lncRNA MEG8 sponging miR-181a-5p contributes to M1 macrophage polarization by regulating SHP2 expression in Henoch Schonlein purpura rats

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Research

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Abstract

Background: Long noncoding RNAs (lncRNAs) are important regulatory molecules in various biological and pathological processes. We herein aimed to explore whether maternally expressed gene 8 (MEG8) promotes M1 macrophage polarization in Henoch-Schonlein purpura (HSP) rats and to investigate the underlying mechanism.

Methods: Relative MEG8 and miR-181a-5p expression and suppressor of SH2 domain-containing tyrosine phosphatase 2 (SHP2) RNA level were examined using quantitative reverse transcription polymerase chain reaction. Expression of SHP2 and the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) pathway-related proteins was detected using western blot. Luciferase activity assay was performed to test whether miR-181a-5p could bind to MEG8 or SHP2. The macrophage phenotype was determined using flow cytometry analysis and enzyme-linked immunosorbent assay.

Results: The macrophage polarization toward the M2 phenotype was observed in

peripheral blood from HSP rats. Furthermore, MEG8 and SHP2 expression were down-regulated but miR-181a-5p was up-regulated in monocyte-derived macrophages from HSP rats compared with the control group. Furthermore, MEG8 acted as a sponge for miR-181a-5p to facilitate SHP2 expression. Moreover, miR-181a-5p mimic and SHP2 knockdown significantly reversed the MEG8 overexpression-mediated suppression of the JAK2/STAT3 signaling and promotion of M1 polarization.

Conclusion: lncRNA MEG8 sponging miR-181a-5p contributes to M1 macrophage polarization by regulating SHP2 expression in Henoch Schonlein purpura rats.

Background

Henoch-Schonlein purpura (HSP) is a vasculitis involving the small vessels of the joints, kidneys, gastrointestinal (GI) tract, and the skin. HSP can also involve the central nervous system (CNS) and the lungs. It is an acute immunoglobulin A (IgA)-mediated disorder that is typically self-limited and managed with supportive care [1]. Environmental, genetic, infection and immune factors appear to contribute to the etiology of HSP [2]. HSP is a very rare disorder that typically affects children; however, the disorder can also be seen in adults and adolescents. The majority of children present before the age of 10. It is often more severe and likely to cause long-term renal disease in adults. It is the most common vasculitis among children, affecting 10 to 20 children per 100,000 every year [3].

Macrophages are widely distributed innate immune cells that play an indispensable role in a variety of physiologic and pathologic processes, including organ development, host defense, acute and chronic inflammation, and tissue homeostasis and remodeling [4]. Activated macrophages are subdivided according to their differentiation as M1 (classically activated) or M2 (alternatively activated) macrophages [5]. M1 macrophages are activated by Granulocyte-macrophage Colony Stimulating Factor (GM-CSF) and exert proinflammatory properties, M1-like polarized macrophages exhibited a high level of

phagocytic activity, and markers that best characterized them were CD64 and CD80, which are characterized by their ability to guide acute inflammatory responses. Indeed, they are able to produce proinflammatory cytokines such as IL-1, IL-6, IL-12, TNF-α, and IFN-γ; The M2-like polarized population is particularly involved during parasitic, helminthic, and fungal infections. They are induced in response to Th2 responses. Macrophage colony-stimulating factor (M-CSF), IL-4, IL-10, IL-13, or a combination of these factors are able to polarize macrophages toward the M2-like phenotype. M2-like macrophages are mainly identified based on the expression of CD206 and CD209, a C-type lectin [6].

miRNAs are short noncoding RNAs of approximately 22 nucleotides (nt), which have been highly conserved during evolution and play crucial roles in immune and inflammatory responses including Henoch-Schonlein purpura. Upon binding to the 3'-untranslated region (UTR) of target mRNAs, miRNAs negatively regulate gene expression by increasing mRNA degradation or/and by repressing the translation process. 96 miRNAs play pivotal roles in the regulation of macrophage development and functions [7]. The lncRNAs are a large class of nonprotein-coding transcripts that are more than 200 nt in length and are involved in various physiologic and pathologic processes. 106 lncRNA mediated transcriptional or posttranscriptional regulation is an important mechanism for epigenetic programming [8].107 Studies have shown that lncRNAs play essential roles inmacrophage activation and polarization [9]. Evidences indicated that MEG8 may function as a regulator in atherosclerosis VSMCs lesions, kidney injury, tumor progression and epithelial-mesenchymal transition of hepatocytes [10]. Nevertheless, the potential role of MEG8 in Henoch-Schonlein purpura has not yet been elucidated. Using bioinformatics analysis, we noted that MEG8 harbors putative binding sites of miR-181a-5p (starBase). Meanwhile, SH2 domain-containing tyrosine phosphatase 2 (SHP2) was identified as a putative target of miR-181a-5p by harboring a miR-181a-5p binding sequence in the 3'-UTR of its mRNA (TargetScan). Furthermore, SHP2 negatively modulates the Janus kinase 2/signal transducer and activator of transcription 3 (JAK2/STAT3) signaling

