitrogen). At 24–48 h after transfection, the cells were stimulated with IFN- $\alpha$  (1000 U/mL, PBL Interferon Source) for 6 h. The RhoA/ROCK inhibitor Y27632 (30,60,90  $\mu$ M, Beyotime) was added 45 min before stimulation with IFN- $\alpha$  (1000 U/mL, PBL Interferon Source). IFN- $\alpha$  was used to activate IFNAR as a type I IFN stimulus.

#### Dual-luciferase reporter gene assay

An ISRE-luciferase construct (pISRE-TA-Luc) was used for monitoring the induction of the STAT1 and STAT2 components of the janus kinase (JAK)/STAT-mediated signal transduction pathways (Supplement Fig. 1). The pISRE-TA-Luc plasmid contains five copies of an ISRE enhancer element located upstream of the minimal TA promoter. Upon binding of the STAT1 and STAT2 heterodimer to the cis-acting ISRE enhancer element, transcription is induced and the luciferase reporter gene is activated. HEK-293 T cells were cultured in a 96-well plate and co-transfected with siRhoA (200 nM) or RhoA expression plasmids (4 mg/mL), or their controls together with a mixture of Renilla vector (10 ng, Promega) and the ISRE-luciferase reporter gene vector (100 ng, Clontech) for 24 h. IFN- $\alpha$  (1000 U/mL) then was added for an additional 6 h of incubation. The cells were harvested and lysed, and luciferase activity was measured using the Dual-Luciferase Reporter Assay System (Promega) and CYTATION3 (BioTek) instrumentation. The ratio of firefly luciferase activity to Renilla luciferase activity was calculated for each well. All experiments were performed in triplicate.

#### RNA extraction and real-time PCR (RT-PCR)

Total RNA was isolated using the RNeasy Mini kit (Qiagen). To quantify mRNA expression, complementary DNA (cDNA) was synthesized from 500 ng of total RNA with the SuperScriptIII RT Reagent kit (Invitrogen) and amplified by real-time PCR (iTaq™ Universal SYBR Greensupermix; Bio-RAD). The endogenous expression of GAPDH was used as the internal control. The relative expression levels were calculated using the  $2^{-\Delta\Delta Ct}$  method. All of the experiments were performed with a ViiA7 Real-Time PCR System (Applied Biosystems).

The primers for RT-PCR were as follow: for GAPDH, forward 5'-CTCCTCCTGTTCGACAGTCA-3' and reverse 5'-CAATACGACCAAATCCGTTG-3'; for RhoA, forward 5'-TCTTCGGAATGATGAGCAC-3' and reverse 5'-CTT TGGTCTTTGCTGAACAC-3'; for IFIT3, forward 5'-TGA GGTCACCAAGAATTCCCTG-3' and reverse 5'-CAA TCTGGTTACACACTCTATCTTC-3'; for OAS1, forward 5'-GAAGGCAGCTCACGAAAC-3' and reverse 5'-TTCTTAAAGCATGGGTAATTC-3', for MX1, forward 5'-GGGTAGCCACTGGACTGA-3' and reverse 5'-AGG TGGAGCGATTCTGAG-3'.

#### Calculation of type I IFN scores

Type I IFN scores were calculated according to the relative expression (RE) of the type I IFN-inducible genes MX1, OAS1, IFIT3, and CXCL10 (Supplement Fig. 2). The mean  $\pm$  SD expression level of these genes in the healthy control (hc) group (mean<sub>hc</sub> and SD<sub>hc</sub>) were used to standardize the expression levels per gene for each study subject [10, 27]. The type I IFN scores were calculated by summing up the individual RE of each gene after normalization to the healthy control value as follows:  $\sum(\text{RE}_{\text{subject}} - \text{Mean}_{\text{hc}}) / \text{SD}_{\text{hc}}$ . An IFN-score was regarded as positive when it was higher than the mean + 2SD of HC values [11].

#### Enzyme-linked immunosorbent assay (ELISA)

The CXCL10 concentrations of plasma samples and cell culture supernatants were analyzed by specific ELISAs (R&D Systems) in accordance with the manufacturer's instructions.

#### Western blotting

HEK-293 T cells were seeded into 6-well plates and  $5 \times 10^5$  HEK-293 T cells per well transfected with siRhoA (200 nM) or RhoA over-expression plasmids (4 mg/mL), or their controls; 48 h after incubation, the cells were treated with IFN- $\alpha$  (1000 U/mL) for an additional 6 h or the RhoA/ROCK inhibitor Y27632 (60  $\mu$ M, Beyotime), which was added for an additional 45 min before adding IFN- $\alpha$ . The cells then were harvested and lysed at different time points and subjected to sodium dodecyl sulfate–polyacrylamide gel electrophoresis and transferred onto polyvinylidene difluoride membranes for immunoblotting followed by protein detection with the SuperSignal West Femto Maximum Sensitivity Substrate (Pierce). The visualized proteins were scanned and the signal intensities quantified using Image J. The specified primary antibodies were directed against RhoA (Santa Cruz Biotechnology, diluted 1:200), total and phosphorylated STAT1 and STAT2, (Proteintech, diluted 1:3000), and  $\beta$ -actin (Abcam, diluted 1:5000). The secondary antibodies were horseradish-peroxidase (HRP)-linked

anti-mouse IgG antibody (Proteintech 1:10,000) and HRP-linked anti-rabbit IgG antibody (Proteintech, diluted 1:10,000).

