MicroRNA-126 accelerates IgE-mediated mast cell degranulation associated with the PI3K/Akt signaling pathway by promoting Ca^{2+} influx

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Abstract. Mast cells (MCs) have been reported to serve a crucial role in allergic diseases, including asthma, allergic rhinitis and anaphylaxis. A previous study revealed that microRNA-126 (miR-126) was associated with airway hyper-responsiveness induced by house dust mites, however the molecular mechanisms were unclear. The present study aimed to investigate the effect of miR-126 on immunoglobulin E (IgE)-regulated MC degranulation and explore its underlying mechanisms. miR-126 expression was quantified using a rat model *in vivo* and in rat peritoneal mast cells (RPMCs) *in vitro*. Overexpression or downregulation of miR-126 was established by transfection with miR-126 mimics or miR-126 inhibitors and MC degranulation was subsequently evaluated. The effect of miR-126 on protein kinase B (Akt) and phosphorylated Akt protein expression was examined by western blot analysis. The phosphoinositide 3-kinase (PI3K) inhibitor (LY294002) was used to determine the role of the PI3K/Akt signaling pathway. In addition, cytosolic calcium (Ca^{2+}) levels were measured by a fura-2 assay. The results demonstrated that miR-126 expression was upregulated in the ear tissues of rats with allergic contact dermatitis and IgE-activated MCs. The overexpression of miR-126 in RPMCs was established following miR-126 mimic transfection. The release of β-hexosaminidase and histamine, markers of MC degranulation, were significantly increased in cells with miR-126 overexpression. The phosphorylation of Akt was significantly increased following transfection with miR-126 mimics in stimulated cells, however the signaling activation was abrogated by LY294002. In addition, Ca^{2+} influx was significantly promoted in stimulated RPMCs overexpressing miR-126. These results indicate that miR-126 accelerated IgE-mediated MC degranulation associated with the PI3K/Akt signaling pathway by promoting Ca^{2+} influx. This suggests that miR-126 may be a promising therapeutic target for the treatment of allergic skin diseases.

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Introduction

Mast cells (MCs) are local tissue-resident cells, which are frequently located within the skin, respiratory tract and gastrointestinal mucosa (1). Increasing evidence indicates that MCs serve a crucial role in allergic diseases, including bronchial asthma, allergic rhinitis, atopic dermatitis and hypersensitivity (2-5). MCs are activated when antigen cross-linking of immunoglobulin E (IgE) binds to the high-affinity receptor (FcεRI), which results in the phosphorylation of Syk tyrosine kinase, calcium (Ca^{2+}) influx, and the activation of protein kinase C, mitogen-activated protein kinases and nuclear factor (NF)-κB (6). Following MC degranulation, inflammatory mediators, including proteases, β-hexosaminidase, histamine and inflammatory cytokines, such as tumor necrosis factor-α and interleukin-6 are released and extensively associated with the pathogenesis of allergic diseases (7). There is a positive correlation between disease severity and the number of MCs (8), therefore regulation of MC activation is considered as a promising and alternative treatment option for MC-associated diseases.

MicroRNAs (miRs) are a group of small, non-coding, single-stranded RNAs, which regulate gene expression at a post-transcriptional level by targeting the 3'-untranslated region (3'-UTR) of specific mRNAs for degradation or

translational repression (9). A previous study indicated that miR-126 was overexpressed in a murine model of asthma, which was induced by house dust mites (10). In addition, the overexpression of miR-126 in bone marrow-derived MCs may promote FcεRI-mediated cytokine production (11). These findings suggested a possible association between miR-126 overexpression and the activation of MC degranulation. However, the role of miR-126 in MC degranulation and the underlying molecular mechanisms are required further clarification.

The present study aimed to investigate the effect of miR-126 on MC activation triggered by IgE and explored the underlying molecular mechanisms. The results revealed that miR-126 accelerated IgE-mediated MC degranulation, which was associated with the phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway and enhanced Ca^{2+} influx.