pathway, activation of which is known to mediate M2 polarization of macrophages. Besides, It has also been reported that SHP2 induces M1 polarization.Therefore, we speculated that lncRNA MEG8 might act as a ceRNA by sponging miR-181a-5p to facilitate SHP2 expression, and thereby suppresses the JAK2/STAT3 pathway, then promotes M1 subtype macrophage polarization in Henoch-Schonlein purpura.

Materials And Methods

Ethical approval

All experimental procedures for animal usage were in accordance with the guidelines for care and use of laboratory animals and were approved by the Institutional Animal Care and Use Committee of Harbin Medical University.

Animal models

Ten- to twelve-week-old wistar rats, weighting 250-300g, were selected, which obtained from Animal Research Center of Harbin Medical University (Harbin, China).

To develop the HSP animal model, rats were sensitized by injection (intraperitoneal, i.p.) of 1ml saline containing 1 mg ovalbumin (OVA; Sigma) and 10 mg alum (Sigma) once per week for 2 weeks. Subsequently, rats each received OVA physiological saline liquor (15 mg/kg) by external jugular vein injection, and also received OVA physiological saline liquor (0.3%; 1 ml; 5 injection points) through intradermal injection. Meanwhile, rats in the control group received equal amounts of physiological saline. The method for HSP model rats was previously described [11].

Twenty-four hours after HSP model establishment, all rats were anesthetized by injecting with dose (40mg/kg) of pentobarbital sodium (Sigma). Subsequently, Blood samples were collected from the posterior orbital venous plexus of the inner canthus of rats for further analysis.

Isolation and culture of rat monocyte-derived macrophages

Isolation and culture of rat monocyte-derived macrophages were performed as previously described [12]. Peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll (Solarbio Life Sciences, Beijing, China) density gradient. Monocytes were obtained from PBMCs using anti-CD14 magnetic beads (Miltenyi Biotec, Auburn, CA), according to the manufacturer's instruction. $CD14^+$ monocytes

were differentiated to macrophages during 7 days of culture in RPMI 1640 (Sigma-Aldrich, St. Louis, MO), containing 10% fetal bovine serum (Sigma-Aldrich) and 50 ng/ml macrophage colony-stimulating factor (Sigma-Aldrich) for 7 days. Half of the culture medium was changed every 3 days unless otherwise indicated.

Flow cytometry and M1/M2 macrophage-related cytokines detection

The phenotypes of rat monocyte-derived macrophages were qualified using flow cytometry analysis according to a previous study [13]. Briefly, macrophages were stained with the following fluorochrome-labeled antibodies: anti-CD80-PE (eBioscience, Thermo Fisher Scientific, Inc., Waltham, MA) and anti-

CD206-MR5D3 (Novus Biologicals Inc., Littleton, CO). After staining for surface markers, macrophages were fixed and analyzed with an Accuri C6 flow cytometer (Becton Dickinson, Franklin Lakes, NJ), and the results were analyzed using FlowJo software (Treestar, Inc., Ashland, OR). The concentrations of IL-12, IFN-γ, IL-4, and TGF-β in rat monocyte-derived macrophages from control or Henoch Schonlein purpura rat were measured using their commercial ELISA kits (R&D Systems,

Minneapolis, MN) according to the manufacturer's instructions.