**Statistical analysis**

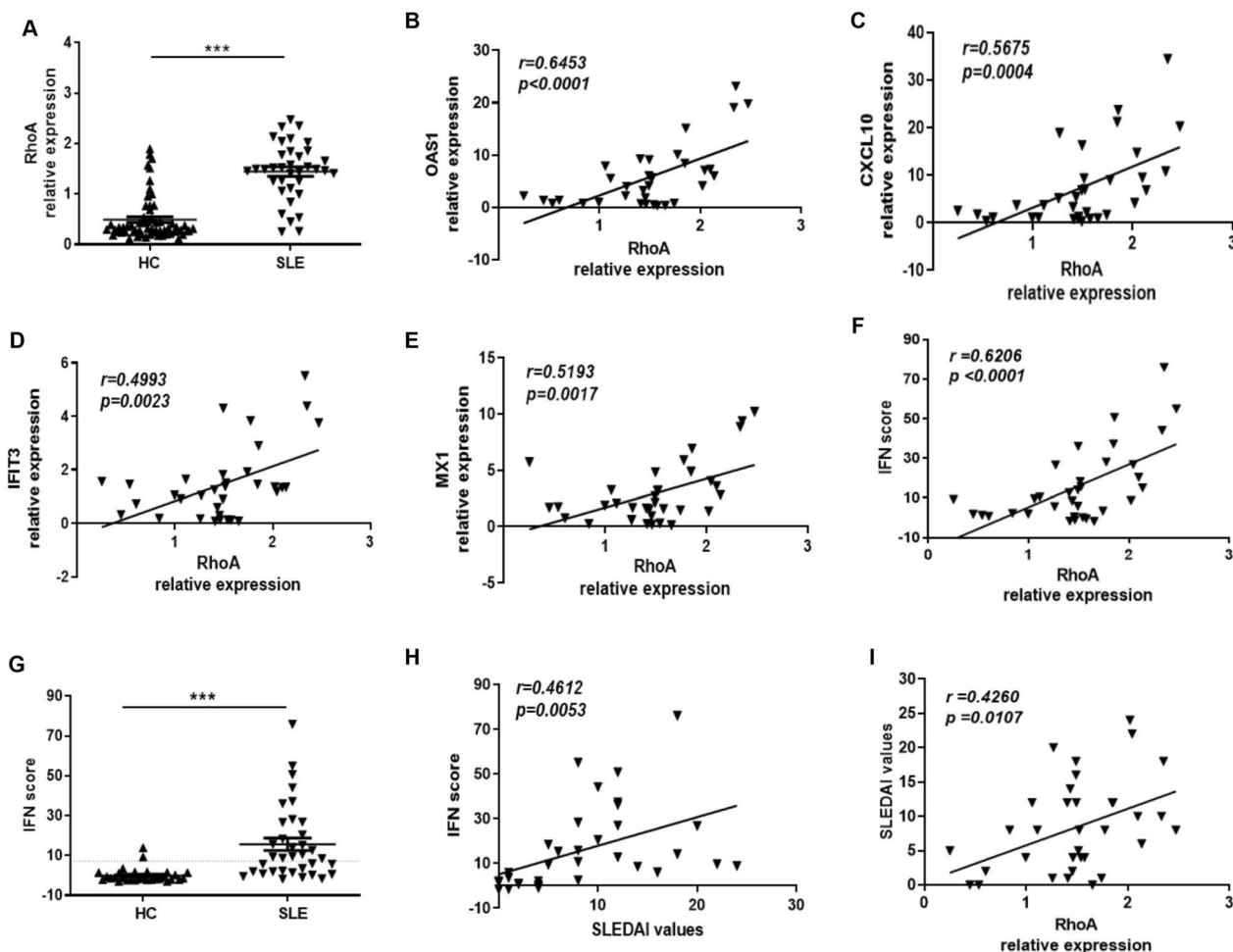
Descriptive data are presented as mean ± standard deviations (SDs). Continuous variables were analyzed using the Student’s *t*-test or nonparametric Mann–Whitney *U*-test depending on the normality of the distribution for comparisons of two groups. The Shapiro–Wilk test was used to assess whether the distribution of data follows a normal distribution. Correlations were calculated using the Spearman *r* test. Data were analyzed with

Prism 8 (GraphPad Software, Inc, San Diego, CA, USA) and *P* values of 0.05 or less were considered statistically significant.

**Results**

**PBMC RhoA expression correlates positively with type I IFN scores in SLE patients**

We investigated differences in the expression of RhoA mRNA in PBMCs between patients with SLE and healthy controls by quantitative, real-time PCR (RT-PCR) and observed a 3-fold higher level of RhoA mRNA in the lupus group (Fig. 1A). (The characteristics of the two studied cohorts are shown in the Supplementary



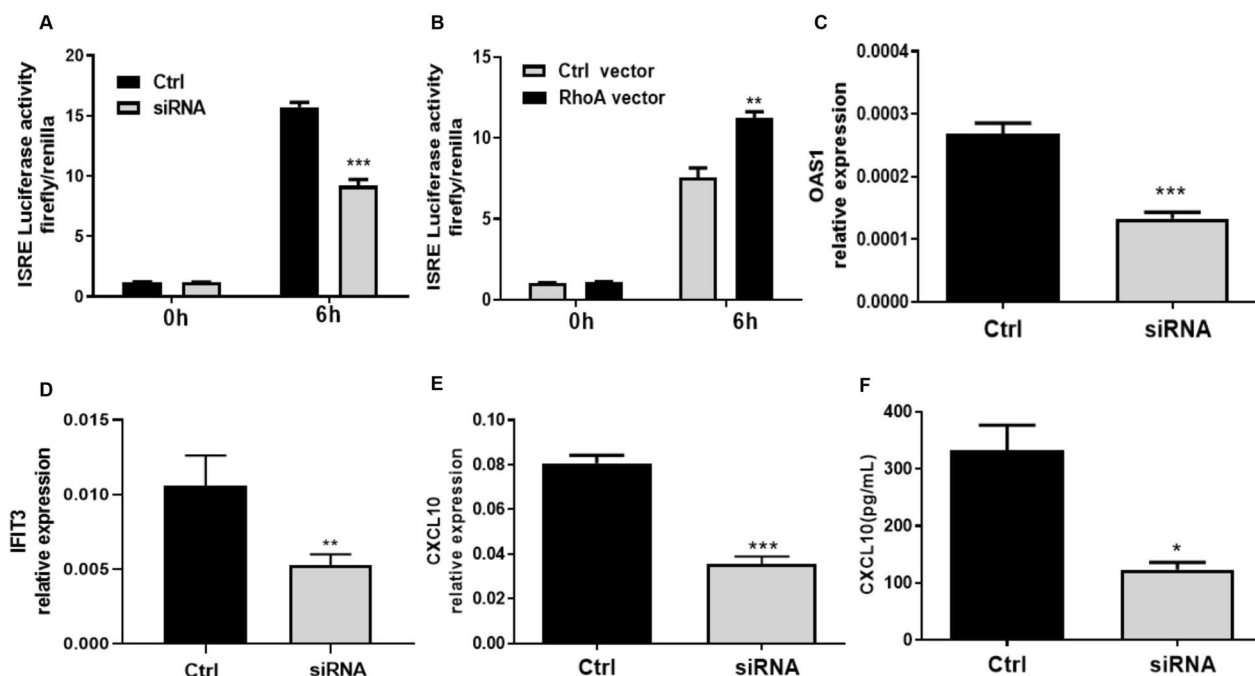
**Fig. 1** Elevated RhoA expression correlates positively with type I IFN-inducible gene expression and IFN scores. **A** Significantly higher expression of RhoA mRNA in the PBMCs of SLE patients (*n*=36) than in healthy controls (HC, *n*=60). **B–E** Correlations between RhoA expression and the type I IFN-inducible genes OAS1, CXCL10, IFIT3, and MX1 in SLE patients. **F** Type I IFN scores calculated by integrating the relative expression of the OAS1, CXCL10, IFIT3, and MX1 genes in the SLE and HC groups. **G** RhoA was highly expressed in high-type I IFN-scoring patients. The dotted line represents the mean ± 2SD of the HC values. **H** Positive correlation between type I IFN scores and SLEDAI values. **I** Positive correlation between RhoA expression levels and SLEDAI values. Bars in **A** and **G** show the mean ± SEM. Each symbol represents an individual sample. Expression is defined as the relative expression of the gene of interest in comparison to GAPDH. \**p*<0.05, \*\*\**p*<0.001 **A** and **G** by Mann–Whitney *U* test and **B–F, H, and I** by Spearman rank correlation test

Table 1). We examined the relationship between RhoA expression in the PBMCs of the SLE group with four well-characterized type I IFN inducible genes: OAS1, CXCL10, IFIT3, and MX1, and observed a significant positive correlation with RhoA expression (Fig. 1B–E). Additionally, RhoA expression correlated with a type I IFN score calculated on the basis of the relative expression of these genes (Fig. 1F). As expected, this score was elevated in the SLE versus healthy control group (Fig. 1G) and correlated with the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) values in our studied population (Fig. 1H). We further observed a positive correlation between RhoA expression (Fig. 1I) and SLEDAI values, while there was no correlation with steroid dosage (Supplementary Fig. 3).

