

Original Article

miR-125a-3p decreases levels of interleukin-17 and suppresses renal fibrosis via down-regulating TGF- β 1 in systemic lupus erythematosus mediated Lupus nephritic mice

Ying Zhang, Xin Chen, Ying Deng

Department of Nephrology, Hanzhong Central Hospital, Hanzhong 723000, Shaanxi, China

Received February 5, 2019; Accepted February 10, 2019; Epub March 15, 2019; Published March 30, 2019

Abstract: Lupus nephritis (LN) is an autoimmune disorder mediated by systemic lupus erythematosus (SLE). Micro RNAs also called as miRs act potentially in the development and progression of SLE. MiR-125a-3p is reported to be down-regulated in patients of SLE. Aim of the study was to evaluate the function of miR-125a-3p and its effect on regulation of fibrosis and interleukin (IL)-17 in LN. The Renal physiology in MRL/MPJ-Fas^{lpr}/J mice was done by Hematoxylin and eosin staining; expression of miR-125a-3p in renal tissues by RT-PCR, Immunoblotting analysis was done expression of proteins. For *in vitro* studies, rat mesangial SV40MES13 cells were used and were transfected with vectors. Luciferase activity was done for studying the potential binding of miR-125a-3p with IL-17. It was found that, the expression of miR-125a-3p in kidney tissues of experimental mice were towards lower side versus the control; on the contrary the levels of IL-17 were up-regulated in experimental mice. Luciferase activity suggested that miR-125a-3p binds potentially on the 3'UTR region of IL-17. The assay also suggested up-regulation of miR-125a-3p and suppressed levels of IL-17 in SV40MES13 cells. The up-regulation of miR-125a-3p suppressed the levels of collagen I/II and transforming growth factor- β 1 (TGF- β 1) in SV40MES13 cells. MiR-125a-3p could be an important factor in the pathogenesis of LN which causes decrease in expression of IL-17 by potentially binding to the 3'UTR region causing suppression of fibrosis via down-regulating TGF- β 1 in the SV40MES13 rat mesangial cells.

Keywords: Lupus nephritis, systemic lupus erythematosus, miR-125a-3p, IL-17

Introduction

Lupus nephritis (LN) is diagnosed to be a complicated autoimmune disorder engendered majorly by systemic lupus erythematosus (SLE), the condition is characterized by damage in multiple organ, majorly in the Kidney, joints, brain and skin [1]. Major of SLE subjects develop LN [2, 3]. LN has been featured by many types of glomerulonephritis having fibrotic or inflammatory responses. Numbers of factors contribute in the development of LN, still the exact genetic pathway remains unclear. Thus it makes worthy to evaluate the genes and the mechanism involved in LN for overcoming nephritis and renal fibrosis. The condition of LN is accompanied with inflammation via a progressive suppression in function of kidney mediated by developing fibrosis. A deposition

of collagen I which is a matrix protein is regarded as a prime factor of fibrosis [4].

The condition of SLE is associated with deregulation of innate and adaptive immune response, cytokines play an important role in these systems [5]. Deregulation in production of cytokines play an important role in immune dysfunction and leads to inflammatory response in tissues causing organ damage. Inflammatory cytokines such as, interferons (type 1 and 2), interleukins (IL) such as IL-6, IL-1, IL-21, immunomodulatory cytokines such as IL-10, the tumor necrosis factor- α (TNF- α), transforming growth factor- β (TGF- β) have been identified to play important role in SLE [6]. The studies have reported various molecular and biological mediators contributing in the pathogenesis of SLE [7], exploiting these has been aimed for devel-

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

oping new therapeutic biomarkers and strategies. Though numbers of factors have been discovered contributing in the development of SLE, the target genes and the involved cascades are still not understood. Thus, it was worth to investigate the possible mechanism and the involved genes for improving the therapy and diagnosis of SLE. Recently IL-17 has been identified to play role in autoimmunity [8]. IL-17 have been identified to be associated with various autoimmune disorders including rheumatoid arthritis [9], sclerosis [10, 11], and SLE [12]. IL-17 was found to play a prime role in animal models LN mice [13, 14] and has been hence assumed to play important role in human SLE [9]. The levels of IL-17 have been found to be upregulated in subjects reported for SLE compared to normal [15-17].

MicroRNAs (miRNAs) are single-stranded endogenously produced, non-coding RNAs having 21-25 nucleotides in length. miRs are responsible for regulating expression of genes via binding to 3' untranslated region (3'UTR). miRs have been implicated in various cellular processes such as cellular immunity [18], inflammation [19], cell proliferation, apoptosis and differentiation [20]. Also, the miRs have been linked to repairing of DNA, oxidative stress, neural development [21, 22]. Evidently, it is affirmed that number of disorders including SLE are regulated by miRs [23]. Numbers of studies have emerged suggesting miRs to act potentially in the progression and development of SLE [24-27]. Antecedently a study conducted on profile of mRNAs in SLE patients identified 8 miRs to be deregulated in SLE patients, among them miR-16, miR-21, miR-451 and miR-223 were overexpressed in the subjects, whereas miR-125a-3p, miR-155 and miR-146a were downregulated. MiR-125a-3p was potentially involved in the progression and development of SLE [28].

The present work evaluates the role of miR-125a-3p and IL-17 on inflammation and fibrosis in MRL/MPJ-Fas^{lpr}/J mice (*in vivo*) and in SV40MES13 mesangial cells (*in vitro*). The study suggested that, the levels of miR-125a-3p were downregulated in MRL/MPJ-Fas^{lpr}/J mice compared to control mice, on contrary the levels of IL-17 were overexpressed in MRL/MPJ-Fas^{lpr}/J mice. Above this, miR-125a-3p influenced the levels of IL-17 via binding on the

3'UTR region of IL-17 directly. We also determined that miR-125a-3p suppressed the fibrosis via *Transforming growth factor beta 1* or TGF- β 1 in SV40MES13 cells. The main objective of the work was to investigate the role of miR-125a-3p in LN and to find whether miR-125a-3p governs the levels of IL-17 in LN.

