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MicroRNA-130b Ameliorates Murine Lupus Nephritis Through Targeting the Type I Interferon Pathway on Renal Mesangial Cells

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Objective. **Type I interferon (IFN) is a critical pathogenic factor during the progression of lupus nephritis (LN). Although microRNAs (miRNAs) have been shown to control the IFN response in immune cells in LN, the role of miRNAs in resident renal cells remains unclear. We undertook this study to investigate the role of microRNA-130b (miR-130b) in the IFN pathway in renal cells as well as its therapeutic effect in LN.**

Methods. **Kidney tissues from patients and (NZB × NZW)F1 lupus-prone mice were collected for detecting miR-130b levels. Primary renal mesangial cells (RMCs) were used to determine the role of miR-130b in the IFN pathway. We overexpressed miR-130b by administering miR-130b agomir in a mouse model of IFNα-accelerated LN to test its therapeutic efficacy.**

Results. **Down-regulated miR-130b expression was observed in kidney tissues from patients and lupus-prone mice. Further analysis showed that under-expression of miR-130b correlated negatively with abnormal activation of the IFN response in LN patients. In vitro, overexpressing miR-130b suppressed signaling downstream from the type I IFN pathway in RMCs by targeting IFN regulatory factor 1 (IRF-1). The opposite effect was observed when endogenous miR-130b expression was inhibited. The inverse correlation between *IRF1* and miR-130b levels was detected in renal biopsy samples from LN patients. More importantly, in vivo administration of miR-130b agomir reduced IFNα-accelerated progression of LN, with decreased proteinuria, lower levels of immune complex deposition, and lack of glomerular lesions.**

Conclusion. **MicroRNA-130b is a novel negative regulator of the type I IFN pathway in renal cells. Overexpression of miR-130b in vivo ameliorates IFNα-accelerated LN, providing potential novel strategies for therapeutic intervention in LN.**

Systemic lupus erythematosus (SLE) is a heterogeneous systemic autoimmune disease characterized by a wide range of clinical manifestations (1). Lupus nephritis (LN) is considered one of the most serious complications of SLE and the major indicator of a poor prognosis (2,3). The most common symptoms of LN are glomerular and tubulointerstitial inflammation, which

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are thought to be initiated by the renal deposition of immune complexes (4–6). The consequent activation of a cascade of inflammatory responses and the production of type I interferon (IFN) are crucial for the development of the disease (7,8). Nevertheless, many strains of lupus-prone mice need a long time to develop LN and have instability of disease onset, which largely hinder understanding of the pathogenesis of the disease and development of effective therapeutic interventions. In contrast, the administration of exogenous IFNα to lupus-prone mice shortens the time to develop clinical manifestations and relatively synchronizes onset of the disease to ensure highly repeatable LN progression, making IFNα-accelerated LN a useful model with which both to study the pathogenesis of LN and to explore novel therapeutic treatment for this disease (9).

The renal deposition of immune complexes activates immune cells, including plasmacytoid dendritic cells, via Toll-like receptors (TLRs) and STATs, regulating the expression of downstream genes in the type I IFN pathway (10). Many recent studies have shown that immune complexes can also directly activate resident renal cells to produce inflammatory mediators that promote accumulation of various kinds of immune cells (2,11). The renal mesangial cell (RMC) is one of the renal resident cells that play a critical role in the pathogenesis of LN (12,13). Studies have shown that RMCs express TLRs and when stimulated with TLR-3 ligands, they produce type I IFN (14,15). Notably, type I IFN produced by resident renal cells, including RMCs, may aggravate autoimmune kidney injury (16,17). However, the mechanism regulating type I IFN signaling in RMCs remains largely unknown.

Current therapies for LN primarily involve nonspecific immunosuppression, such as with cyclosporin A and mycophenolate mofetil (18). However, most of them have adverse effects in patients, including hypertension (19). Therefore, many researchers are currently working to overcome this problem by developing more targeted and effective treatments.

MicroRNAs (miRNAs) are single-stranded noncoding RNAs that regulate gene expression through messenger RNA (mRNA) degradation and translational repression (20). Because they are important genetic regulators, miRNAs also play pathophysiologic roles in the evolution of many diseases (21,22), and accumulating evidence has shown that miRNAs are involved in the pathogenesis of lupus. For instance, down-regulated microRNA-146a (miR-146a) contributes to the abnormal activation of the type I IFN pathway in the peripheral blood mononuclear cells of lupus patients (23), and miR-150 promotes renal fibrosis by reducing the expression of suppressor of cytokine signaling 1 in LN, consequently increasing the profibrotic molecules in local renal cells (24). These studies suggest that miRNAs are potential therapeutic targets in autoimmune diseases.