**RhoA enhances ISRE-luciferase activity and selected type I IFN-induced genes**

We hypothesized that RhoA may upregulate the type I IFN signaling pathway by increasing the activity of interferon response elements (ISRE) in target genes. As a major member of the type I interferon (IFN) family, IFN- $\alpha$  was used to stimulate the induction of

ISRE-luciferase activity and type I IFN-induced genes. We transfected cultured HEK-293 T cells with an ISRE-luciferase reporter plasmid and simultaneously treated them with a siRNA targeting RhoA or a RhoA expression plasmid. After 6 h of IFN- $\alpha$  stimulation, we quantified the activity of ISRE. The successful silencing or increased expression of RhoA in HEK293T cells was verified by RT-PCR, which showed that inhibition or overexpression of RhoA led to a 2-fold decrease or a 10-fold increase in RhoA expression, respectively (Supplementary Fig. 2). We observed that genetic knockdown of RhoA reduced, and forced RhoA expression increased, ISRE-luciferase activity, respectively (Fig. 2A, B). In addition, experimental inhibition or augmentation of RhoA expression had commensurate effects on the expression of OAS1 and IFIT3 (Fig. 2B–D). As we found low expression of CXCL10 in HEK-293 T cells, we examined the expression of this chemokine in humans in THP-1 monocytes. As expected, siRNA-induced RhoA knockdown decreased type I IFN-induced CXCL10 mRNA and protein expression levels in THP-1 cells (Fig. 2E, F). We also identified that transfection with RhoA siRNA reduced RhoA mRNA and protein levels in THP-1 monocytes



**Fig. 2** RhoA regulates the type I IFN-stimulated response element (ISRE) and downstream gene expression. **A** ISRE activity of HEK293T cells transfected with ISRE-luciferase and a Renilla reporter plasmid together with a negative control (Ctrl) or RhoA-targeted siRNA (each at 200 nM). **B** ISRE activity of HEK293T cells transfected with RhoA-overexpression or control plasmids (each at 100 ng). At 24 h after transfection, the cells were left unstimulated (0 h) or stimulated with IFN- $\alpha$  (1000 U/mL, 6 h). Data in **A** and **B** are expressed as fold-change based on relative luciferase activities (ratio of firefly luciferase to Renilla luciferase). **C–E** The relative expression of OAS1, IFIT3, and CXCL10 mRNAs were determined by quantitative PCR. **F** The CXCL10 levels in culture supernatants were determined by enzyme-linked immunosorbent assay. Data were assessed in HEK293T (**C, D**) and THP1 cells (**E, F**) at 24 h after transfection of the negative control (Ctrl) or siRNA (200 nM) stimulated cells with IFN- $\alpha$  (1000 U/mL) for 6 h. Bars show the mean  $\pm$  SEM of 3 independent experiments. \* $p < 0.05$ , \*\* $p < 0.01$  and \*\*\* $p < 0.001$  by Student's  $t$ -test

(Supplementary Fig. 4). Taken together, these findings indicate that RhoA is a positive regulator of ISRE activity and the transcription of the type I IFN-induced genes OAS1, IFIT3, and CXCL10.

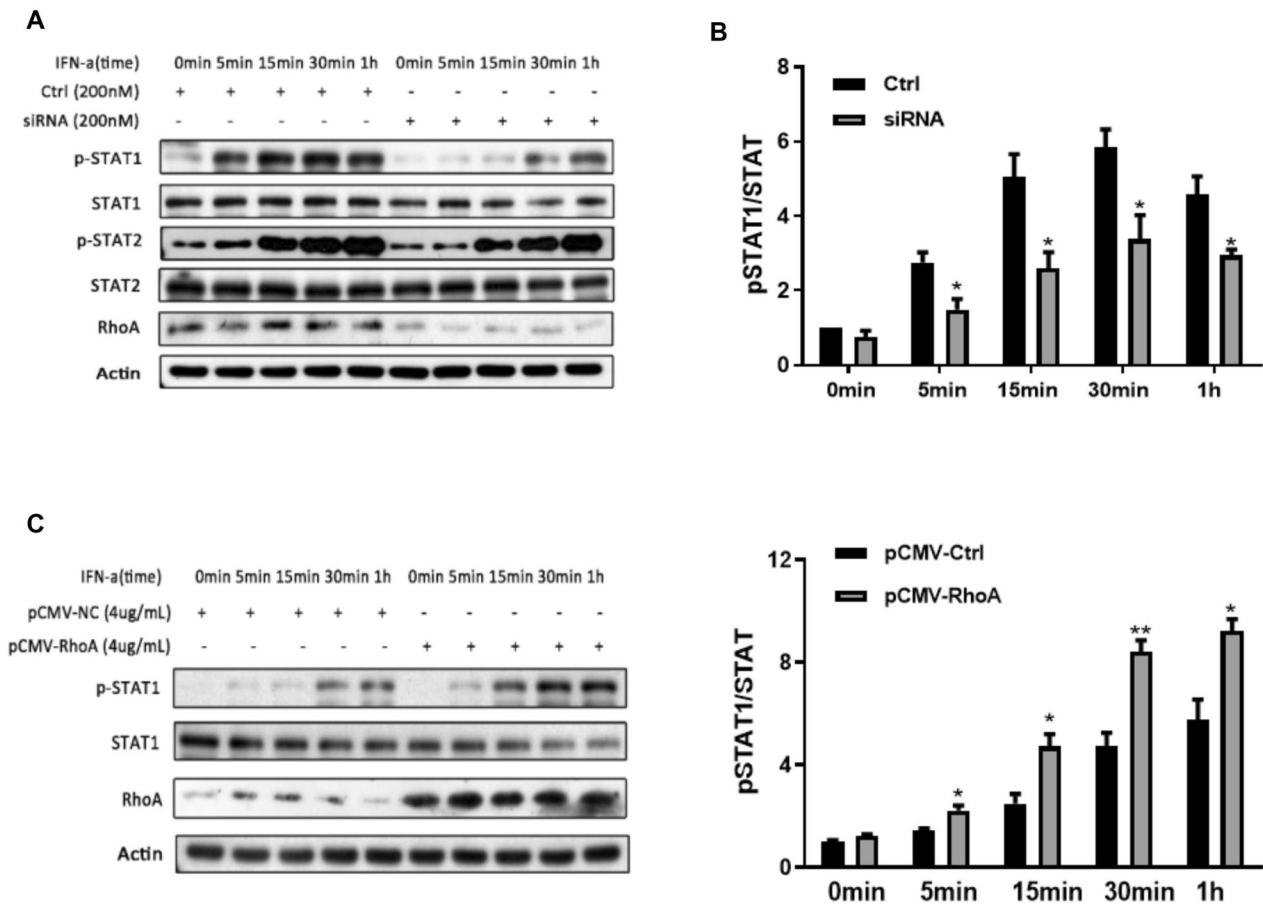
**RhoA promotes STAT-1 phosphorylation by type I IFN**