Materials and methods

Animals. A total of 12 male 8-week-old Sprague-Dawley rats (weight, 200.2±15.4 g) were purchased from the Hubei Research Center of Laboratory Animals (Wuhan, China) and housed in specific pathogen-free conditions. The rats were divided into three groups as follows: i) Normal (n=4); ii) allergic contact dermatitis (ACD) model (n=4); and iii) isolation of rat peritoneal mast cells (RPMCs; n=4). The rats were housed 4 per cage and maintained at a temperature of 22±1˚C in a relative humidity of 55±10%, under a 12 h light/dark cycle for 1 week prior to the start of the experiments. Water and a standard diet were provided *ad libitum* throughout the study. The study protocol was approved by the Ethics Committee of the Animal Care and Use Committee of the Central Hospital of Wuhan (approval no. SCXK2015-0018; Wuhan, China).

Establishing 2,4-dinitrofluorobenzene (DNFB)-induced ACD. Briefly, the rats were sensitized by topically applying 25 μl 0.5% DNFB (acetone:olive oil, 4:1; Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) on the shaved hind flank on days 1 and 2. Following 5 days the rats were challenged with 10 μl 0.5% DNFB on the left ear to induce ACD.

Isolation of RPMCs. At the end of the experiment, on day 8, rats in the RPMC group were anesthetized with 7% intraperitoneal chloral hydrate (350 mg/kg; Sigma-Aldrich; Merck KGaA). Following its administration, no symptoms of chemical peritonitis were observed. Dulbecco's minimal essential medium (DMEM; Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) with 10% fetal bovine serum (Invitrogen; Thermo Fisher Scientific, Inc.) containing penicillin (100 U/ml), streptomycin (100 μg/ml) and heparin (5 U/ml) (Gibco; Thermo Fisher Scientific, Inc.) was injected into the peritoneal cavity. Once the lavage was completed, the rats (252.1±10.6 g) were sacrificed via CO_2 inhalation (the flow rate of CO_2 displaced >30% of the chamber volume/minute) and cervical dislocation was used to sacrifice the animals. Cells of the peritoneal lavage fluid were collected by centrifugation at 400 x g for 15 min at room temperature and resuspended in 1 ml serum-free DMEM. The macrophages were separated from the MCs by differential centrifugation using a Percoll solution (Sigma-Aldrich; Merck KGaA) as previously described (12).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Total RNA was extracted from the RPMCs using TRIzol reagent (Invitrogen; Thermo Fisher Scientific, Inc.). RT was conducted using a TaqMan MicroRNA Reverse Transcription kit (Thermo Fisher Scientific, Inc.) at 42˚C for 30 min followed by 75˚C for 5 min. qPCR was performed using a TaqMan MicroRNA assay (Thermo Fisher Scientific, Inc.). The following primer sequences were used for the PCR: miR-126, forward 5'-CGTACCGTGAGTAATAATG-3' and reverse 5'-AACTGGTGTCGTGGAG-3'; and U6, forward 5'-CAAGGATGACACGCAAAT-3' and reverse 5'-TGGTGTCGTGGAGTCG-3'. The PCR protocol was 95˚C for 10 min, followed by 40 cycles of 95˚C for 10 sec, 60˚C for 30 sec and 72˚C for 30 sec followed by final extension at 72˚C for 5 min and storage at 4˚C. Each sample was analyzed in duplicate. The expression of U6 was used as the endogenous control for miRNAs analysis. The data were quantified using the $2^{-\Delta\Delta Cq}$ method (13).

Transfection. RPMCs were transfected with miR-126 mimic (5'-UCGUACCGUGAGUAAUAAUCCG-3'), negative control (5'-UUCUCCGAACGUGUCACGUTT-3'), miR-126 inhibitor (5'-CAUUAUUACUUUUGGUACGCG-3') and its control (5'-CAGUACUUUUGUGUAGUACAA-3') all supplied by Shanghai GenePharma Co., Ltd. (Shanghai, China) using Lipofectamine® 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The final concentration of oligonucleotides was 50 nM. Following 24 h incubation at 37˚C, the cells were processed for further experiments.

Analysis of β-hexosaminidase release. RPMCs were activated for a degranulation assay. Following challenge with dinitrophenyl (DNP)-human serum albumin (HSA) (100 ng/ml) (Sigma-Aldrich; Merck KGaA) to sensitized cells, the cell supernatant was mixed with an equal volume of substrate solution (1 mM 4-nitrophenyl-N-acetyl-β-D-glucosaminide in 0.1 M citrate buffer, pH 4.5) for 1 h at 37˚C. The reaction was stopped with carbonate buffer (0.1 M Na_2CO_3/$NaHCO_3$). The absorbance was measured with a microplate reader at 405 nm.