Materials and methods

Animals

For the study 16-17 weeks old female MRL/MPJ-Fas^{lpr}/J mice weighing 20-22 g (n = 10) and similarly aged BALB/C female mice (n = 10) (pathogen free) were obtained from the animal house of Hanzhong central hospital, Hanzhong, Shaanxi, China. All the animal studies received ethical clearance from the animal ethical committee of Hanzhong central hospital, Hanzhong, Shaanxi, China, the approval number was HCH/SLE/2018/R12. The animal experiments were carried out at laboratories of Hanzhong central hospital, Hanzhong, Shaanxi, China. All the animals were housed under controlled conditions of temperature (22 \pm 2°C) and relative humidity (50 \pm 10%) with 12 h dark and light cycle, the mice were provided with access to chow diet and water ad libitum.

Cell culture

For in vitro studies, the mouse mesangial cells SV40MES13 (ATCC[®] CRL-1927[™]) were obtained from ATCC, USA. The cells were utilized for transfection for studying the function of miR-125a-3p in SLE. The mouse mesangial cells were subjected to culture in DMEM media, added with FBS (10%) previously inactivated by heating, penicillin (100 units/mL), glutamine (2 mmol/L) and streptomycin (100 mg/ml) all obtained from Sigma Aldrich USA.

Hematoxylin-eosin staining

The renal tissue samples were subjected to hematoxylin and eosin staining, for the same the kidneys were harvested and fixed using xylene (Sigma Aldrich USA) and fixed in paraffin wax. The tissue sections of 5 μ m thickness were obtained using microtome, the sections were then deparaffinized using ethanol, followed by hematoxylin and eosin staining (Sigma Aldrich USA) for histological evaluation. The tissue sections were subjected to light micro-

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

scope study (Olympus, Japan). The extent of vascular and glomerular damage was done using the semi-quantitative scoring system as discussed earlier [29].

Real-time PCR analysis

The level of miR-125a-3p in renal tissue samples of MRL/MPJ-Fas^{lpr}/J and BALB/C mice was evaluated by RT-PCR. The total RNA was isolated using a RNA isolation kit (Thermo Fisher USA) following the provided instructions. The synthesis of cDNA was done from isolated total RNAs using a reverse transcription kit (Thermo Fisher USA). The cDNA were amplified for PCR analysis. The RT-PCR primers utilized in the study were as, miR-125a-3p 5'-TCCCTGAGACCCTAACTTGTGA-3. The reverse transcription was carried using the forward and reverse primers along with cDNA template in various conditions of temperature followed by PCR amplification. The results of PCR amplification were studied by changing the number of cycles for each of the cDNA. For PCR analysis U6 was selected as loading control.

Immunoblotting analysis

The expression of protein was done by immunoblotting experiments, the total protein contents were estimated using a protein estimation kit (Thermo Fisher USA) in accordance to provided instructions. For the study the anti-TGF- β 1, anti-collagen I/III was obtained from Santa Cruz Biotech (USA), anti-IL-17, anti- β -actin were procured from cell signaling technologies (USA). The proteins were separated on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (10%) and were channelized to polyvinylidene difluoride (PVDF) membranes (ThermoFisher USA). The filters were exposed to I^{vy} antibodies for 12 hours at 4°C. The PVDF membranes were treated with horseradish peroxidase-conjugated goat anti-rabbit/mouse immunoglobulin-G II^{vy} antibody for 1 hour at 37°C. The expressions of proteins were normalized using β -actin as loading control.

Construction of vector

For constructing vectors, the cDNA from the hospital lab were used. The primers used for the study were as follows, miR-125a-3p: 5'-TCCCTGAGACCCTAACTTGTGA-3, the 3'-un-

translated region of IL-17 was produced by amplification of genomic DNA sequence i.e ATCCCTCAAAGCTCAGCGTGTC. After PCR, the resultants were cloned in the pUM-T vector followed by verification by sequencing. The PCR analysis was carried using a one step RT-PCR system (Invitrogen USA). The clone plasmids, pmiRGLO-3'UTR and pcDNA3.1-miR-125a-3p were separated with QIAGEN Plasmid Kits (Qiagen USA), the extraction was done by adding fragments of T-miR-125a-3p/pcDNA3.1, pmiRGLO and T-3'UTR.

Interference of RNA

The miR-125a-3p over-expressing rat mesangial SV40MES13 cells (ATCC[®] CRL-1927[™]), the cells were cloned with plasmids. The anti-miR-125a-3p expressing SV40MES13 cells were created by transfecting them with miR-125a-3p inhibitor and a siRNA control. All the transfecting vectors were used with Lipofectamine-2000 reagent (Invitrogen USA) in the ratio of 1:1, the transfection was done by incubating the resultant for 20 min. The obtained complex was mixed with SV40MES13 cells followed by incubation of 4 hours at room temperature. The cells were plated into plates (6 well) using DMEM (ThermoFisher USA) added with heat-inactivated FBS (10%) (Sigma Aldrich USA), for 24 hours at room temperature. The cells were stimulated using P8139 i.e a cell stimulator (50 ng/ml) (Sigma-Aldrich USA) along with Ionomycin (1 μ mol/L) (Sigma-Aldrich USA). The cells were harvested after 24 hours of incubation.

Luciferase activity

The Luciferase activity was evaluated using Luciferase assay system (Promega USA) following the supplied instructions. Briefly, the cultured cells received transfection using Lipofectamine 2000 reagent (Invitrogen, USA) with vectors i.e. miR-125a-3p plasmids, miR mutant control. The activities of firefly and renilla luciferase were evaluated after 24 hours of transfection with the help of Luciferase assay system (Promega USA).

Immunohistochemical studies

The tissues sections of 5 μ m thickness obtained by embedding in paraffin were rehydrated using xylene solution followed by treatment

with ethanol for de-paraffinizing the sections. The tissue sections were blocked after retrieving the antigen using goat serum (5%) for 20 min. The sections were subjected to immunostaining by incubating for 12 at room temperature using I^{vy} antibodies i.e IL-17 and F4/80 obtained from cell signaling technologies (USA) and Bio-Rad (USA) respectively. The tissue slides were washed using phosphate buffer saline (PBS) at least 3 times followed by incubation with I^{vy} antibodies at room temperature for 20 min. The tissue slides again rinsed using PBS followed by incubation for 10 min in DAB Peroxidase (HRP) Substrate Kit (Vector laboratories, USA). The slides were counter stained using Hematoxylin (Sigma-Aldrich USA), the slides were viewed under light microscope (Olympus Japan).