In this study, miR-130b expression was significantly reduced in renal biopsy samples from LN patients compared with those from control patients. Interestingly, we found that miR-130b negatively regulates type I IFN signaling in primary RMCs by targeting IFN regulatory factor 1 (IRF-1) expression, which is up-regulated in the kidneys of LN patients. Furthermore, in vivo administration of miR-130b agomir attenuated IFNα-accelerated LN in (NZB $\times$ NZW)F1 mice. Taken together, our findings demonstrate that miR-130b plays a previously unrecognized role in the pathogenesis of LN by negatively regulating the type I IFN pathway, suggesting that miR-130b is a promising therapeutic target for the treatment of LN.

MATERIALS AND METHODS

Human study subject samples. Human kidney samples used in this study were obtained from Shanghai Renji Hospital after informed consent was obtained from all patients. All LN patients fulfilled the American College of Rheumatology 1982 revised criteria for SLE (25). Clinical SLE activity was assessed with the SLE Disease Activity Index (26). Samples from the control group were obtained from the renal cortex adjacent to renal tumor. Additional clinical information about the subjects is listed in Supplementary Table 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract. The study was approved by the Research Ethics Board of Shanghai Renji Hospital.

Cell culture, stimulation, and transfection. Mouse primary RMCs were isolated and cultured as previously described (27). Human primary RMCs were purchased from ScienCell Research Laboratories. The mouse SV40 MES 13 cell line was obtained from the cell bank of Shanghai Institutes for Biological Sciences at the Chinese Academy of Sciences.

Both mouse and human primary RMCs were stimulated with type I IFN (1,000 units/ml; PBL InterferonSource). Small interfering RNAs (siRNAs), miRNA mimic, and miRNA inhibitor were synthesized by GenePharma. The sequences of the siRNAs, mimic, inhibitor, and control are shown in Supplementary Table 2, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract. Primary RMCs were transfected with these RNAs using Lipofectamine RNAiMAX (Invitrogen) according to the manufacturer's instructions.

Quantitative real-time polymerase chain reaction (PCR). RNA was extracted from cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized with a PrimeScript RT Reagent kit (Takara). To determine the quantities of mRNAs expressed, the cDNAs were amplified with real-time PCR using a SYBR Premix Ex Taq RT-PCR kit (Takara), and the expression of *RPL13a* and *Gapdh* was

determined as the internal control for humans and mice, respectively. The primer sequences for all genes are shown in Supplementary Table 3, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract. The quantities of miRNAs were determined with specific TaqMan probes using a TaqMan MicroRNA Assay kit (Applied Biosystems). *RNU6* and *sno202* were used as the internal controls for humans and mice, respectively.

Western blotting analysis. Lysates were resolved by electrophoresis, transferred to PVDF membranes, and probed with antibodies directed against phosphorylated (Tyr) STAT-1, STAT-1, phosphorylated (Tyr) STAT-2, STAT-2, JAK-1, IRF-1, IRF-3, and IRF-7 (all from Cell Signaling Technology), Tyk-2 and β-tubulin (both from Abcam), and Flag-(M2) (Sigma). To overexpress IRF-1, we used pcDNA3.1-Flag vector (Addgene) to clone cDNA of *IRF1*.

Enzyme-linked immunosorbent assay (ELISA). Protein levels of CCL5 or CXCL10 in cell supernatants were measured with a CCL5 or CXCL10 DuoSet ELISA kit (R&D Systems) according to the manufacturer's instructions.

Luciferase assays. For the IFN-stimulated response element (ISRE)–Luc reporter assay, mesangial cells were transfected with a mixture of 100 ng of ISRE-Luc vector (Clontech), 20 ng of *Renilla* vector (Promega), and miR-130b mimic or inhibitor using Lipofectamine 2000 (Invitrogen). For the 3′-untranslated region (3′-UTR) reporter assay, the psiCHECK-2 vector (Promega) was used to clone the 3′-UTR of *IRF1*. Mesangial cells were transfected with a mixture of 50 ng of the 3′-UTR-Luc reporter vector and miR-130b mimic or inhibitor.

Mouse model. *LN accelerated in (NZB × NZW)F1 mice with IFNα-expressing adenovirus.* C57BL/6, NZB, and NZW mice were purchased from The Jackson Laboratory, and a colony of (NZB × NZW)F1 mice was developed in a specific pathogen–free barrier facility. Glomerulonephritis was accelerated in the (NZB × NZW)F1 mouse model by administration of IFNα-expressing adenovirus, as described previously (28). Briefly, 8–10-week-old (NZB × NZW)F1 mice were treated with a single intravenous (IV) injection of 10^9 particles of IFNα5 adenovirus (ViGene Biosciences) or a control adenovirus.