Histamine release assay. To determine MC degranulation, the levels of histamine in homogenates from the left ear of ACD rats and the culture medium of RPMCs were measured. Histamine concentrations were detected using an ELISA kit (KT60094; Kamiya Biomedical Company, Seattle, WA, USA) according to the manufacturer's protocol.

LY294002 assay. RPMCs were transfected with miR-126 mimics or its NC and the cells were sensitized overnight at 37˚C with anti-DNP IgE (0.5 μg/ml; Sigma-Aldrich; Merck KGaA). Cells were subsequently treated with DNP-HSA (100 ng/ml) at 37˚C for 4 h in the presence or absence of LY294002 (10 μM; Sigma-Aldrich; Merck KGaA). Following, β-hexosaminidase and histamine release were detected.

Western blot analysis. RPMCs were obtained and lysed with a lysis buffer (20 mM Tris-HCl, pH 7.5; 150 mM NaCl; 1 mM Na_2EDTA; 1 mM EGTA; 1% Triton; 2.5 mM sodium pyrophosphate; 1 mM β-glycerophosphate; 1 mM Na_3VO_4 and 1 μg/ml leupeptin) containing protease and phosphatase

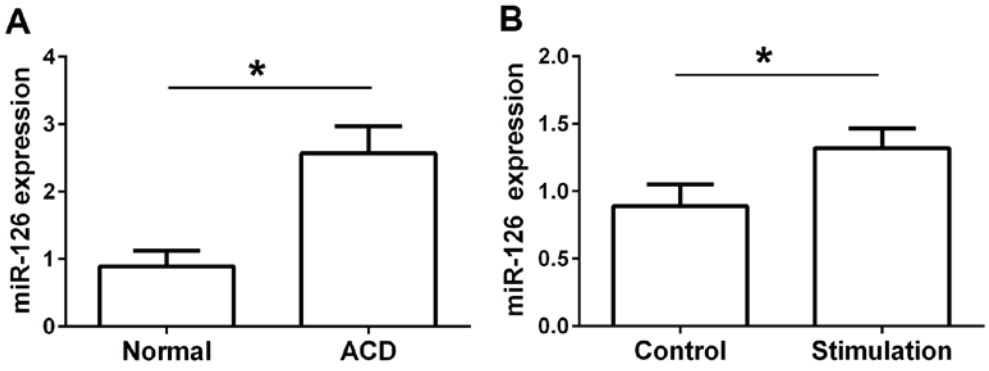

Figure 1. miR-126 expression in rats with 2,4-dinitrofluorobenzene-induced ACD and IgE-activated MCs. (A) miR-126 expression in the left ear tissues of ACD rats. (B) Rat peritoneal MCs were sensitized with anti-DNP IgE (0.5 μg/ml) and stimulated with DNP-HSA (100 ng/ml) for 4 h. The expression of miR-126 was analyzed. Data are presented as the mean ± standard deviation (n=4); *P<0.05. miR, microRNA; IgE, immunoglobulin E; ACD, allergic contact dermatitis; MC, mast cell; DNP, dinitrophenyl; HAS, human serum albumin.

inhibitors (no. 9803; Cell Signaling Technology, Inc., Danvers, MA, USA). Supernatants were analyzed for protein content using a BCA Protein Assay kit (no. 7780; Cell Signaling Technology, Inc.). Proteins (20 μg) were separated by 12% SDS-PAGE gels and transferred onto polyvinylidene difluoride membranes. Following blocking overnight at 4˚C with 5% skimmed milk, the membranes were incubated with rabbit anti-rat primary antibodies against Akt (cat. no. SC-8312; 1:200), phosphorylated (p)-Akt(S473) (cat. no. SC-135651; 1:100) and GAPDH (cat. no. SC-25778; 1:1,000; all Santa Cruz Biotechnology, Inc., Dallas, TX, USA). Following 3 washes with Tris-buffered saline with Tween-20 buffer, the membranes were incubated at room temperature for 2 h with the appropriate horseradish-peroxidase-conjugated goat anti-rabbit secondary antibodies (cat. no. SC-2004; 1:5,000; Santa Cruz Biotechnology, Inc.). The proteins were visualized using an enhanced chemiluminescence detection reagent (Pierce; Thermo Fisher Scientific, Inc.).