Real-time polymerase chain reaction analysis

Total RNA was extracted from rat monocyte-derived macrophages using RNeasy Plus Mini Kit (Invitrogen Co, USA) according to the manufacturer's instructions, and cDNA was generated through reverse transcription using the PrimeScript RT Master Mix kit (TaKaRa Bio Inc, Japan). Equal amounts of cDNA were diluted and amplified through real-time polymerase chain reaction (PCR) using All-in-one qPCR Mix (TaKaRa Bio Inc) in a 20 μl reaction volume containing 10 μl of 2×All-in-one qPCR Mix (TaKaRa Bio Inc), 1 μl of 2 mmol/L forward primer, 1 μl of 2 mmol/L reverse primer, 1 μl of cDNA, and 6 μl of nuclease-free water. After an initial denaturation step for 10 min at 95°C, the conditions for cycling were 40 cycles of 10 s at 95°C, 20 s at 60°C, and 15 s at 72°C. For the normalization of each sample, glyceraldehyde 3-phosphate dehydrogenase (GAPDH, TaKaRa Bio Inc) primers were used to measure the amount of GAPDH cDNA. The primers used are listed below (Table 1). Relative expression levels of mRNA, lncRNA, and miRNA was determined using the $2^{-\Delta\Delta Ct}$ method [14]. Relative MEG8 and miR-181a-5p expression were normalized to 18S and the mRNA level of SHP2 was normalized to GAPDH.

Western blotting analysis

The protein levels in rat monocyte-derived macrophages were detected by Western blotting analysis. Briefly, the cells from different groups were lysed by Radio-Immunoprecipitation Assay lysis buffer (Beyotime Bio Inc, Shanghai, China). The protein concentrations were determined by BCA protein estimation kit (Beyotime Bio Inc). Then, equal amounts of protein samples were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (Beyotime Bio Inc), and transferred to polyvinylidene difluoride membranes (Beyotime Bio Inc). After blocking with 5% non-fat dry milk, the membranes were incubated with primary antibodies at 4°C overnight. Then the corresponding secondary antibodies conjugated with horseradish peroxidase were added. ECL detection reagent (7 Sea Biotech, Shanghai, China) was used to visualize the blots. The results were quantified with Gel-Pro-Analyzer (Media Cybernetics, USA).

Plasmid construction and cell transfection

To overexpress MEG8 in rat monocyte-derived macrophages, the synthetic MEG8 sequence was subcloned into the pcDNA3.1 vector (Invitrogen), generating a pcDNA3.1-MEG8 vector. An empty pcDNA3.1 vector used as a control. Cells were transfected with these plasmids using Lipofectamine 2000 (Invitrogen) according to

the manufacturer's instructions. To knockdown MEG8 and SHP2, MEG8 small interfering RNA (si-MEG8), si-SHP2, and the corresponding scramble controls were

purchased from (GenePharma, Shanghai, China) and transfected into cells using Lipofectamine RNAiMAX Reagent (Invitrogen). hsa-miR-181a-5p mimic, mimic negative control (NC), hsa-miR-181a-5p inhibitor, and NC inhibitor (GenePharma) were transfected into cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Cells were harvested 48 hour post-transfection. The overexpression and knockdown efficiency was determined using RT-PCR analysis [15].

Luciferase activity assay

The fragments of MEG8 and 3’-UTR of SHP2 containing the predicted wild-type (WT) binding sites of miR-181a-5p or mutated miR-181a-5p binding sites (MUT) were amplified using PCR and inserted into a pMIR-REPORT luciferase reporter vector (Ambion, Austin, TX), named as MEG8-WT, MEG8-MUT, SHP2-WT, and SHP2-MUT. When the luciferase reporter assay is performed, cells were cotransfected with 200 ng constructed luciferase reporter vectors, 25 ng pRL-TK (expressing Renilla luciferase as the internal control) and 20μM miR-181a-5p mimic or mimic NC using Lipofectamine 2000. A luciferase reporter assay system (Promega Corporation, Fitchburg, WI) was applied to analyze the luciferase activities at 24 hr posttransfection.

Statistical analysis

All statistical analyses were performed using SPSS version 20.0 software (SPSS, Inc., Chicago). The unpaired Student t test was used to analyze differences between only two value sets. One-way analysis of variance was used to analyze differences among three or more groups. The data are presented as the mean ± standard deviation from at least three independent experiments. $P < 0.05$ was considered statistically significant.