In vivo prevention and treatment experiment. (NZB × NZW)F1 mice were injected IV with 24 n*M* miR-130b agomir (RiboBio) or control agomir in Entranster in vivo Transfection Reagent (Engreen) on 3 consecutive days 3 and 5 weeks after IFNα5 adenovirus injection for the prevention assay or 5 and 7 weeks after IFNα5 adenovirus injection for the treatment assay. Urine was measured 3, 5, 7, and 9 weeks after IFNα5 adenovirus injection. Mice were euthanized, and kidneys were obtained 7 weeks after IFNα5 adenovirus injection in the prevention assay or 9 weeks after IFNα5 adenovirus injection in the treatment assay. All experiments complied with the relevant laws and institutional guidelines, as overseen by the Animal Studies Committee of the Institute of Health Sciences.

Evaluation of renal disease. *Proteinuria.* Mice were held in metabolic cages, and their urine was collected over a 24-hour period. Urinary protein was measured with a BCA Protein Assay kit (Tiangen Biotech).

Kidney histology. Kidneys were fixed in 4% paraformaldehyde and embedded in paraffin. Sections were cut and stained with periodic acid–Schiff, and lesion severity was graded in a blinded manner by a pathologist. The severity of renal lesions in mouse LN was graded from 0 to 3 using the activity index described for humans (including glomerular cellularity, mesangial matrix expansion, crescent formation, infiltrating leukocytes, interstitial mononuclear cell infiltrates in the medulla and cortex, hyaline deposits, and fibrinoid necrosis). The antibody to IRF-1 used for immunohistochemistry (Cell Signaling Technology) was diluted 1:100. For IgG staining, frozen sections were stained with phycoerythrin-conjugated anti-mouse IgG2a monoclonal antibody (diluted 1:50; BD PharMingen) and anti-C3 monoclonal antibody (diluted 1:100; Santa Cruz Biotechnology).

Statistical analysis. Three independent experiments were performed to confirm the reproducibility of each experiment in vitro. Values are the mean ± SEM. Differences between groups were analyzed for statistical significance with Student's *t*-test. The relationship between miR-130b and *IRF1* was tested with Pearson's correlation and linear regression. *P* values less than 0.05 were considered significant.

RESULTS

Reduced expression of miR-130b in LN. To better understand the roles of miRNAs in the development of LN, our laboratory previously screened for dysregulated miRNAs in kidney biopsy samples from SLE patients with nephritis using miRNA expression microarrays. We found that expression of several miRNAs (e.g., miR-130b and miR-23b) was significantly lower in the kidneys of LN patients than in those of control patients ($P < 0.05$) (Han X: unpublished observations). The reduced levels of miR-130b were confirmed with a single quantitative PCR validation (Figure 1A). Similarly, we observed that miR-130b expression was significantly decreased in the kidneys of (NZB × NZW)F1 lupus-prone mice compared with control mice (Figure 1B). Furthermore, in a mouse model of IFNα-accelerated glomerulonephritis (28), renal miR-130b expression was progressively down-regulated with increasing severity of disease. More intriguingly, we also detected a reduction of miR-130b expression in primary RMCs at the late stage of IFNα treatment in (NZB × NZW)F1 mice (Figure 1C).

Since down-regulation of miR-130b might reflect defects in regulation of the inflammatory response, and since type I IFN plays a key role in LN in humans and mice, we tested whether underexpression of miR-130b in kidneys is associated with activation of the type I IFN pathway. IFN scores are used to determine activation of the type I IFN pathway in LN patients, as previously described (23). Interestingly, our data showed a negative correlation between miR-130b levels and IFN activation (Figure 1D). Collectively, our data indicate that miR-130b is probably involved in the development of LN.