ELISA

The serum of MRL/MPJ-Fas^{lpr}/J mice and the cell supernatant of SV40MES13 cells were collected and rinsed using PBS at least 2 times. The fraction content of IL-17 in both cell supernatant of SV40MES13 cells and in serum of mice was estimated using a Mouse IL-17 ELISA Kit (Biocompare USA) following provided instructions. The absorbance of samples was recorded at 450 nm using a microplate reader (ThermoFisher USA). The content of IL-17 was expressed as pg/ml.

Statistical analysis

All the data presented were mean values \pm standard deviation (SD) (n = 3). The comparison of results statistically was done by Bonferroni's multiple comparison tests or by performing ANOVA. Values of $P < 0.05$ were regarded as significant.

Results

The expression of miR-125a-3p was down-regulated while IL-17 was up-regulated in MRL/MPJ-Fas^{lpr}/J mice compared to BALB/C mice

Initially, the MRL/MPJ-Fas^{lpr}/J and the control mice (BALB/C) (n = 10) were evaluated for establishing the histological variations among the LN and the BALB/C control mice. The histology of the isolated kidney from MRL/MPJ-Fas^{lpr}/J and the BALB/C mice were done by hematoxylin and eosin (HE) staining (**Figure 1**). The

kidney histology after HE staining of BALB/C mice showed normal appearing glomerulus and tubules, whereas the kidneys of MRL/MPJ-Fas^{lpr}/J mice showed multiple lesions, sclerosis in many glomeruli, with tubules showing atrophy, marked dilation, large deposition of mesangial matrix and formation of crescent (**Figure 1A**). The histological scoring was done to assess the renal injury (**Figure 1B**). The kidney sections from the experimental mice were also analyzed for periodic acid-Schiff (PA) staining using the scoring system (**Figure 1C, 1D**). The results of PA in the MRL/MPJ-Fas^{lpr}/J mice showed enlarged appearing glomeruli due expansion of mesangial matrix, proliferation in intra capillary spaces along with hyper-cellularity was seen, compared to control mice (**Figure 1C and 1D**).

We further studied the function of miR-125a-3p in MRL/MPJ-Fas^{lpr}/J mice, for the same the kidney tissues of MRL/MPJ-Fas^{lpr}/J as well as control mice were subjected to RT-PCR study (**Figure 1E**). The results showed that the miR-125a-3p levels in the kidney tissues of MRL/MPJ-Fas^{lpr}/J were towards lower side by at least 2 times compared to the BALB/C mice (0.51 ± 0.12 for MRL/MPJ-Fas^{lpr}/J mice compared to 1.00 ± 0.11 BALB/C mice) (** $P < 0.01$). It has been established earlier that IL-17 plays an important role and is up-regulated in SLE [12]. We next evaluated the levels of IL-17 in serum of both the group of mice i.e. MRL/MPJ-Fas^{lpr}/J and BALB/C mice by ELISA test. The results of ELISA showed that the levels of IL-17 in serum of both MRL/MPJ-Fas^{lpr}/J mice were at least 5 times higher compared to BALB/C mice (**Figure 1F**). Next, the influx of macrophages was studied by F4/80 immunohistochemistry of kidney tissues (**Figure 1G**). The results showed that the basement of glomerulus, the tubules and interstitial tissues of MRL/MPJ-Fas^{lpr}/J mice had significantly higher number of F4/80 positive cells compared to kidney tissues of BALB/C mice. The outcomes of immunohistochemistry (IHC) for expression of IL-17 in MRL/MPJ-Fas^{lpr}/J mice showed a positive expression of IL-17 compared to BALB/C mice, suggesting infiltration of inflammatory cells in the tissues of MRL/MPJ-Fas^{lpr}/J mice. Protein expression of IL-17 in MRL/MPJ-Fas^{lpr}/J and control mice was studied by immunoblotting evaluation (**Figure 1H**). The results of protein expression were parallel to IHC, the levels of IL-17 in kidney tissues of MRL/MPJ-Fas^{lpr}/J mice were atleast 3 folds