The two serine-threonine kinases ROCK1 and ROCK2 are important downstream effectors of Rho GTPases that phosphorylate downstream signaling intermediates. RhoA activation is known to induce the tyrosine phosphorylation of the signal transducer and activator of transcription (STAT) proteins [28, 29]. To further clarify the mechanism by which RhoA regulates type I IFN signaling, we tested whether the activating function of RhoA is attributable to the promotion of STAT-1/2 phosphorylation activity. SiRNA genetic knockdown of RhoA reduced and RhoA overexpression increased STAT-1 phosphorylation, respectively, as revealed by Western

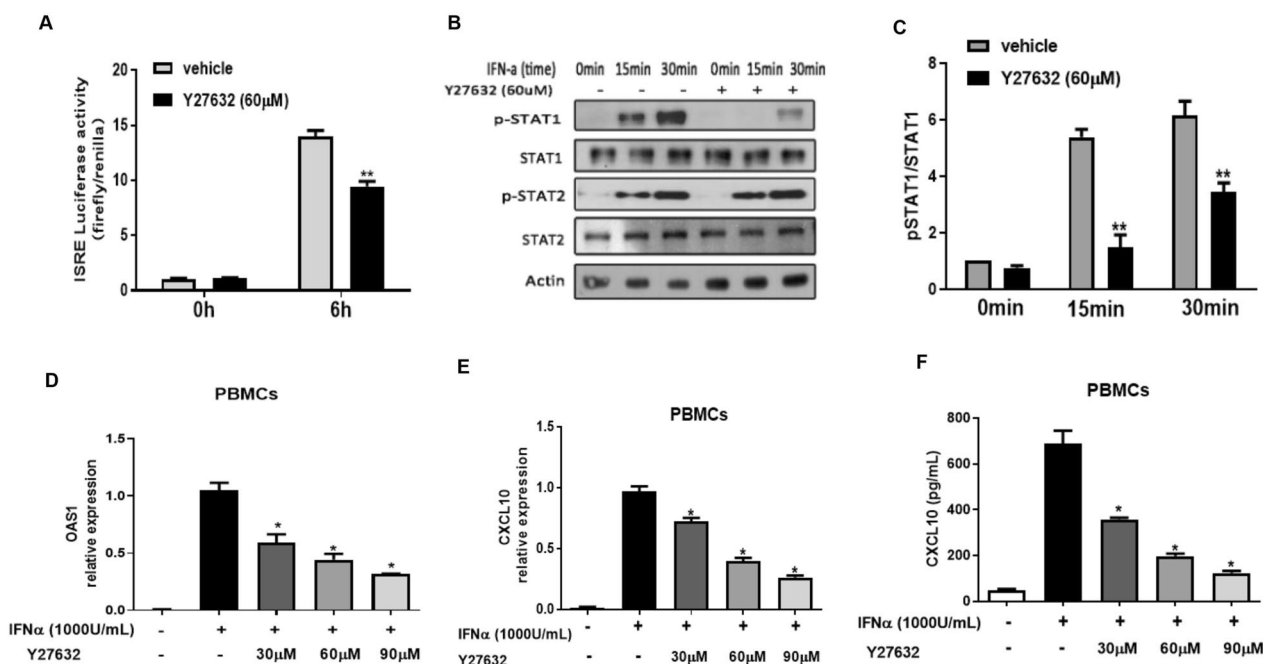
blotting (Fig. 3A–D). By contrast, the phosphorylation of STAT-2 was not affected by experimental changes in RhoA expression.

**Pharmacologic RhoA/ROCK inhibition reduces type I IFN signaling**

We next tested if the small molecule RhoA/ROCK inhibitor Y27632 influenced the phosphorylation of STAT-1, ISRE–luciferase activity, and the expression levels of ISGs in a manner similar to the knockdown of RhoA. We treated ISRE–luciferase transfected HEK293T cells with Y27632 (60 mM) before stimulation with IFN- $\alpha$  (1000 U/mL). Both ISRE–luciferase activation and STAT-1 phosphorylation were decreased upon the addition of Y27632 (Fig. 4A–C). We also examined PBMCs treated with Y27632 (60 mM, 45 min), followed by 6 h of stimulation with 1000 units/ml of IFN- $\alpha$ . We found that the phosphorylation of STAT-1 was decreased by the prior addition



**Fig. 3** RhoA regulates type I IFN-induced STAT-1 phosphorylation. Western blot analysis (A, C) and densitometry histograms (B, D) of cell lysates of cultured HEK293T cells ( $5 \times 10^5$  cells per well) transfected with siRNA (200 nM) targeting RhoA mRNA (A) or a RhoA expression plasmid vector (4  $\mu$ g/mL) (C), and their controls (a negative control siRNA or pCMV-NC) stimulated with IFN- $\alpha$  (1000 U/mL) for different times, as shown. The staining density histograms are from three independent experiments and values are expressed as the ratios of phosphorylated STAT-1 protein to total STAT-1. The immunoblot of cells transfected with a negative control siRNA (B) or the pCMV-NC vectors (D) were set at a value of 1. \* $p < 0.05$ , \*\* $p < 0.01$ . by Student's *t*-test



**Fig. 4** The RhoA/Rock Inhibitor Y27632 downregulates type I IFN signaling. **A** HEK293T cells were co-transfected with ISRE-luciferase and Renilla reporter plasmids for 24 h, then cultured with or without Y27632 (60 µM, 45 min) before the addition of IFN-α (1000 U/mL, 6 h). Luciferase activity was measured by dual luciferase assay. **B** PBMCs from healthy controls were cultured in the presence or absence of Y27632 (60 µM, 45 min) and stimulated with IFN-α (1000 U/mL) for different times. Western blots show whole-cell lysates harvested at the indicated times. **C** Histograms show the ratios of phosphorylated to total STAT-1 at the indicated times. The ratio of pSTAT-1/STAT-1 in the absence of Y27632 and IFN-α at 0 min was set at 1. **D** Relative expression of OAS1 and **E** CXCL10 mRNA in PBMCs cultured with Y27632 (0, 30, 60, and 90 µM, for 45 min) and stimulated with IFN-α (1000 U/mL) for 6 h. Results are the relative expression levels of OAS1 and CXCL10 mRNA normalized to endogenous GAPDH mRNA levels. **F** CXCL10 levels in PBMC culture supernatants quantified by ELISA. Bars show the mean ± SEM of 3 individual healthy donors; \**p* < 0.05, \*\**p* < 0.01, **A** and **C** versus vehicle by Student's *t*-test

of Y27632 (Fig. 2B, C). Furthermore, there was a dose-dependent decrease in the ability of PBMCs to express OAS1 and CXCL10 mRNA as well as produce CXCL10 protein (Fig. 2E, F). Taken together, these studies indicate that similar to the genetic knockdown of RhoA, Y27632 decreases the consequences of type I IFN stimulation on RhoA-mediated signaling by reducing ISRE-luciferase activity, STAT-1 phosphorylation, and the expression of the type I IFN-induced genes OAS1 and CXCL10.