Cytosolic Ca^{2+} measurement. Following treatment with anti-DNP IgE (0.5 μg/ml; Sigma-Aldrich) at 37˚C for 24 h, cells were washed three times in Tyrode's buffer (135 mM NaCl; 5 mM KCl; 1.8 mM $CaCl_2$; 1.0 mM $MgCl_2$; 5.6 mM glucose; 20 mM HEPES; and 1 mg/ml bovine serum albumin at pH 7.4) and co-incubated with 2 μM fura-2/AM (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C for 30 min. The fura-2/AM was discarded and the cells were resuspended in Tyrode's buffer and stimulated with 100 ng/ml DNP-HSA at 37˚C for 4 h. Measurements of Ca^{2+} influx were made using a Leica DMI 6000B fluorescence microscope (Leica Microsystems GmbH, Wetzlar, Germany) controlled with SlideBook software (Intelligent Imaging Innovations, Inc.; Denver, CO, USA). Fluorescence emission at 505 nm was monitored while alternating excitation between 340-380 nm at a frequency of 0.5 Hz. Ca^{2+} influx is presented as 340/380 nm ratio.

Statistical analysis. Data are presented as the mean ± standard deviation. Statistical analyses were conducted using SPSS 16.0 software (SPSS, Inc., Chicago, IL, USA). The results were analyzed using one-way analysis of variance and Duncan's post-hoc test. P<0.05 was considered to indicate a statistically significant difference.

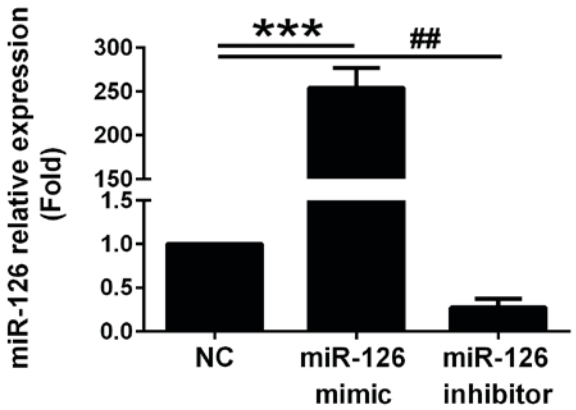

Figure 2. miR-126 is overexpressed in transfected MCs. Rat peritoneal MCs were transfected with miR-126 mimics, the NC and miR-126 inhibitors. Following 24 h, the expression of miR-126 was examined by reverse transcription-quantitative polymerase chain reaction and analyzed using the $2^{-\Delta\Delta Cq}$ method. Data are presented as the mean ± standard deviation (n=4); ***P<0.001 and $^{\#\#}$P<0.01. miR, microRNA; MC, mast cell; NC, negative control.

Results

miR-126 is increased in IgE-stimulated RPMCs. To investigate the effect of miR-126 on MC degranulation, miR-126 expression was analyzed in a rat model of hypersensitivity *in vivo* and in isolated RPMCs *in vitro*. The results revealed that the expression of miR-126 was significantly increased in rats with DNFB-induced ACD compared with the normal group (Fig. 1A). In comparison with the control group, the expression of miR-126 was also significantly increased in the IgE-stimulated RPMCs (Fig. 1B).

miR-126 is overexpressed in RPMCs transfected with miR-126 mimics. To assess the effect of miR-126 on MC degranulation, RPMCs were transfected with miR-126 mimics to construct a model of miR-126 overexpression. miR-126 expression was significantly increased at 24 h following transfection with the miR-126 mimics (Fig. 2). Conversely, miR-126 expression was clearly decreased following transfection with the miR-126 inhibitor (Fig. 2).