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

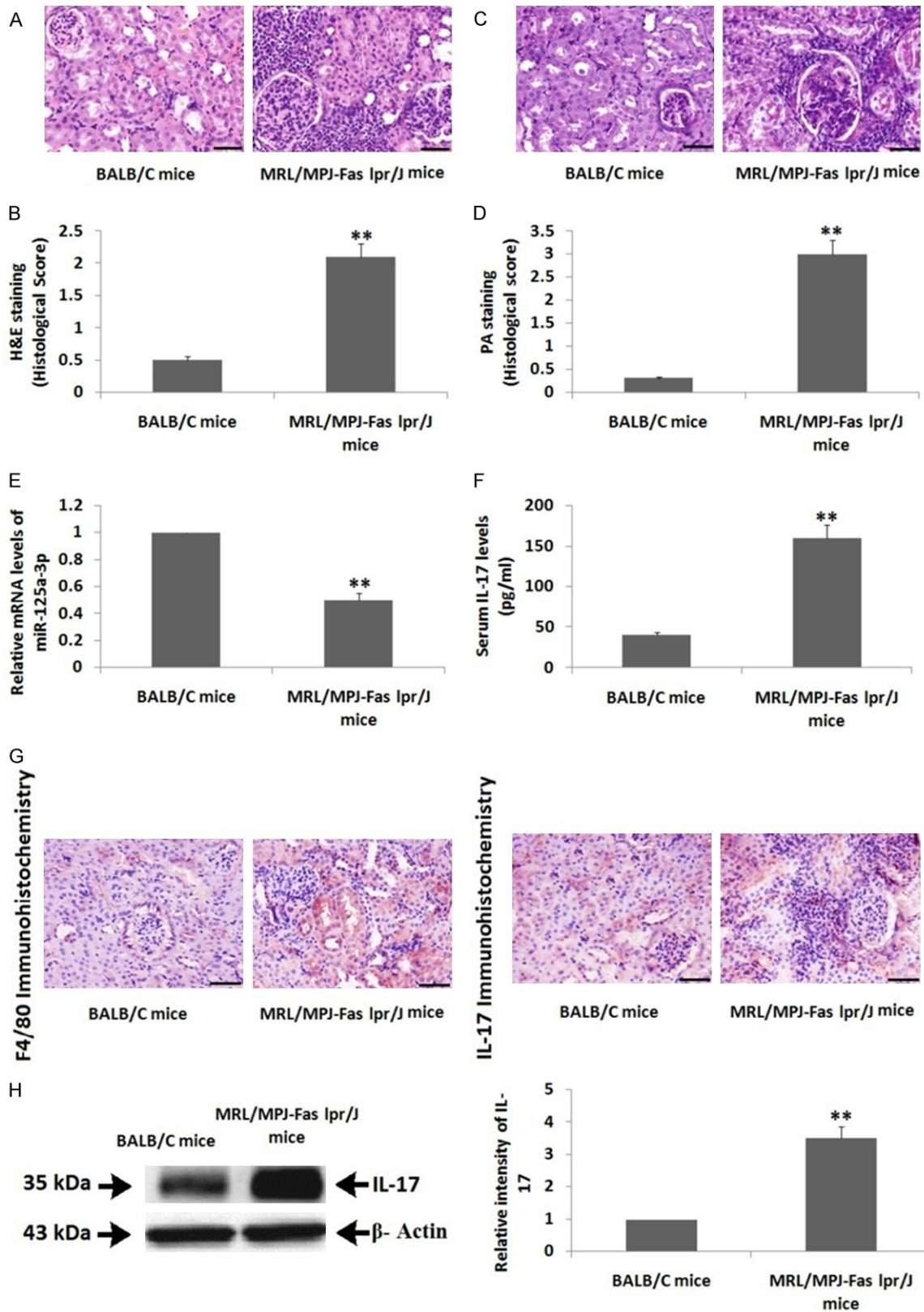


Figure 1. The levels of IL-17 and miR-125a-3p in MRL/MPJ-Fas *lpr/J* and BALB/C mice. (A-C) Histological study of kidney tissue sections of MRL/MPJ-Fas *lpr/J* and BALB/C mice. The tissue sections were obtained and were subjected to H&E (A) and PA staining and (C). (B) (H&E) and (D) (PA) show the results of semi-quantitative analysis of

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

histology studies. (E) Shows the results of RT-PCR analysis for relative expression of miR-125a-3p in MRL/MPJ-Fas^{lpr}/J and BALB/C mice. (F) The results of ELISA analysis for serum levels of IL-17 of MRL/MPJ-Fas^{lpr}/J and BALB/C mice. (G) Results of Immunohistochemistry for F4/80 and IL-17 in kidney tissue sections from MRL/MPJ-Fas^{lpr}/J and BALB/C mice. (H) Western blot analysis for expression of protein levels of IL-17 and Relative expression of IL-17 against β -actin as loading standard (H). **P < 0.01 compared to BALB/C mice. The data presented are mean \pm SD.

higher compared to the levels in BALB/C mice (3.5 ± 0.7 in MRL/MPJ-Fas^{lpr}/J mice compared to 1.00 ± 0.00 in BALB/C mice, **P < 0.01) (Figure 1H). The results of experiment hence suggested that the expression of miR-125a-3p in the renal tissues of MRL/MPJ-Fas^{lpr}/J mice were downregulated compared to the levels in control mice, and the levels of IL-17 which is an inflammatory cytokine were significantly over-expressed in MRL/MPJ-Fas^{lpr}/J compared to BALB/C mice.

MiR-125a-3p governs the levels of IL-17 in SV40MES13 cells

Numbers of reports have been published suggesting the involvement of miRs in development of LN [25]. In a study earlier, miR-125a-3p governed the expression of IL-17 by binding to 3'UTR of IL-17 in cancer cells of bone cancer [30]. In the study we found that the expression of IL-17 was up-regulated while that of miR-125a-3p was suppressed in MRL/MPJ-Fas^{lpr}/J mice (Figure 1E and 1H), in direction of finding the underlying mechanism for up-regulation of IL-17 in renal tissue of MRL/MPJ-Fas^{lpr}/J mice, we cloned the 3'UTR of IL-17 into the reporter plasmids. The reporter luciferase plasmids were used further for transfection along with miR-125a-3p or the mutant which were selected as control in SV40MES13 cells. We selected SV40MES13 cells for the study as the mouse mesangial cells are reported to be over-responsive against immune response in LN [31]. The results of our study suggested that expression of miR-125a-3p caused a significant suppression in the Luciferase activity of IL-17 by binding to 3'UTR (Figure 2A), whereas the mutations of miR-125a-3p binding sites abolished the suppression of luciferase activity (Figure 2A), suggesting that miR-125a-3p decreases the levels of IL-17 by binding to the 3'UTR of IL-17 mRNA directly.

In addition to the above findings, the over-expression of miR-125a-3p caused a significant suppression in the expression of IL-17. To further look into the involved mechanism of miR-125a-3p in the development of LN, the

miR-125a-3p mimics as well as inhibiting vectors were transfected in the SV40MES13 cells, followed by RT-PCR analysis for studying the levels of mRNA levels of miR-125a-3p. The levels of IL-17 were evaluated in the SV40MES13 cell supernatants, the results showed that levels of IL-17 were on significantly lower side in supernatants of cells transfected with miR-125a-3p vector compared to that with empty vector (NC) (Figure 2B) (**P < 0.01). Conversely, upon inhibiting miR-125a-3p reversed the levels of IL-17 in the cell supernatants after transfecting the cells with miR-125a-3p inhibitor (Figure 2C). The outcomes suggested that the expression of IL-17 in the cell supernatant of SV40MES13 cells transfected with anti-miR-125a-3p vector were two times higher compared to that treated with NC i.e transfected with empty vector (**P < 0.01) (Figure 2D). The expression of protein IL-17 in SV40MES13 cells after transfecting them with mimic or inhibitor was done by immunoblotting analysis (Figure 2D, 2E). We observed that, the levels of IL-17 in miR-125a-3p mimic transfected cells was at least 2 times lower compared to the NC cells. Parallel to the findings of ELISA analysis, the inhibition of miR-125a-3p elevated the levels of IL-17 in anti-miR-125a-3p cells (Figure 2E). All together, the outcomes suggested that miR-125a-3p ameliorated the levels of IL-17 via binding to the 3'UTR region of IL-17 in the SV40MES13 cells.