**RhoA/ROCK inhibition attenuates CXCL10 and OAS1 expression in type I IFN scoring high SLE PBMCs**

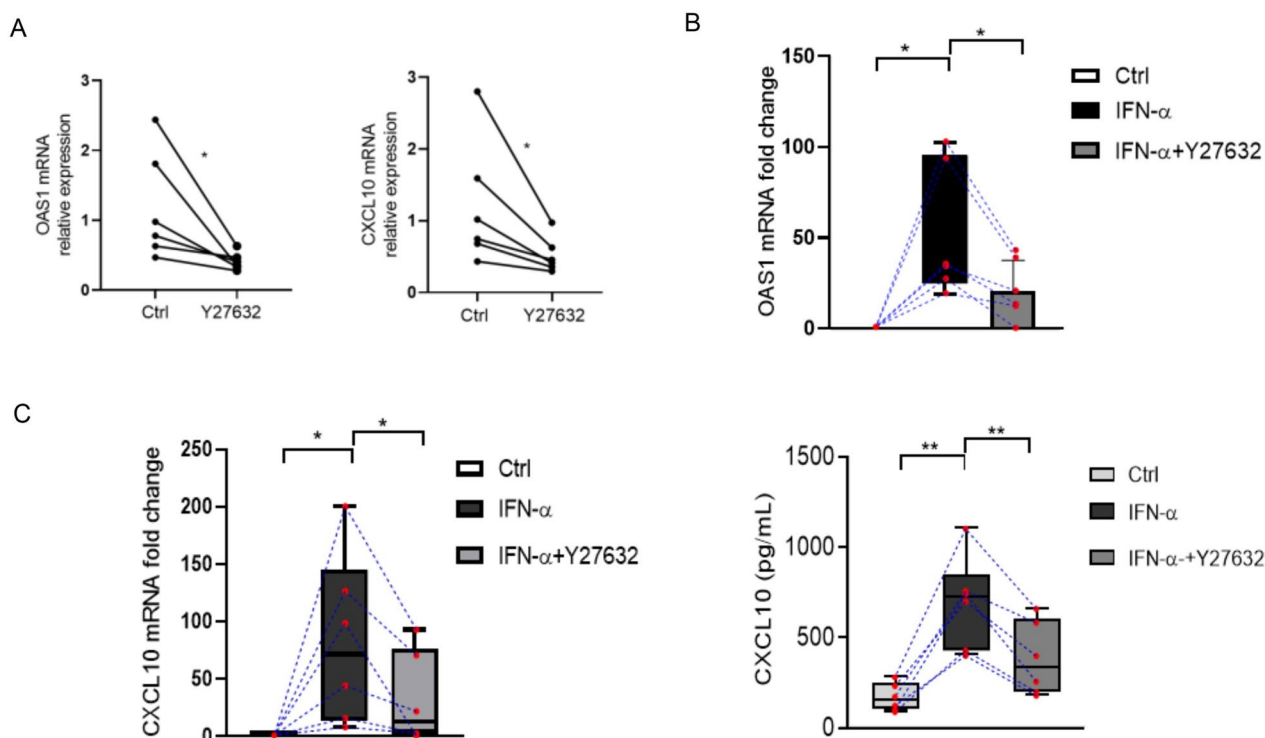
We next examined if pharmacologic inhibition of RhoA could reduce the type I IFN signaling pathway in PBMCs obtained from lupus patients with high type I IFN scores (see Supplementary Table 2 for patient characteristics). We incubated PBMCs with Y27632 (60 mM, 6 h) and observed a reduction in the expression of OAS1 and CXCL10 mRNA when compared to medium alone (Fig. 5A). The addition of IFN-α (1000 U/ml, 6 h) to these PBMCs increased both baseline OAS1 and CXCL10 mRNA expression, and CXCL10 protein production, but

these increases were nevertheless significantly reduced by prior treatment with Y27632 (60 mM, 45 min).

**Discussion**

Systemic lupus erythematosus is an autoimmune disease provoked by aberrant and sustained type I IFN responses and elevated levels of inflammatory cytokines, leading to tissue inflammation and critical end-organ damage [30]. Despite improvements in treatment during the past several decades, complete control of lupus disease activity is rarely achieved [31]. Elevated levels of type I IFN and its gene expression signature correlate with measures of disease activity and clinical flares, leading to therapeutic efforts targeting IFN-α, the type I IFN receptor, and downstream signaling molecules [32]. Anifrolumab, a monoclonal antibody targeting the type I IFN receptor, has been shown to reduce disease activity in SLE patients [13], although not without attendant risks of excessive immunosuppression and infection [33].

The interferon signaling pathway is complex, involving the regulation of the expression of multiple genes. Different interferon-related genes may be selected in various studies to calculate a type I IFN score [9]. For example,



**Fig. 5** The RhoA/ROCK inhibitor Y27632 reduces type I IFN signaling in lupus PBMCs. **A** Relative expression of OAS1 and CXCL10 mRNA in PBMCs obtained from SLE patients ( $n=6$ ) and incubated in medium alone or with Y27632 (60  $\mu\text{M}$ , 6 h). **B** OAS1 and **C**. CXCL10 mRNA measurements in lysates of lupus PBMCs ( $n=6$ ) cultured with or without Y27632 (60  $\mu\text{M}$ , 45 min) and stimulated with IFN- $\alpha$  (1000 U/mL). Results are the relative expression levels of OAS1 and CXCL10 mRNA normalized to endogenous GAPDH mRNA levels. **D** CXCL10 levels in culture supernatants quantified by ELISA. Bars show the mean  $\pm$  SEM of 6 individual PBMC samples. \* $p < 0.05$  by Student's  $t$ -test

Marticorena et al. derived a score from the expression of five type I IFN-inducible genes (LY6E, OAS1, IFIT1, ISG15, and MX1) [34]. Other studies have calculated IFN scores using different gene sets, such as IFN3-scores (IFI27, IFIT3, and CXCL10) [35], and IFN12-scores (M1.2: IP10, IFI44L, IFIT3, LY6E, MX1, and SERPING1; M3.4: IFITM1, IRF7, and STAT1; M5.12: C1QA, IFI16, and IRF9) [11]. At present, there is no established consensus by which interferon-stimulated genes (ISGs) should be used to construct an IFN score. Generally, the selection of an interferon signature serves as a biomarker for SLE and assists in assessing disease activity. In our study, we evaluated the expression of four type I IFN-inducible genes (OAS1, CXCL10, IFIT3, and MX1) in SLE patients and healthy controls based on the prior work of Chiche, L et al. and Wu L et al. [35, 36]. Consistent with previous findings, we observed a significant upregulation of these four genes in SLE patients compared to the healthy control group, as shown in Supplementary Fig. 2. Accordingly, we selected MX1, OAS1, IFIT3, and CXCL10 for the type I IFN score calculation following an algorithm previously introduced in the literature [10]. Both the type I IFN score and the individual

gene expression levels exhibited good diagnostic accuracy in distinguishing between SLE patients and healthy subjects. The area under the curve (AUC) for the type I IFN score was 0.8967. As expected, the type I IFN score was elevated in the SLE group when compared to the healthy control group and correlated with SLEDAI values in our study population.