Results

Low MEG8 expression and M2 polarization in Henoch Schonlein purpura

Flow cytometry analysis showed that the percentage of $CD80^+$ macrophages (an M1 macrophage marker) was significantly lower, whereas the percentage of $CD206^+$ macrophages (an M2 macrophage marker) in peripheral blood from Henoch Schonlein purpura rat was higher than that in the control group (Figure 1A,B). Furthermore, enzyme-linked immunosorbent assay (ELISA) results demonstrated that concentrations of M1 macrophage cytokines IL-12 (Figure 1C) and IFN-γ(Figure 1D) were significantly lower, whereas concentrations of M2 macrophage cytokines IL-4 (Figure 1E) and TGF-β(Figure 1F) were higher in rat monocyte-derived macrophages from Henoch Schonlein purpura rat than those in the control group. These data indicated that Henoch Schonlein purpura rat existed M1 polarized to M2 macrophages and M2 polarization was exhibited. In addition, RT-qPCR analysis revealed that MEG8 expression was significantly decreased in the Henoch Schonlein purpura group compared with the control group (Figure 1G).

MEG8 overexpression promotes M1 polarization and suppressed JAK2/STAT3 signaling

We next investigated the functional significance of MEG8 in macrophage response. To this end, the monocyte-derived macrophages from Henoch Schonlein purpura rat were transfected with pcDNA3.1-MEG8 to overexpress MEG8. The results of RT-qPCR confirmed the overexpression efficiency of MEG8 (Figure 2A). Flow cytometry analysis showed that MEG8 overexpression significantly increased

the percentage of CD80+ macrophages, whereas decreased the percentage of CD206+ macrophages in HSP rat macrophages compared with HSP+ vector group (Figure 2B,C). Furthermore, ELISA results demonstrated that MEG8 overexpression significantly elevated the levels of M1-related cytokines IL-12 and IFN-γ, whereas reduced those of M2-related cytokines IL-4 and TGF-βin HSP macrophages compared with HSP+vector group (Figure 2D-G). These data suggested that MEG8 overexpression promoted the polarization of HSP rat-derived macrophages from M2 to M1 subtype. Moreover, the JAK2/STAT3 pathway involved in the induction of M2 polarization of macrophages [16]. Our results showed that the JAK2/STAT3 pathway was activated in HSP rat-derived macrophages, as evidenced by an increase in phosphorylation of JAK2 and STAT3 in HSP group compared with the control group (Figure 2H,I). Importantly, MEG8 overexpression potently decreased the increased phosphorylation levels of JAK2 and STAT3 in HSP group (Figure 2H,I) indicating the suppression of the JAK2/STAT3 pathway by MEG8 overexpression.

MEG8 acts as a ceRNA by sponging miR-181a-5p to facilitate SHP2

We then sought to determine the mechanism by which MEG8 promotes M1 polarization and suppressed JAK2/STAT3 signaling. Consistent with the lower MEG8 expression in HSP, the mRNA level of SHP2 was lower in HSP macrophages than that in the control group. In contrast, miR-181a-5p expression in HSP macrophages was higher than that in the control group (Figure 3A).

We next investigated the interaction among MEG8, miR-181a-5p, and SHP2. Luciferase reporter assay showed that miR-181a-5p mimic led to a marked decrease in luciferase activity in MEG8-WT reporter compared with the mimic NC group, whereas had no obvious effect on the luciferase activity in MEG8-MUT reporter. These data verified the direct binding between MEG8 and miR-181a-5p (Figure 3B). Furthermore, we also observed decreased luciferase activity in HEK293T cells cotransfected with WT SHP2 3'-UTR luciferase reporter plasmids and miR-181a-5p mimic (Figure 3C), suggesting that 3'-UTR of SHP2 is directly targeted by miR-181a-5p. In addition, MEG8 overexpression significantly inhibited miR-181a-5p expression, whereas induced SHP2 expression at both mRNA (Figure 3D), protein (Figure 3E) levels. In contrast, MEG8 knockdown exerted the opposite effects. Moreover, miR-181a-5p overexpression with a miR-181a-5p mimic significantly inhibited, whereas miR-181a-5p inhibition elevated expression of both MEG8 and SHP2 (Figure 3F,G). Collectively, these results indicated that MEG8 acted as a sponge for miR-181a-5p to facilitate SHP2 expression.

miR-181a-5p overexpression reverses the MEG8-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling

Flow cytometry analysis showed that the percentage of CD80+ macrophages was significantly decreased, whereas the percentage of CD206+ macrophages was increased in the MEG8 + miR-181a-5p mimic group compared with the MEG8 + mimic NC group (Figure 4A,B). Furthermore, ELISA results demonstrated that the concentrations of IL-12 and IFN-γwere significantly reduced, whereas the concentrations of IL-4 and TGF-βwere elevated in the MEG8+miR-181a-5p mimic

group compared with the MEG8+mimic NC group (Figure 4C–F). These data suggested that miR-181a-5p overexpression reversed the MEG8 overexpression-mediated promotion of M1 polarization. Moreover, the phosphorylation level of JAK2 and STAT3 was significantly increased in

the MEG8+miR-181a-5p mimic group compared with the MEG8+mimic NC group (Figure 4G-I). These findings indicated that miR-181a-5p overexpression significantly reversed the MEG8-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling pathway.

SHP2 knockdown reverses the MEG8-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling

SHP2 has been reported to induce M1 polarization. Furthermore, SHP2 negatively modulates the JAK2/STAT3 signaling pathway in fibrotic tissue remodeling [17]. So we explored whether SHP2 involved in the MEG8 overexpression-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling.

Flow cytometry analysis showed that the percentage of CD80+ macrophages was significantly decreased, whereas the percentage of CD206+ macrophages was increased in the MEG8+SHP2 siRNA group compared with the MEG8+scramble group (Figure 5A,B). Furthermore, ELISA results demonstrated that the concentrations of IL-12 and IFN-γwere significantly reduced, whereas the concentrations of IL-4 and TGF-βwere elevated in the MEG8+SHP2 siRNA group compared with the MEG8+scramble group (Figure 5C–F). These data suggested that SHP2 knockdown reversed the MEG8 overexpression-mediated promotion of M1 polarization. Moreover, the phosphorylation level of JAK2 and STAT3 was significantly increased in the MEG8+SHP2 siRNA group compared with the MEG8 + scramble group (Figure 5G-I). These findings indicated that SHP2 knockdown significantly reversed the MEG8-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling pathway.

Discussion

Henoch-Schonlein purpura is a systemic vasculitis characterised by the deposition of immunoglobulin A (IgA)-containing immune complexes in the walls of small vessels (arterioles, capillaries and venules), which belongs to the group of nongranulomatous, predominantly small vessel vasculitides [18]. Inflammatory processes are commonly divided in different stages: initiation, inflammation, resolution and finally, tissue integrity restoration. Along these lines, macrophages play an important role during the initiation and resolution phases of inflammatory processes. Consequently, these cells have been classified in a simplified manner into different and opposite functional states (pro-inflammatory and anti-inflammatory), characterized by also diametrically opposed phenotypes [19]. Thus, it was suggested that, the restoration of homeostasis and the tissue repair might involve macrophage differentiation by switching gene expression towards different cell programs [20].

Currently, it is known that functional polarization of macrophages only into M1/M2 groups is an over simplified description of macrophage heterogeneity and plasticity, indeed, it is necessary to consider a

continuum of functional states [21]. Overall, the current available data indicate that macrophage polarization is a multifactorial process in which a huge number of factors can be involved producing different activation scenarios [22]. Once a macrophage adopts a phenotype, it still retains the ability to continue changing in response to new environmental influences. The reversibility of polarization has a critical therapeutic value, especially in diseases in which an M1/M2 unbalance plays a pathogenic role [23]. In our study, we found that HSP rat existed M1 polarized to M2 macrophages and M2 polarization was exhibited, MEG8 overexpression promoted the polarization of rat-derived macrophages from M2 to M1 subtype. It indicated that the potential therapeutic role of MEG8 played in Henoch Schonlein purpura.

lncRNAs play an important role in the regulation of gene expression and in various biological processes. Abnormal expression of lncRNAs is involved in the pathogenesis of various diseases [24]. Recent studies have suggested that lncRNAs potentially impact the expression levels of miRNA targets by competing for miRNAs [25]. Evidences have shown that MEG8 contributed to epigenetic progression of EMT of lung and pancreatic cancer cells, and suppressed the proliferation and migration of both the vascular smooth muscle cell (VSMC) and trophoblast cells [26,27]. In addition, MEG8 resulted in down-regulated expression of α-SMA, Colla1, Smad2 and Smad3 and formation of myofibroblast, thus alleviating the progression of kidney fibrosis [28]. However, the function and mechanism of MEG8 in Henoch-Schonlein purpura are unclear. From our results, Consistent with the lower MEG8 expression in HSP, the mRNA level of SHP2 was lower in HSP macrophages than that in the control group. In contrast, miR-181a-5p expression in HSP macrophages was higher than that in the control group. miR-181a-5p inhibition elevated expression of both MEG8 and SHP2. To sum up, these data indicated that MEG8 acted as a sponge for miR-181a-5p to facilitate SHP2 expression, which reveal a previously unknown regulatory network from past research.