MiR-125a-3p suppressed the levels of fibrogenic factors in rat mesangial cells

Transforming growth factor- β (TGF- β) is one of the prime mediators of renal fibrosis responsible to induce the aggregation of collagen I and III which are the extracellular matrix proteins; these proteins are responsible to impair the kidney function. Earlier in a report, miRs showed a downstream effect on TGF- β mediated fibrosis of kidney [32]. In order to evaluate the role of miR-125a-3p on the levels of transforming growth factor- β , the levels of TGF- β 1 which is the prime subunit of TGF- β along with the collagen I and III were assessed against the

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

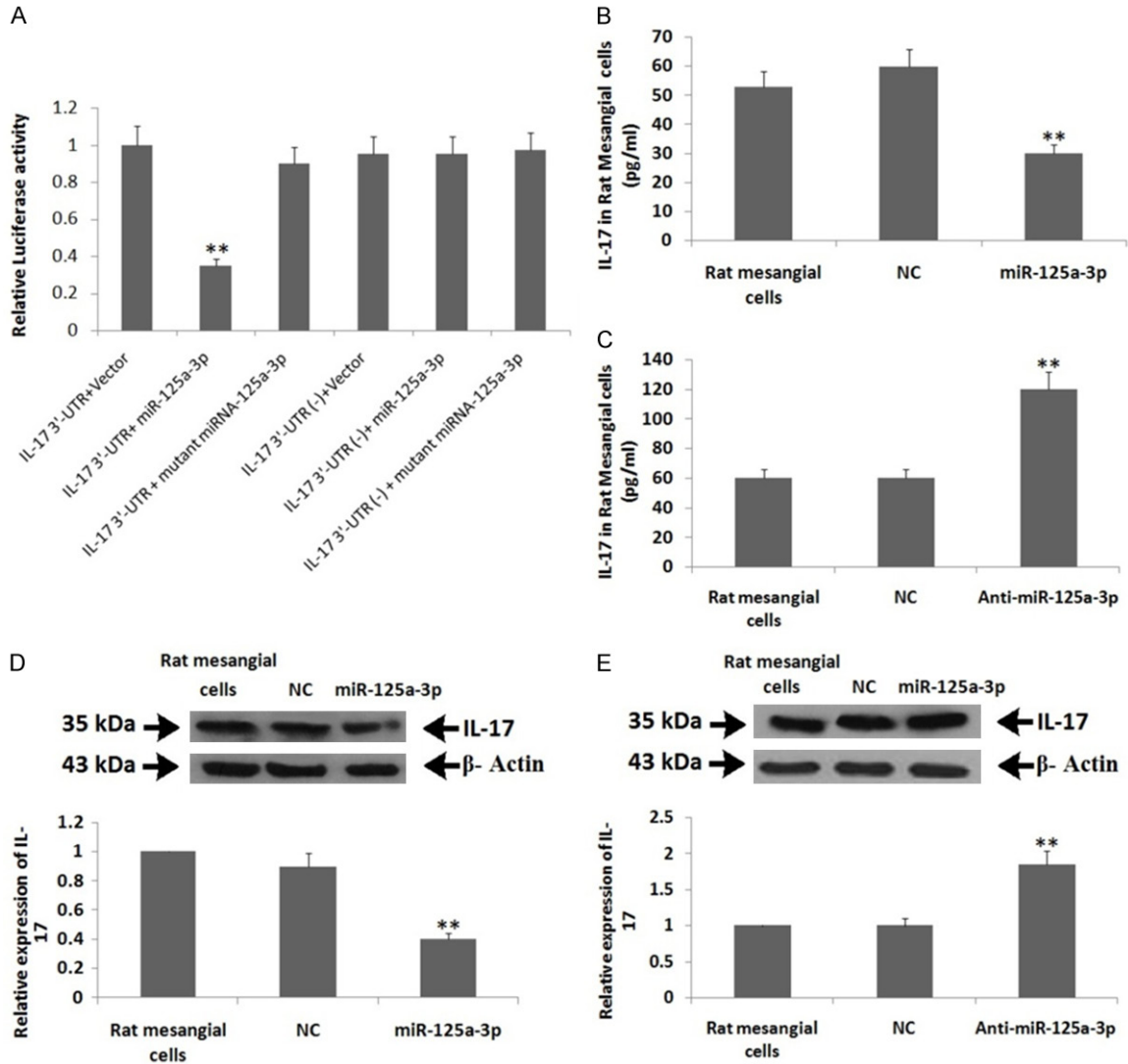


Figure 2. Levels of IL-17 are regulated by miR-125a-3p via binding directly to the 3'UTR region of IL-17 in rat mesangial cells. A: The luciferase activity decreased significantly after miR-125a-3p transfection the rat mesangial cells transfected with luciferase reporter vector bearing the 3'UTR of IL-17 against the cells transfected with miRNA control. **P < 0.01 compared to IL-17 3'-UTR+vector. B: Up-regulation of miR-125a-3p suppressed the levels of IL-17 in supernatants of rat mesangial cells. The cells received transfection of miR-125a-3p mimic and empty vectors. The levels of IL-17 in rat mesangial cells were evaluated by ELISA test. C: Inhibition of miR-125a-3p elevated the levels of IL-17 in cell supernatants. The cells received transfection of miR-125a-3p inhibitor or empty vector and the supernatant were subjected for ELISA analysis for levels of IL-17. D: Up-regulation of miR-125a-3p suppressed the protein levels of IL-17 in rat mesangial cells. The cells received transfection with miR-125a-3p mimic and empty vectors. The levels of IL-17 in rat mesangial cells were evaluated by Western blot analysis followed by results for quantitative analysis. E: Blockade of miR-125a-3p elevated the protein levels of IL-17 in rat mesangial cells. The cells received transfection with miR-125a-3p inhibitor and empty vectors. The levels of IL-17 in rat mesangial cells were evaluated by western blot analysis. **P < 0.01 versus NC group. NC = Rat mesangial cells transfected with empty vector; miR-125a-3p were the cells transfected with a miR-125a-3p mimic vector; anti-miR-125a-3p, were the cells transfected with a miR-125a-3p inhibitor vector. All the data are presented as mean \pm SD.

levels in empty vector transfected SV40MES13 cells i.e. NC cells and the miR-125a-3p vector transfected SV40MES13 cells i.e. the miR-125a-3p cells, results of immunoblotting analysis suggested that, up-regulation of miR-125a-