RhoA is a member of the Rho subfamily of GTPases that is rapidly activated by a diverse array of biochemical signals to regulate numerous biological processes, including cytoskeletal reorganization, cell proliferation and differentiation, and apoptosis [37–39]. The RhoA/ROCK pathway has been implicated in lupus pathology in a prior study that showed that increased phosphorylation of the ezrin, radixin, and moesin (ERM) proteins interact with the macrophage migration inhibitory factor (MIF) co-receptor CD44 to promote the adhesion, migration and inflammatory response of T lymphocytes [40]. Genetic knockdown of RhoA further was observed to suppress the apoptosis of renal tubular epithelial cells in mice with lupus nephritis [41]. The RhoA/ROCK inhibitor, Y27632, also attenuated disease development in lupus-prone mice by diminishing T cell production of



IL-17 and IL-21 [42], and it reduced serum the levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$  and interleukin-6 (IL-6) while increasing the levels of IL-10 [43]. Additionally, pharmacologic RhoA/ROCK inhibition reduced the production of anti-dsDNA antibody levels and the responsiveness of B cells to B-cell activating factor receptor (BAFF/BAFFR) [38, 44], suggesting a role in the differentiation of autoantibody-producing B lymphocytes in SLE [45]. Our previous work has shown that the expression of RhoA is significantly higher in lupus T cells and that targeting RhoA can reduce their production of IL-2 [22]. In the present study, we examined the influence of the RhoA GTPase in type I IFN signaling and the expression of a subset of type I IFN pathway genes. We confirmed RhoA to be highly expressed in the PBMC population of SLE patients when compared to healthy controls, especially in those lupus patients with high type I IFN scores. Our study also showed that genetic reduction in RhoA expression or pharmacologic inhibition of RhoA activity reduced activation of the type I IFN pathway, supporting its potential as a therapeutic target for the treatment of SLE.

Once stimulated, type I IFN binds to the IFNARs to initiate downstream signaling via activation of the janus kinase (JAK)/STAT pathway, phosphorylation and activation of STAT-1 and STAT-2, and transcription of type I IFN-stimulated genes [32]. There has been growing evidence to indicate that RhoA mediates phosphorylation of STAT-3 and STAT-5 in several cell types via ROCK [29] and that targeted RhoA-ROCK inhibition modulates STAT-3 phosphorylation to shift toward a pathologic Th17/Treg imbalance in patients with lupus [28]. We found RhoA siRNA to reduce type I IFN-stimulated phosphorylation of STAT-1 but not STAT-2, leading to a downregulation of the type I IFN response. We confirmed RhoA inhibition to reduce expression of the type I IFN-responsive genes IFIT3, OAS1, and CXCL10, which are up-regulated in SLE patients. Conversely, the forced overexpression of RhoA upregulated ISRE reporter expression. The RhoA/ROCK inhibitor in turn reduced type I IFN-induced STAT-1 phosphorylation and ISRE reporter gene expression, as well as OAS1 and CXCL10 in human lupus PBMCs. These observations extend the observations of Badr and co-workers regarding RhoA activation by type I interferon [25], and together support a model for a positive feedback loop whereby type I IFN stimulation of RhoA contributes to the sustained and pathologic overactivation type I IFN signaling. Conceivably, interruption of this feedback pathway by the pharmacologic targeting RhoA could provide a means to reset type I IFN activation in a manner that would be therapeutically beneficial in lupus but preserve necessary anti-infective responses.

Type I IFN also can regulate downstream gene transcription levels through non-classical pathways involving CRK-like protein (CRKL), phosphatidylinositol 3 kinase (PI3K), and mitogen-activated protein kinase (MAPK) [32]. Our study indicates that RhoA is a major effector of the JAK/STAT pathway upon IFNAR activation, with excessive activation of RhoA potentially leading to the activation of the IFNARs and enhancement of type I IFN signaling. The existence of non-classical pathways suggests that type I IFN may modulate cellular activity through other mechanisms that contribute to their biological activity [46]. Therefore, we have reason to believe that RhoA is sufficient but not necessary for IFNAR-mediated cell activation and that there exist mechanisms of type I IFN signaling pathways independent of RhoA. Therefore, it is important to note that the specific signaling mechanisms and downstream effects require further research.

## Conclusions

The present findings describe a positive regulatory role of RhoA in the activation of the type I IFN response pathway. The potential therapeutic implications of reducing RhoA expression levels or using the RhoA/ROCK inhibitor Y27632 in attenuating aberrant type I IFN signaling in SLE should be considered in follow-up studies.

## Abbreviations

SLE	Systemic lupus erythematosus
ISRE	IFN-stimulated response element
IFN	Interferon
IFNAR	IFN-a/b receptor
ISGs	IFN-stimulated genes
STAT-1	Signal transducer and activator of transcription 1
PBMCs	Peripheral blood mononuclear cells
CXCL10	C-X-C motif chemokine ligand 10
OAS1	2'-5'-Oligoadenylate synthetase 1
MX1	MX dynamin like GTPase 1
IFIT3	Interferon-induced protein with tetratricopeptide repeats 3
SLEDAI	Systemic Lupus Erythematosus Disease Activity Index
JAK	Janus kinase

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13075-024-03263-3>.

**Additional file 1: Supplementary Fig. 1.** Plasmid construct. A schematic figure of the plasmid construct is pISRE-TA-Luc for monitoring the induction of the STAT1 and STAT2 components of JAK/STAT-mediated signal transduction pathways.

**Additional file 2: Supplementary Fig. 2.** Increased type I IFN-inducible gene expression in SLE patients. Increased type I IFN-inducible gene (MX1, OAS1, IFIT3 and CXCL10) expression in SLE patients compared to the healthy control group. Area under the receiver operating characteristic curves (AUC) for detection of SLE by references to the expression levels of MX1, OAS1, IFIT3, CXCL10 and the IFN score. Statistical significance was determined using Student's ttest; the p-values are all less than 0.001.

**Additional file 3: Supplementary Fig. 3.** Correlation between RhoA expression levels and steroid dosage in SLE patients. Each symbol represents an individual patient sample. The dosage of steroids presented refers to prednisone or its equivalent.

**Additional file 4: Supplementary Fig. 4.** The expression changes of RhoA after transfection. Quantitative PCR analysis of RhoA expression in HEK-293T 24 hours post-transfection of siRNA targeting RhoA mRNA at 200 nM (A) or a RhoA expression plasmid vector at 4 ug/mL (B), along with their respective controls (a negative control siRNA or pCMV-NC). Quantitative PCR (C) and immunoblot (D) analysis carried out to evaluate the expression changes of RhoA in THP1 cells after transfection with RhoA siRNA or control siRNA directed against RhoA. (E) The histograms represent the ratios of RhoA protein to actin. The immunoblot results of cells transfected with a negative control (Ctrl) were assigned a value of 1. The results are presented as mean  $\pm$  SEM. Statistical significance was determined using Student's t-test, with  $**p < 0.01$  and  $***p < 0.001$  denoting significance.