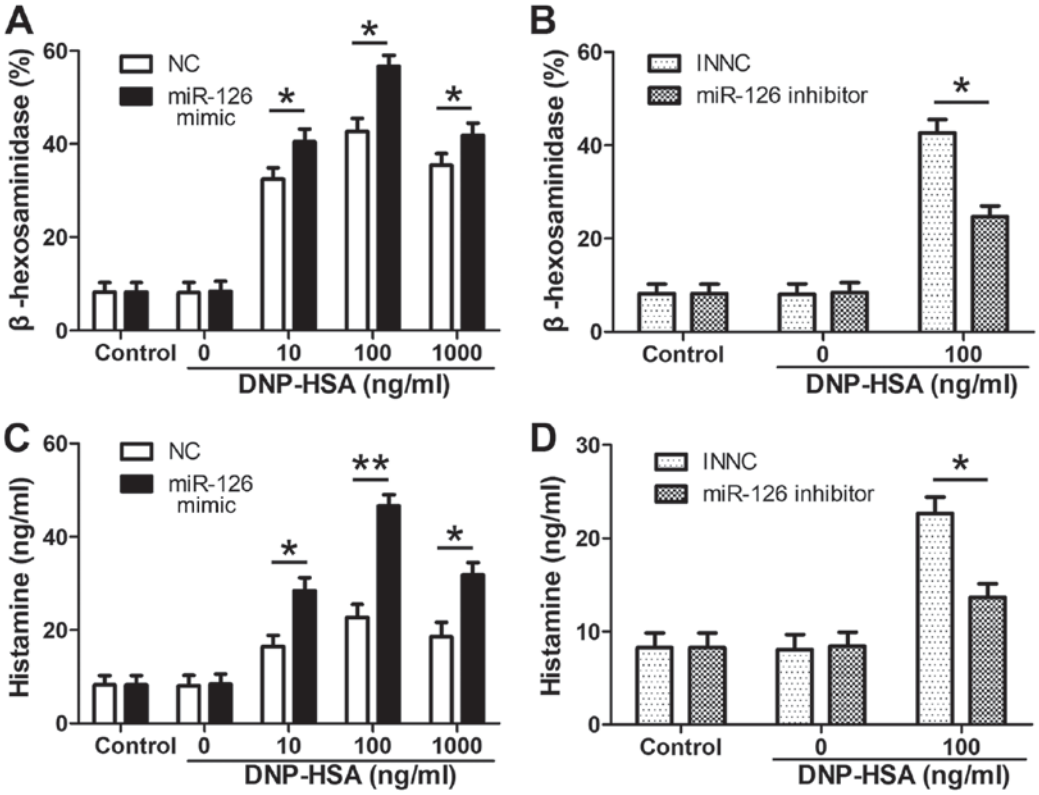

Figure 3. Effect of miR-126 on MC degranulation. Rat peritoneal MCs were transfected with miR-126 mimics and their NC, as well as the miR-126 inhibitor and its NC. Following sensitization with anti-DNP immunoglobulin E (0.5 μg/ml), the cells were treated with the indicated concentrations of DNP-HSA for 4 h. The release of β-hexosaminidase was detected in the (A) miR-126 mimics and (B) miR-126 inhibitor groups. The release of histamine was detected in the (C) miR-126 mimics and (D) miR-126 inhibitor groups. Data are presented as the mean ± standard deviation (n=4); *P<0.05 and **P<0.01. miR, microRNA; MC, mast cell; NC, negative control; INNC, inhibitor negative control; DNP, dinitrophenyl; HAS, human serum albumin.

Effect of miR-126 on MC degranulation. β-hexosaminidase and histamine are of MC degranulation (14); their release was detected to evaluate the level of MC degranulation. RPMCs were transfected with miR-126 mimics, miR-126 inhibitor and NCs. Following sensitization with anti-DNP IgE, cells were treated with the indicated concentrations of DNP-HSA for 4 h. The release of β-hexosaminidase was detected (Fig. 3). β-hexosaminidase release was significantly upregulated in the miR-126 mimic group compared with the negative control group (Fig. 3A), where as it was significantly downregulated in the miR-126 inhibitor group (Fig. 3B). Similar results were observed for histamine release in the miR-126 mimic group (Fig. 3C) and the miR-126 inhibitor group (Fig. 3D).

miR-126 enhances PI3K/Akt signal activation in IgE-mediated MCs. To clarify if the PI3K/Akt signaling pathway was associated with the effect of miR-126 on MC degranulation, RPMCs were transfected with miR-126 mimics and the negative control and the cells were either stimulated with DNP IgE and DNP-HSA or not stimulated. The protein expression of Akt and p-Akt was then determined by western blot analysis. Compared with the negative control group, the phosphorylation of Akt was significantly increased in the miR-126 mimic group in the stimulated cells (Fig. 4). However, there were no significant differences observed between the two groups of non-stimulated cells.