3p caused a significant decrease in the levels of TGF- β 1 and also of collagen I and III in the miR-125a-3p vector transfected cells compared to SV40MES13 cells or the NC (**Figure 3A**). The results suggested miR-125a-3p sup-

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

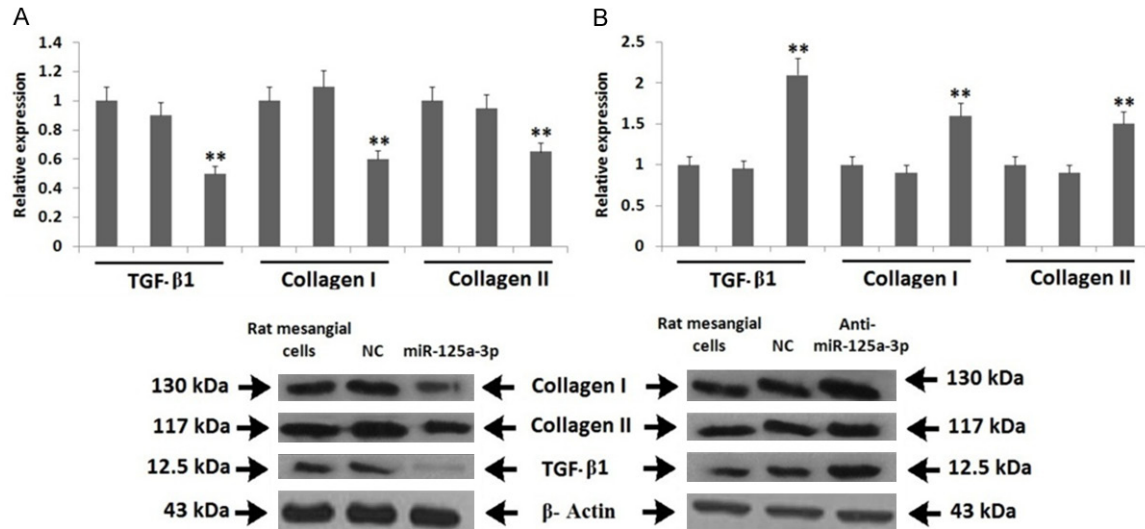


Figure 3. MiR-125a-3p modulated the levels of fibrosis factors in rat mesangial cells. A: Up-regulation of miR-125a-3p suppressed the levels of fibrosis factors in rat mesangial cells. The cells received transfection of miR-125a-3p and control vectors. Western blot analysis was done for levels of collagen I, collagen III and TGF-β1 in rat mesangial cells. B: Blockade of miR-125a-3p elevated the levels of fibrosis factors rat mesangial cells. The cells received transfection with miR-125a-3p inhibitor or empty vectors. Western blot analysis was done for evaluating levels of collagen I, collagen III and TGF-β1 in rat mesangial cells. **P < 0.01 compared to NC group. NC = Rat mesangial cells transfected with empty vector, miR-125a-3p = Rat mesangial cells transfected with a miR-125a-3p mimic; anti-miR-125a-3p = Rat mesangial cells transfected with a miR-125a-3p inhibitor.

pressed the expression of TGF-β1 and also of collagen I and III in rat mesangial cells.

The outcomes suggested that the suppression of fibrogenic factors was involved with up-regulation of miR-125a-3p in the mesangial cells. In order to affirm the role of miR-125a-3p in the suppression of fibrogenic factors the SV40-MES13 cells received transfection of anti-miR-125a-3p, inhibitor, empty vector i.e NC were utilized to compare the levels of fibrogenic factors. We observed that the levels of TGF-β1 along with fibrogenic factors were elevated in cells transfected with anti-miR-125a-3p vector (**Figure 3B**). Also it was found that TGF-β1 stimulated the up-regulation of both the fibrogenic factors i.e. collagen I and collagen III. The results suggested that miR-125a-3p regulated the levels of both collagen I and III via suppressing the levels of TGF-β1 in mesangial cells.

Discussion

Expression patterns of cytokines in LN would be of low importance for disease manifestations as the condition is clinically heterogeneous. Previously some of the reports had focused on deregulation of adaptive immunity in LN, however miRs have been identified to be

the potential regulators in development and progression of disease. Furthermore, a report recently has presented the potential of miRs such as mir-16, miR-21, mir-451 and miR-223 were up-regulated, whereas miR-155, miR-125a-3p and miR-146a were down-regulated in LN [28]. However, more studies are needed in search of new therapies. The present study evaluated the expression of miR-125a-3p and IL-17 in SV40MES13 rat mesangial cells as well as in MRL/MPJ-Fas^{lpr}/J mice to explicate the role of miR-125a-3p in the progression of LN. It was evidenced that, the expression of miR-125a-3p was decreased whereas the levels of IL-17 were over-expressed in MRL/MPJ-Fas^{lpr}/J mice compared to BALB/C mice respectively (**Figure 1**). Also, the results of luciferase activity suggested that miR-125a-3p modified the expression of IL-17 by potentially binding to the 3'UTR region of IL-17, while up-regulation of miR-125a-3p decreased the levels of IL-17 as evidenced by protein expression by immunoblotting analysis in the SV40MES13 rat mesangial cells (**Figure 2**). We also found that, miR-125a-3p decreased the levels of fibrosis factor and the transfection of miR-125a-3p inhibitor vector caused an elevation in levels of IL-17 along with fibrosis factor (**Figures 2 and 3**). Our

findings suggested that miR-125a-3p as a novel candidate which plays an important role in the development and progression of LN.

We discovered that, the levels of miR-125a-3p were downregulated in kidneys of MRL/MPJ-Fas^{lpr}/J mice compared to BALB/C mice (**Figure 1C**). In a study reported earlier, 8 miRs were found to be deregulated in SLE subjects [28], among which miR-125a-3p was involved in cascades related to SLE. Thus, we supposed that the regulation of miR-125a-3p is essential for the development and progression of SLE in MRL/MPJ-Fas^{lpr}/J mice.