**Additional file 5: Supplementary Table 1.** Demographic, clinical features of SLE patients and healthy controls. **Supplementary Table 2.** Demographic and clinical features of SLE patients for the PBMC studies.

### Acknowledgements

We thank the patients and rheumatologists at the Second Affiliated Hospital of Xiamen Medical College who participated in this study.

### Authors' contributions

WF and RB contributed to conception and design of the study. WF and LKY performed the statistical analysis. WF and RB wrote the first draft and revised the manuscript. All authors contributed to the article and approved the submitted version. The authors have declared no conflicts of interest.

### Funding

This work was supported by the National Natural Science Foundation of China (Grant Number 81901669), Natural Science Foundation of Fujian Province (NO.2023J011644), Medical Innovation Project of Fujian Provincial Health Commission (NO.2022CXB025), and Xiamen Science and Technology (Medical and Health) Project (NO.3502Z20184061). LL and RB are supported by the ACR Rheumatology Research Fund and NIH grant 1R01-AR-078334.

### Availability of data and materials

No datasets were generated or analysed during the current study.

### Declarations

#### Ethics approval and consent to participate

The study complies with the current National Health and Family Planning Commission of China ethical standards and was reviewed and approved by the hospital ethics committee (No 2022051 and No 20190301007). All participants signed an informed consent form.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

#### Author details

<sup>1</sup>Department of Rheumatology and Immunology, the Second Affiliated Hospital of Xiamen Medical College, Xiamen Medical College, Xiamen 361021, China. <sup>2</sup>Department of Rheumatology, Zhongshan Hospital of Xiamen University, School of Medicine, Xiamen University, Xiamen 361000, China. <sup>3</sup>Department of Nephrology, the Second Affiliated Hospital of Xiamen Medical College, Xiamen Medical College, Xiamen 361021, China. <sup>4</sup>Department of Internal Medicine, Yale University School of Medicine, New Haven, CT 06520, USA.

Received: 20 November 2023 Accepted: 8 January 2024

Published online: 20 January 2024

### References

- Durcan L, O'Dwyer T, Petri M. Management strategies and future directions for systemic lupus erythematosus in adults. *Lancet*. 2019;393:2332–43.
- Jiang J, Zhao M, Chang C, Wu H, Lu Q. Type I interferons in the pathogenesis and treatment of autoimmune diseases. *Clin Rev Allergy Immunol*. 2020;59:248–72.
- Rönnblom L. The importance of the type I interferon system in autoimmunity. *Clin Exp Rheumatol*. 2016;34:21–4.
- Gallucci S, Meka S, Gamero AM. Abnormalities of the type I interferon signaling pathway in lupus autoimmunity. *Cytokine*. 2021;146: 155633.
- Chyuan IT, Tzeng HT, Chen JY. Signaling pathways of type I and type III interferons and targeted therapies in systemic lupus erythematosus. *Cells*. 2019;8:963.
- Oke V, Gunnarsson I, Dorschner J, Eketjäll S, Zickert A, Niewold TB, Svenungsson E. High levels of circulating interferons type I, type II and type III associate with distinct clinical features of active systemic lupus erythematosus. *Arthritis Res Ther*. 2019;21:107.
- Postal M, Vivaldo JF, Fernandez-Ruiz R, Paredes JL, Appenzeller S, Niewold TB. Type I interferon in the pathogenesis of systemic lupus erythematosus. *Curr Opin Immunol*. 2020;67:87–94.
- Eloranta ML, Rönnblom L. Cause and consequences of the activated type I interferon system in SLE. *J Mol Med (Berl)*. 2016;94:1103–10.
- Baechler EC, Batliwalla FM, Karypis G, Gaffney PM, Ortmann WA, Espe KJ, Shark KB, Grande WJ, Hughes KM, Kapur V, Gregersen PK, Behrens TW. Interferon-inducible gene expression signature in peripheral blood cells of patients with severe lupus. *Proc Natl Acad Sci U S A*. 2003;100:2610–5.
- Feng X, Wu H, Grossman JM, Hanvivadhanakul P, FitzGerald JD, Park GS, Dong X, Chen W, Kim MH, Weng HH, Furst DE, Gorn A, McMahon M, Taylor M, Brahn E, Hahn BH, Tsao BP. Association of increased interferon-inducible gene expression with disease activity and lupus nephritis in patients with systemic lupus erythematosus. *Arthritis Rheum*. 2006;54:2951–62.
- Lambers WM, de Leeuw K, Doornbos-van der Meer B, Diercks GFH, Bootsma H, Westra J. Interferon score is increased in incomplete systemic lupus erythematosus and correlates with myxovirus-resistance protein A in blood and skin. *Arthritis Res Ther*. 2019;21:260.
- Bezalel S, Guri KM, Elbirt D, Asher I, Stoeber ZM. Type I interferon signature in systemic lupus erythematosus. *Isr Med Assoc J*. 2014;16:246–9.
- Tanaka Y, Tummala R. Anifrolumab, a monoclonal antibody to the type I interferon receptor subunit 1, for the treatment of systemic lupus erythematosus: an overview from clinical trials. *Mod Rheumatol*. 2021;31:1–12.
- Houssiau FA, Thanou A, Mazur M, Ramitterre E, Gomez Mora DA, Misterska-Skora M, Perich-Campos RA, Smakotina SA, Cerpa Cruz S, Louzri B, Croughs T, Tee ML. IFN-alpha kinoid in systemic lupus erythematosus: results from a phase IIb, randomised, placebo-controlled study. *Ann Rheum Dis*. 2020;79:347–55.
- Crow MK, Olfieriev M, Kirou KA. Targeting of type I interferon in systemic autoimmune diseases. *Transl Res*. 2015;165:296–305.
- Nacionales DC, Kelly-Scumpia KM, Lee PY, Weinstein JS, Lyons R, Sobel E, Satoh M, Reeves WH. Deficiency of the type I interferon receptor protects mice from experimental lupus. *Arthritis Rheum*. 2007;56:3770–83.
- Ricker E, Chowdhury L, Yi W, Pernis AB. The RhoA-ROCK pathway in the regulation of T and B cell responses. *F1000Res*. 2016;5:F1000 Faculty Rev-2295.
- Su VL, Calderwood DA. Signalling through cerebral cavernous malformation protein networks. *Open Biol*. 2020;10:200263.
- Shahbazi R, Baradaran B, Khordadmehr M, Safaei S, Baghbanzadeh A, Jigari F, Ezzati H. Targeting ROCK signaling in health, malignant and non-malignant diseases. *Immunol Lett*. 2020;219:15–26.
- Etienne-Manneville S, Hall A. Rho GTPases in cell biology. *Nature*. 2002;420:629–35.
- Stirzaker RA, Biswas PS, Gupta S, Song L, Bhagat G, Pernis AB. Administration of fasudil, a ROCK inhibitor, attenuates disease in lupus-prone NZB/W F1 female mice. *Lupus*. 2012;21:656–61.