Our research also found that miR-181a-5p overexpression and SHP2 knockdown significantly reversed the MEG8-mediated promotion of M1 polarization and suppression of JAK2/STAT3 signaling pathway. JAK2/STAT3 pathway was activated in HSP rat-derived macrophages, as evidenced by an increase in phosphorylation of JAK2 and STAT3 in the HSP group compared with the control group. Recently, miR-181a-5p was shown to be an essential regulator of inflammation in macrophages and dendritic cells by targeting IL-β and c-Fos. Of note, miR-181a-5p expression is significantly decreased in monocytes from obese patients and patients with coronary artery disease (CAD) [29]. SHP2 directly influences the activation of the MAPK pathway, which plays an important role in proliferation of macrophages [30]. Tao et al [31] reported that SHP2 inactivation augmented IL-4-mediated M2 polarization. Further studies revealed that SHP2 deletion in macrophages promoted the association of Janus kinase 1 (JAK1) with IL-4Rαand enhanced IL-4-mediated JAK1 signal transducer and activator of transcription 6 activation, thereby resulting in M2 skewing of macrophages [32]. Zhao L et al [33] found that selective inhibition of SHP2 in mice led to an attenuated inflammatory response by skewing macrophages toward alternatively activated M2 macrophage polarization. Elsa Quintana et al [34] showed that inhibition of SHP2 causes direct and selective depletion of pro-tumorigenic M2 macrophages and promotes anti-tumor immunity, highlighting an investigational therapeutic approach for some RAS pathway-driven cancers. Ariella Zehender et al [35] demonstrated that the activating effects of SHP2 on TGFβ-induced JAK2/STAT3

activation directly translate into stimulatory effects on fibroblasts. Fibroblasts overexpressing SHP2 are more susceptible to the profibrotic effects of TGFβ, whereas the stimulatory effects of TGFβ on myofibroblast differentiation and collagen release are reduced in SHP2 knockout fibroblasts. Selective inactivation of SHP2 in fibroblasts also reduced the pro-fibrotic effects of TGFβ signaling in vivo. A recent report demonstrated that SHP2 is required for M1 polarization of macrophages in the context of Haemophilus influenza infection and that inactivation of SHP2 may promote M2 polarization [36]. These data suggest that inhibition of SHP2 is a promising investigational therapeutic approach.

Conclusion

In conclusion, our results support the notion that MEG8 acts as a ceRNA of miR-181a-5p to facilitate SHP2 expression, which in turn causes suppression of the JAK2/STAT3 pathway and thereby the macrophage polarization from M2 toward M1 subtype. These findings indicate the potential therapeutic effects of MEG8 in

Henoch Schonlein purpura rats.

Declarations

Ethics approval and consent to participate

The study is approved by the ethical committee of Harbin medical University of technology committee.

Consent for publication

Not applicable

Availability of data and materials

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

Competing interests

No competing interests, financial or otherwise, declared by the authors.

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Authors' contributions

Jiang Ming-yu drafted the manuscript. Ren Ming-yong significantly contributed to all the figure designs used throughout the manuscript. Yin Ming-ying critically reviewed the manuscript. Dai Ji-cheng conceived the concept of work. All authors read and approved the final manuscript.

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Abbreviations

MEG8: maternally expressed gene 8

IFN-γ: Interferon γ

TGF-β: transforming growth factor β

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

JAK2: Janus kinase 2

STAT3: signal transducer and activator of transcription 3

mRNA: messenger RNA

NC: negative control

RT-qPCR: quantitative reverse transcription polymerase chain reaction

siRNA: small interfering RNA

SHP2: the Src homology phosphotyrosylphosphatase 2

UTR: untranslated region

WT: wild type

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Table

Due to technical limitations, table 1 is only available as a download in the supplementary files.