IL-17 is involved in autoimmunity [8], and related disorders which include rheumatoid arthritis [9], sclerosis [10, 11] and SLE [12]. Also IL-17 has been identified to play prime role in LN [13, 14]. The present study it was found that, IL-17 was over-expressed in MRL/MPJ-Fas^{lpr}/J mice (**Figure 1D**). Regulation of IL-17 is involved with several factors; Fli-1 is a transcriptional factor has been found to be regulating renal IL-17 expression in adult MRL/MPJ-Fas^{lpr}/J mice [33]. To find whether IL-17 was the favorable target of miR-125a-3p, luciferase activity was measured and the results showed that miR-125a-3p binds to the 3'UTR region of IL-17 (**Figure 2A**), whereas the results of immunoblotting studies showed that increased levels of miR-125a-3p down-regulated the expression of IL-17 significantly (**Figure 2B**) in SV40MES13 rat mesangial cells. These outcomes suggested that miR-125a-3p is a potential regulator involved the regulating the expression of IL-17 by binding to the 3'UTR region directly.

MicroRNAs are regarded as the potential regulators of fibrosis of organs [34]. Studies have evidenced that TGF- β is potential mediator involved in the development and progression of fibrosis by inducing miRs like miR-192, miR-36, miR-37 and miR-21 and suppressing miR-200 and miR-29 [34], all these are found to play a potential role in renal fibrosis and also causes the amplification of TGF- β leading to fibrosis, whereas miR-200 and miR-29 have been found to act as protectors in fibrosis of kidney by halting the laying down of extracellular matrix [35]. Hence, we postulated that miR-125a-3p could suppress the levels of fibrosis factor via the TGF- β cascade. In our research, we found that up-regulation of miR-125a-3p caused a suppression in the expression of TGF- β 1 and colla-

gen I/II i.e the fibrogenic factors, whereas its inhibition by a miR-125a-3p inhibitor elevated the content of collagen I/II and TGF- β 1 levels in rat mesangial cells (**Figure 3**). The findings of present work showed that miR-125a-3p modulated the levels of fibrosis factors via blocking the TGF- β 1 in rat mesangial cells, the findings were parallel to the findings reported earlier [36]. In a study earlier, miR-144 was associated in regulation of TGF- β 1 levels in fibrotic hepatic tissues [37]. Therefore, we confirm that miR-125a-3p may be responsible for regulation of fibrogenic factors via the TGF- β 1 cascade in the SV40MES13 rat mesangial cells. Thus our findings conclude that elevated IL-17 leads to fibrosis in SLE, up-regulation of miR-125a-3p could suppress it. The established mechanism needs further evaluation for improving overall outcomes in treating the disease.

Conclusion

In conclusion, the present study is the first evidence that miR-125a-3p could be an important factor in the pathogenesis of LN which causes decrease in expression of IL-17 by potentially binding to the 3'UTR region causing suppression of fibrosis via down-regulating TGF- β 1 in the SV40MES13 rat mesangial cells. Our findings suggested a new insight for the complex mechanisms involved in the pathogenesis of SLE mediated LN.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Ying Deng, Department of Nephrology, Hanzhong Central Hospital, Hanzhong 723000, Shaanxi, China. Tel: 0086-0916-2681141; Fax: 0086-0916-2681141; E-mail: amburaq@yahoo.com

References

- [1] Tsokos GC. Systemic lupus erythematosus. *N Engl J Med* 2011; 15: 2110-2121.
- [2] Borchers AT, Leibushor N, Naguwa SM, Cheema GS, Shoenfeld Y, Gershwin ME. Lupus nephritis: a critical review. *Autoimmun Rev* 2012; 12: 174-94.
- [3] D'Cruz DP, Khamashta MA, Hughes GR. Systemic lupus erythematosus. *Lancet* 2007; 369: 587-96.
- [4] Tachaudomdach C, Kantachuesiri S, Wongpraphairot S, Worawichawong S, Tankee P,