Effect of the PI3K-inhibitor on miR-126-enhanced MC degranulation. A specific PI3K-inhibitor (LY294002) was

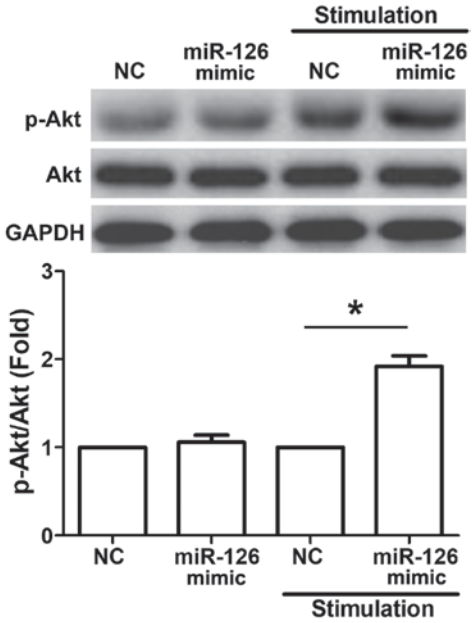

Figure 4. miR-126 activates the PI3K/Akt signaling pathway in IgE-mediated MC degranulation. Rat peritoneal MCs were transfected with miR-126 mimics or its NC. Then the cells were sensitized with anti-DNP IgE (0.5 μg/ml) and stimulated with DNP-HSA (100 ng/ml) for 15 min. The protein expression of Akt and p-Akt were determined by western blot analysis and representative results are reported. Data are presented as the mean ± standard deviation (n=4); *P<0.05. miR, microRNA; MC, mast cell; IgE, immunoglobulin E; NC, negative control; Akt, protein kinase B; PI3K, phosphatidylinositol 3-kinase; p-, phosphorylated; DNP, dinitrophenyl; HAS, human serum albumin.

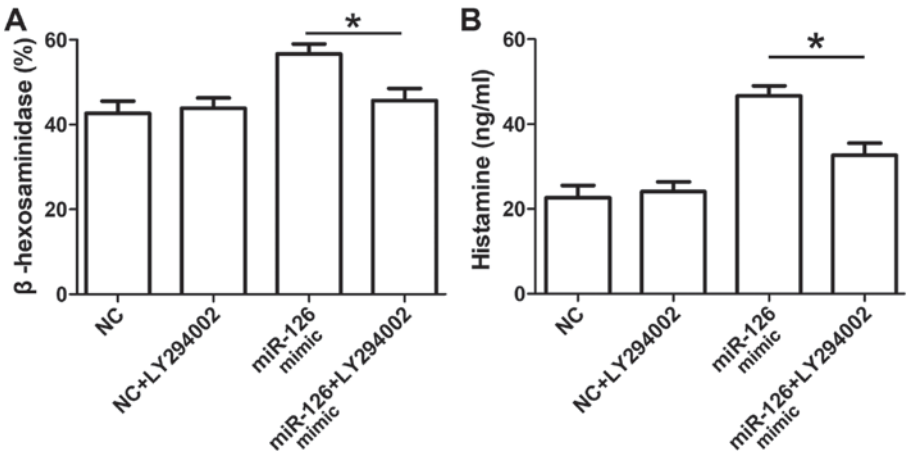

Figure 5. Effect of the PI3K inhibitor (LY294002) in miR-126-improving MC degranulation. Rat peritoneal MCs were transfected with miR-126 mimics or its NC, and the cells were sensitized overnight with anti-DNP immunoglobulin E (0.5 μg/ml). The cells were subsequently treated with DNP-HSA (100 ng/ml) for 4 h in the presence or absence of LY294002 (10 μM). Release of (A) β-hexosaminidase and (B) histamine was measured. Data are presented as the mean ± standard deviation (n=4); *P<0.05. miR, microRNA; MC, mast cell; NC, negative control; PI3K, phosphatidylinositol 3-kinase; DNP, dinitrophenyl; HAS, human serum albumin.