22. Fan W, Liang D, Tang Y, Qu B, Cui H, Luo X, Huang X, Chen S, Higgs BW, Jallal B, Yao Y, Harley JB, Shen N. Identification of microRNA-31 as a novel regulator contributing to impaired interleukin-2 production in T cells from patients with systemic lupus erythematosus. *Arthritis Rheum.* 2012;64:3715–25.
23. Rozo C, Chinenov Y, Maharaj RK, Gupta S, Leuenberger L, Kirou KA, Bykerk VP, Goodman SM, Salmon JE, Pernis AB. Targeting the RhoA-ROCK pathway to reverse T-cell dysfunction in SLE. *Ann Rheum Dis.* 2017;76:740–7.
24. Isgro J, Gupta S, Jacek E, Pavri T, Duculan R, Kim M, Kirou KA, Salmon JE, Pernis AB. Enhanced rho-associated protein kinase activation in patients with systemic lupus erythematosus. *Arthritis Rheum.* 2013;65:1592–602.
25. Badr G, Saad H, Waly H, Hassan K, Abdel-Tawab H, Alhazza IM, Ahmed EA. Type I interferon (IFN-alpha/beta) rescues B-lymphocytes from apoptosis via PI3Kdelta/Akt, Rho-A, NFkappaB and Bcl-2/Bcl(XL). *Cell Immunol.* 2010;263:31–40.
26. Gladman DD, Ibañez D, Urowitz MB. Systemic lupus erythematosus disease activity index 2000. *J Rheumatol.* 2002;29:288–91.
27. Kirou KA, Lee C, George S, Louca K, Papagiannis IG, Peterson MG, Ly N, Woodward RN, Fry KE, Lau AY, Prentice JG, Wohlgemuth JG, Crow MK. Coordinate overexpression of interferon-alpha-induced genes in systemic lupus erythematosus. *Arthritis Rheum.* 2004;50:3958–67.
28. Zanin-Zhorov A, Weiss JM, Nyuydzefe MS, Chen W, Scher JU, Mo R, Depoil D, Rao N, Liu B, Wei J, Lucas S, Koslow M, Roche M, Schueller O, Weiss S, Poyurovsky MV, Tonra J, Hippen KL, Dustin ML, Bazar BR, Liu CJ, Waksal SD. Selective oral ROCK2 inhibitor down-regulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism. *Proc Natl Acad Sci U S A.* 2014;111:16814–9.
29. Corry J, Mott HR, Owen D. Activation of STAT transcription factors by the Rho-family GTPases. *Biochem Soc Trans.* 2020;48:2213–27.
30. Kaul A, Gordon C, Crow MK, Touma Z, Urowitz MB, van Vollenhoven R, Ruiz-Irastorza G, Hughes G. Systemic lupus erythematosus. *Nat Rev Dis Primers.* 2016;2:16039.
31. Lee YH, Choi SJ, Ji JD, Song GG. Overall and cause-specific mortality in systemic lupus erythematosus: an updated meta-analysis. *Lupus.* 2016;25:727–34.
32. Bengtsson AA, Rönnblom L. Role of interferons in SLE. *Best Pract Res Clin Rheumatol.* 2017;31:415–28.
33. Crow MK. Advances in understanding the role of type I interferons in systemic lupus erythematosus. *Curr Opin Rheumatol.* 2014;26:467–74.
34. Landolt-Marticorena C, Bonventi G, Lubovich A, Ferguson C, Unnithan T, Su J, Gladman DD, Urowitz M, Fortin PR, Wither J. Lack of association between the interferon-alpha signature and longitudinal changes in disease activity in systemic lupus erythematosus. *Ann Rheum Dis.* 2009;68:1440–6.
35. Wu L, Qin Y, Xia S, Dai M, Han X, Wu Y, Zhang X, Ma J, Wang Y, Tang Y, Liu Z, Zhu W, Jallal B, Yao Y, Qu B, Shen N. Identification of cyclin-dependent kinase 1 as a novel regulator of type I interferon signaling in systemic lupus erythematosus. *Arthritis Rheumatol.* 2016;68:1222–32.
36. Chiche L, Jourde-Chiche N, Whalen E, Presnell S, Gersuk V, Dang K, Anguiano E, Quinn C, Burtey S, Berland Y, Kaplanski G, Harle JR, Pascual V, Chaussabel D. Modular transcriptional repertoire analyses of adults with systemic lupus erythematosus reveal distinct type I and type II interferon signatures. *Arthritis Rheumatol.* 2014;66:1583–95.
37. Yang JQ, Kalim KW, Li Y, Zhang S, Hinge A, Filippi MD, Zheng Y, Guo F. RhoA orchestrates glycolysis for TH2 cell differentiation and allergic airway inflammation. *J Allergy Clin Immunol.* 2016;137:231–45.e4.
38. Zhang S, Zhou X, Lang RA, Guo F. RhoA of the Rho family small GTPases is essential for B lymphocyte development. *PLoS One.* 2012;7:e33773.
39. Yang JQ, Kalim KW, Li Y, Zheng Y, Guo F. Ablation of RhoA impairs Th17 cell differentiation and alleviates house dust mite-triggered allergic airway inflammation. *J Leukoc Biol.* 2019;106:1139–51.
40. Li Y, Harada T, Juang YT, Kytтары VC, Wang Y, Zidanic M, Tung K, Tsokos GC. Phosphorylated ERM is responsible for increased T cell polarization, adhesion, and migration in patients with systemic lupus erythematosus. *J Immunol.* 2007;178:1938–47.
41. Tian Y, Han YX, Guo HF, Jin HT, Sun C, Qi X, Ma LY, Bo SW. Upregulated microRNA-485 suppresses apoptosis of renal tubular epithelial cells in mice with lupus nephritis via regulating the TGF-β-MAPK signaling pathway by inhibiting RhoA expression. *J Cell Biochem.* 2018;119:9154–67.
42. Biswas PS, Gupta S, Chang E, Song L, Stirzaker RA, Liao JK, Bhagat G, Pernis AB. Phosphorylation of IRF4 by ROCK2 regulates IL-17 and IL-21 production and the development of autoimmunity in mice. *J Clin Invest.* 2010;120:3280–95.
43. Wang Y, Lu Y, Chai J, Sun M, Hu X, He W, Ge M, Xie C. Y-27632, a Rho-associated protein kinase inhibitor, inhibits systemic lupus erythematosus. *Biomed Pharmacother.* 2017;88:359–66.
44. Lundell AC, Nordström I, Andersson K, Lundqvist C, Telemo E, Nava S, Kaipe H, Rudin A. IFN type I and II induce BAFF secretion from human decidual stromal cells. *Sci Rep.* 2017;7:39904.
45. Samotij D, Reich A. Biologics in the treatment of lupus erythematosus: a critical literature review. *Biomed Res Int.* 2019;2019:8142368.
46. Platanias LC. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol.* 2005;5:375–86.

## Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.