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

- Riengrojpitak S, Kitiyakara C. High collagen I gene expression as an independent predictor of adverse renal outcomes in lupus nephritis patients with preserved renal function. *Arch Pathol Lab Med* 2015; 139: 378-87.
- [5] Vincent FB, Morand EF, Mackay F. BAFF and innate immunity: new therapeutic targets for systemic lupus erythematosus. *Immunol Cell Biol* 2012; 90: 293-303.
- [6] Ohl K, Tenbrock K. Inflammatory cytokines in systemic lupus erythematosus. *J Biomed Biotechnol* 2011; 2011: 432595.
- [7] Rahman A, Isenberg DA. Systemic lupus erythematosus. *N Engl J Med* 2008; 358: 929-939.
- [8] Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. *Arch Immunol Ther Exp (Warsz)* 2015; 63: 435-449.
- [9] Chabaud M, Durand JM, Buchs N, Fossiez F, Page G, Frappart L, Miossec P. Human interleukin-17: a T cell-derived proinflammatory cytokine produced by the rheumatoid synovium. *Arthritis Rheum* 1999; 42: 963-970.
- [10] Kurasawa K, Hirose K, Sano H, Endo H, Shinkai H, Nawata Y, Takabayashi K, Iwamoto I. Increased interleukin-17 production in patients with systemic sclerosis. *Arthritis Rheum* 2000; 43: 2455-2463.
- [11] Lock C, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L. Genemicroarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* 2002; 8: 500-508.
- [12] Nalbandian A, Crispin JC, Tsokos GC. Interleukin-17 and systemic lupus erythematosus: current concepts. *Clin Exp Immunol* 2009; 157: 209-215.
- [13] Hsu HC, Yang P, Wang J, Wu Q, Myers R, Chen J, Yi J, Guentert T, Tousson A, Stanus AL, Le TV, Lorenz RG, Xu H, Kolls JK, Carter RH, Chaplin DD, Williams RW, Mountz JD. Interleukin 17-producing T helper cells and interleukin 17 orchestrate autoreactive germinal center development in autoimmune BXD2 mice. *Nat Immunol* 2008; 9: 166-175.
- [14] Jacob N, Yang H, Pricop L, Liu Y, Gao X, Zheng SG, Wang J, Gao HX, Putterman C, Koss MN, Stohl W, Jacob CO. Accelerated pathological and clinical nephritis in systemic lupus erythematosus-prone new zealand mixed 2328 mice doubly deficient in TNF receptor 1 and TNF receptor 2 via a Th17-associated pathway. *J Immunol* 2009; 182: 2532-2541.
- [15] Wong CK, Lit LC, Tam LS, Li EK, Wong PT, Lam CW. Hyperproduction of IL-23 and IL-17 in patients with systemic lupus erythematosus: implications for Th17-mediated inflammation in auto-immunity. *Clin Immunol* 2008; 127: 385-393.
- [16] Wong CK, Ho CY, Li EK, Lam CW. Elevation of proinflammatory cytokine (IL-18, IL-17, IL-12) and Th2 cytokine (IL-4) concentrations in patients with systemic lupus erythematosus. *Lupus* 2000; 9: 589-593.
- [17] Doreau A, Belot A, Bastid J, Riche B, Trescol-Biemont MC, Ranchin B, Fabien N, Cochat P, Pouteil-Noble C, Trolliet P, Durieu I, Tebib J, Kassai B, Ansieau S, Puisieux A, Eliaou JF, Bonnefoy-Berard N. Interleukin 17 acts in synergy with B cell-activating factor to influence B cell biology and the pathophysiology of systemic lupus erythematosus. *Nat Immunol* 2009; 10: 778-785.
- [18] Yang S, Li F, Jia S, Zhang K, Jiang W, Shang Y, Chang K, Deng S, Chen M. Early secreted antigen ESAT-6 of mycobacterium tuberculosis promotes apoptosis of macrophages via targeting the microRNA155-SOCS1 interaction. *Cell Physiol Biochem* 2015; 35: 1276-1288.
- [19] Jiang X, Xue M, Fu Z, Ji C, Guo X, Zhu L, Xu L, Pang L, Xu M, Qu H. Insight into the effects of adipose tissue inflammation factors on miR-378 expression and the underlying mechanism. *Cell Physiol Biochem* 2014; 33: 1778-1788.
- [20] Allegra A, Alonci A, Campo S, Penna G, Petrunaro A, Gerace D, Musolino C. Circulating microRNAs: new biomarkers in diagnosis, prognosis and treatment of cancer (review). *Int J Oncol* 2012; 41: 1897-1912.
- [21] Dou L, Wang S, Sui X, Meng X, Shen T, Huang X, Guo J, Fang W, Man Y, Xi J, Li J. MiR-301a mediates the effect of IL-6 on the AKT/GSK pathway and hepatic glycogenesis by regulating PTEN expression. *Cell Physiol Biochem* 2015; 35: 1413-1424.
- [22] Zhang B, Farwell MA. microRNAs: a new emerging class of players for disease diagnostics and gene therapy. *J Cell Mol Med* 2008; 12: 3-21.
- [23] Fiorucci G, Chiantore MV, Mangino G, Percario ZA, Affabris E, Romeo G. Cancer regulator microRNA: potential relevance in diagnosis, prognosis and treatment of cancer. *Curr Med Chem* 2012; 19: 461-474.
- [24] Wu H, Zhao M, Chang C, Lu Q. The real culprit in systemic lupus erythematosus: abnormal epigenetic regulation. *Int J Mol Sci* 2015; 16: 11013-11033.
- [25] Qu B, Shen N. miRNAs in the pathogenesis of systemic lupus erythematosus. *Int J Mol Sci* 2015; 16: 9557-9572.
- [26] Kaga H, Komatsuda A, Omokawa A, Ito M, Teshima K, Tagawa H, Sawada K, Wakui H. Downregulated expression of miR-155, miR-17, and miR-181b, and upregulated expres-

miR-125a-3p decreases levels of interleukin-17 in lupus nephritic mice

- sion of activation-induced cytidine deaminase and interferon- α in PBMCs from patients with SLE. *Mod Rheumatol* 2015; 25: 865-870.
- [27] Deng X, Su Y, Wu H, Wu R, Zhang P, Dai Y, Chan TM, Zhao M, Lu Q. The role of microRNAs in autoimmune diseases with skin involvement. *Scand J Immunol* 2015; 81: 153-165.
- [28] Wang H, Peng W, Ouyang X, Li W, Dai Y. Circulating microRNAs as candidate biomarkers in patients with systemic lupus erythematosus. *Transl Res* 2012; 160: 198-206.
- [29] Wellmann U, Letz M, Schneider A, Amann K, Winkler TH. An Ig mu-heavy chain transgene inhibits systemic lupus erythematosus immunopathology in autoimmune (NZB 9 NZW)F1 mice. *Int Immunol* 2001; 13: 1461-9.
- [30] Jin H, Li DW, Wang SN, Luo S, Li Q, Huang P, Wang JM, Xu M, Xu CX. miR-125a promotes the progression of giant cell tumors of bone by stimulating IL-17A and b-catenin expression. *Mol Ther Nucleic Acids* 2018; 13: 493-502.
- [31] Peairs A, Dai R, Gan L, Shimp S, Rylander MN, Li L, Reilly CM. Epigallocatechin-3-gallate (EGCG) attenuates inflammation in MRL/lpr mouse mesangial cells. *Cell Mol Immunol* 2010; 7: 123-32.
- [32] Chung AC, Lan HY. MicroRNAs in renal fibrosis. *Front Physiol* 2015; 6: 50.
- [33] Sato S, Zhang X, Fujita Y, Yashiro M, Asano T, Kobayashi H, Watanabe H and Migita K. Transcription factor flt-1 impacts renal IL-17 expression in adult MRL/lpr mouse despite similar interstitial immune cell infiltration into the kidney. Abstract number 998. 2018 ACR/ARHP Annual Meeting.
- [34] Leask A, Abraham DJ. TGF-beta signaling and the fibrotic response. *FASEB J* 2004; 18: 816-27.
- [35] Lesk AM. Molecular biology. Coordination of sequence data. *Nature* 1985; 314: 318-9.
- [36] Roderburg C, Luedde M, Vargas Cardenas D, Vucur M, Mollnow T, Zimmermann HW, Koch A, Hellerbrand C, Weiskirchen R, Frey N, Tacke F, Trautwein C, Luedde T. miR-133a mediates TGF-b-dependent derepression of collagen synthesis in hepatic stellate cells during liver fibrosis. *J Hepatol* 2013; 58: 736-42.
- [37] Liu Z, Yi J, Ye R, Liu J, Duan Q, Xiao J, Liu F. miR-144 regulates transforming growth factor-beta1 induced hepatic stellate cell activation in human fibrotic liver. *Int J Clin Exp Pathol* 2015; 8: 3994-4000.