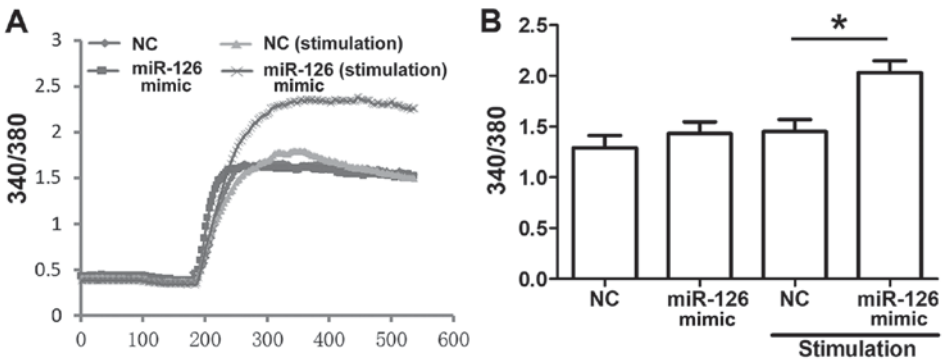

Figure 6. Ca^{2+} influx was associated with the regulation of miR-126 on MC degranulation. Rat peritoneal MCs were transfected with miR-126 mimics or its NC. The cells were subsequently sensitized with anti-DNP immunoglobulin E (0.5 μg/ml) and treated with DNP-HSA (100 ng/ml) for 4 h. (A) The intracellular Ca^{2+} influx was determined and (B) statistically analyzed using the 340/380 ratio. Representative traces of intracellular Ca^{2+} changes were observed. Data are presented as the mean ± standard deviation (n=4); *P<0.05. miR, microRNA; MC, mast cell; NC, negative control; DNP, dinitrophenyl; HAS, human serum albumin.

used to confirm the role of the PI3K/Akt signaling pathway in miR-126-enhanced MC degranulation. Compared with the miR-126 mimic group, the release of β-hexosaminidase (Fig. 5A) and histamine (Fig. 5B) were significantly reduced in the miR-126 mimic + LY294002 groups.

Ca^{2+} influx participates in the regulation of miR-126 on MC degranulation. Ca^{2+} mobilization was indicated as associated with MC degranulation, which responded to the crosslinking of IgE binding to its receptors. Changes in cytosolic Ca^{2+} were examined using a fura-2 assay. Ca^{2+} influx was significantly enhanced in the miR-126 mimic group compared with the NC group when the cells were stimulated (Fig. 6).

Discussion

The association between miRNAs and mast cell activation involved in allergic inflammation attracts increasing attention. A previous study reported a significant upregulation of miR-21, miR-142-3p, miR-142-5p and miR-223 in the skin of mice with allergic contact dermatitis and also in humans sensitized with diphenylcyclopropenone (15). The present study observed that miR-126 was significantly upregulated in ear samples from DNFB-treated rats and IgE-stimulated peritoneal MCs. However, there were certain differences between the two studies, which may attribute to the difference of miRs expression. Rats were used for the ACD model in the present study as opposed to mice and the time of repeated-DNFB application was different to the above study by ~3 weeks. In addition, another previous study detailed that miR-126 was significantly downregulated following the extension of culture time in bone marrow-derived MCs, which suggests that cell type and culture time may affect miR-126 expression (11). miR-126 can be isolated from vascular endothelial cells and serves an important role in maintaining endothelial cell proliferation and migration (16). It may further influence other cell processes, including angiogenesis and apoptosis (17-19). miR-126 positively regulates MC proliferation and cytokine production through suppression of Sprouty-related Ena/VASP homology-1 domain-containing protein (Spred1) (11). In

the present study, the model of miR-126 overexpression in RPMCs was established following miR-126 mimic transfection, which was associated with the activation of MC degranulation and the increased release of β-hexosaminidase and histamine.

The PI3K/Akt signaling pathway is important for cell growth, differentiation, metabolism, survival and apoptosis (20). As the PI3K conformation changes, Ser473 and Thr308 phosphorylation is required for Akt activation (21). A number of previous studies have demonstrated that the PI3K/Akt signaling pathway participated in the regulation of miRNAs on MC degranulation (22-24). miR-155 controlled MC activation by modulating the PI3Kγ signaling pathway and anaphylaxis in mice with cutaneous anaphylaxis (22). Downregulation of miR-223 promoted MC degranulation via the PI3K/Akt pathway by targeting insulin-like growth factor 1 receptor (23). Lentiviral short hairpin RNA against KCa3.1 inhibited the allergic response in allergic rhinitis and suppressed MCs activity via the PI3K/AKT signaling pathway (24). In the present study, the phosphorylation of Akt was increased, accompanied by miR-126 overexpression, which was reversed by LY294002, the specific PI3K inhibitor. These findings suggest that PI3K/Akt signaling was associated with the regulation of miR-126 on MCs.

Ca^{2+} participates in intra- and extracellular signaling pathways and serves a key role in cell destiny (25). Crosslinking of FcεRI complexes at the MC surface initiates cytoplasmic Ca^{2+} (Ca_i^{2+}) mobilization and stimulates Ca^{2+} influx, which is important for MC degranulation (26). In a previous study, miR-214 knockout mice exhibited more severe cardiac injuries, including increased cardiomyocyte loss and larger fibrotic regions, which were associated with high Ca_i^{2+} levels and Ca_i^{2+} overload (27). In addition, the overexpression of miR-25 protected cardiomyocytes against oxidative damage by inactivating the mitochondrial apoptosis pathway, which targets the mitochondrial Ca^{2+} uniporter and reduces H_2O_2-induced elevation of mitochondrial Ca^{2+} concentrations (28). In line with these previous reports, the results of the present study demonstrated that transfection with miR-126 mimics promoted Ca^{2+} influx in IgE-stimulated MCs. The present authors speculate that the exogenous or endogenous stimulation induced the overexpression of miR-126 on MCs, thereby enhancing the Ca^{2+} influx. The decreased Ca^{2+} in the cytoplasm triggered the PI3K/Akt signaling pathway, which contributed to the acceleration of IgE-mediated MC degranulation.

In the present study, the effect of miR-126 on MC degranulation was investigated based on the intrinsic association between PI3K/Akt signaling and Ca^{2+} influx. The results provide additional evidence supporting the possible molecular mechanism of miR-126 on allergic skin inflammation. However, there were certain limitations in the present study that should be mentioned: i) Bone marrow-derived MCs were not used as a rat model was selected. In the majority of cases, bone marrow-derived MCs were isolated from mice and induced differentiation following cytokine stimulation *in vitro* for 4-6 weeks. Due to time and cost considerations, peritoneal MCs were used in the present study, which was suitable for the experimental design; although bone marrow-derived MCs may offer more comprehensive support of the hypothesis. ii) If other signaling pathways or transcription factors, including NF-κB and AP-1 were associated the biological effect of miR-126, further studies are required to investigate their underlying mechanisms and cascade reactions. iii) Although certain targets of miR-126 were reported in different cell types and disease models, including EGFL7 (29), pik3r2 (30), ROCK1 (31), IRS-1 and GOLPH3 (32), to the best of our knowledge, Spred1 was reported as a direct target of miR-126 and was associated with MC activation (11). The present study focused on the direct effect of miR-126 on MC degranulation and its preliminary molecular mechanisms. However, it is possible that the mRNA 3'-UTR may be degraded by miR-126 at a post-transcriptional level and that proteins may regulate the PI3K/Akt signaling pathway and Ca^{2+} influx. Further studies are required to clarify the underlying molecular mechanisms.

In conclusion, miR-126 expression was increased in ACD rats and rat peritoneal MCs. Overexpression of miR-126 inhibited the release of β-hexosaminidase and histamine, attenuated the phosphorylation of Akt and enhanced Ca^{2+} influx. This suggests that miR-126 accelerated IgE-mediated MC degranulation associated with the PI3K/Akt signaling pathway via promoting Ca^{2+} influx. These findings provide an insight into the potential role of miR-126 in association with the treatment of allergic skin diseases.

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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.

Authors' contributions

YB, SW, YY and JL designed the experiments. YB and SW performed the study and wrote the manuscript. YG and WZ participated in establishing the animal model. HJ was involved in analyzing the data. YY and JY revised the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethical Committee of the Animal Care and Use Committee of the Central Hospital of Wuhan (approval no. SCXK2015-0018).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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