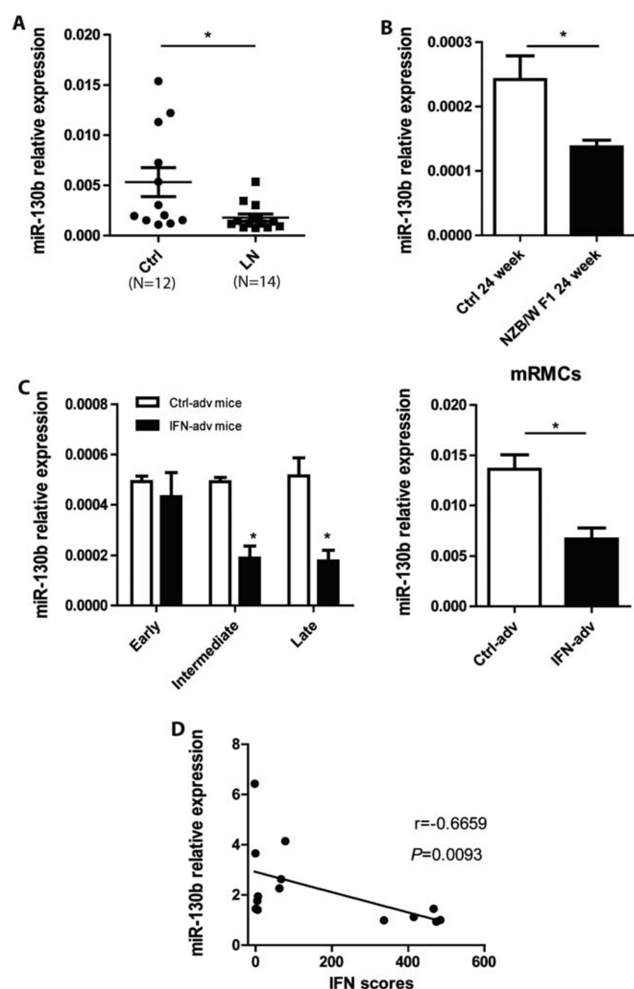

Figure 1. MicroRNA-130b (miR-130b) expression is reduced in kidneys from patients with lupus nephritis (LN) and from (NZB × NZW)F1 lupus-prone mice, and correlates negatively with type I interferon (IFN) pathway activity. **A,** Quantitative polymerase chain reaction validation of miR-130b expression in renal biopsy samples from patients with LN and tumor-adjacent normal tissue samples from patients with kidney cancer (Ctrl). **B,** MicroRNA-130b levels in kidneys from (NZB × NZW)F1 lupus-prone mice compared with those in kidneys from control NZW mice (Ctrl) (n = 5–6 mice per group). **C,** Changes in miR-130b levels in kidneys (left) and primary renal mesangial cells (mRMCs) (at late stage of treatment only; right) from IFNα-treated (NZB × NZW)F1 mice. Early stage = 21 days; intermediate stage = 35 days; late stage = 49 days (n = 3–5 mice per group). Ctrl-adv = control adenovirus; IFN-adv = IFNα-expressing adenovirus. **D,** Correlation between miR-130b levels and activation of the type I IFN pathway in kidneys from 14 LN patients. In **A**, symbols represent individual patients; bars show the mean ± SEM. In **B** and **C**, values are the mean ± SEM. In **A**, **B**, and **C** (right), $* = P < 0.05$; in **C** (left), $* = P < 0.05$ versus control adenovirus–treated mice.

Considering that the level of miR-130b is dysregulated in LN patients and mouse models of LN, and given that few studies have examined the effects of abnormal miR-130b expression on the regulation of the type I IFN pathway or the pathogenesis of LN (29,30), we selected miR-130b for further investigation.

Negatively regulated activation of the type I IFN pathway by miR-130b. As mentioned above, miR-130b expression is reduced in primary RMCs from mice with IFNα-accelerated LN, and miR-130b expression correlates negatively with activation of type I IFN signaling in kidneys. Next, we tested whether miR-130b could intrinsically modulate the type I IFN pathway in RMCs. Overexpression of miR-130b in a mouse mesangial cell line reduced ISRE reporter gene activity stimulated by type I IFN, and silencing endogenous miR-130b with miR-130b inhibitor promoted ISRE-Luc activity (Figure 2A).

We further found that in primary RMCs from C57BL/6 mice, overexpression of miR-130b reduced the expression of several IFN-inducible genes, including *IFIT1*, *CXCL10*, and *CCL5*, at both the mRNA and protein levels. In contrast, silencing endogenous miR-130b consistently increased expression of these genes (Figure 2B) (see Supplementary Figure 1, available on the *Arthritis & Rheumatology* web site at http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract). MicroRNA-130b exerted the same effect on primary RMCs from (NZB × NZW)F1 mice (see Supplementary Figure 2, http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract). Taken together, our data demonstrated that miR-130b suppresses the signaling downstream from type I IFN.

MicroRNA-130b down-regulates the type I IFN pathway by inhibiting STAT-1 tyrosine phosphorylation. The binding of type I IFN to its receptor initiates a signaling cascade that leads to the phosphorylation of STATs and the induction of IFN-stimulated genes. Therefore, to further investigate the molecular mechanism of miR-130b in regulating type I IFN signaling, we used miR-130b mimic and inhibitor to test whether miR-130b affects activation of the JAK/STAT signaling pathway. Tyrosine phosphorylation of STAT-1 induced by type I IFN was significantly decreased in mouse RMCs with overexpressed miR-130b and was increased in RMCs with underexpressed miR-130b, while total STAT-1 protein levels were relatively unaffected. Tyrosine phosphorylation of STAT-2 and total JAK-1, Tyk-2, and STAT-2 protein levels were not influenced by manipulation of miR-130b expression (Figure 2C).

As type I IFN is crucial for the pathogenesis of human and mouse LN, we tested whether miR-130b affected activation of STAT-1 in human and (NZB × NZW)F1 mouse RMCs. We found that miR-130b also suppressed type I IFN signaling by down-regulating

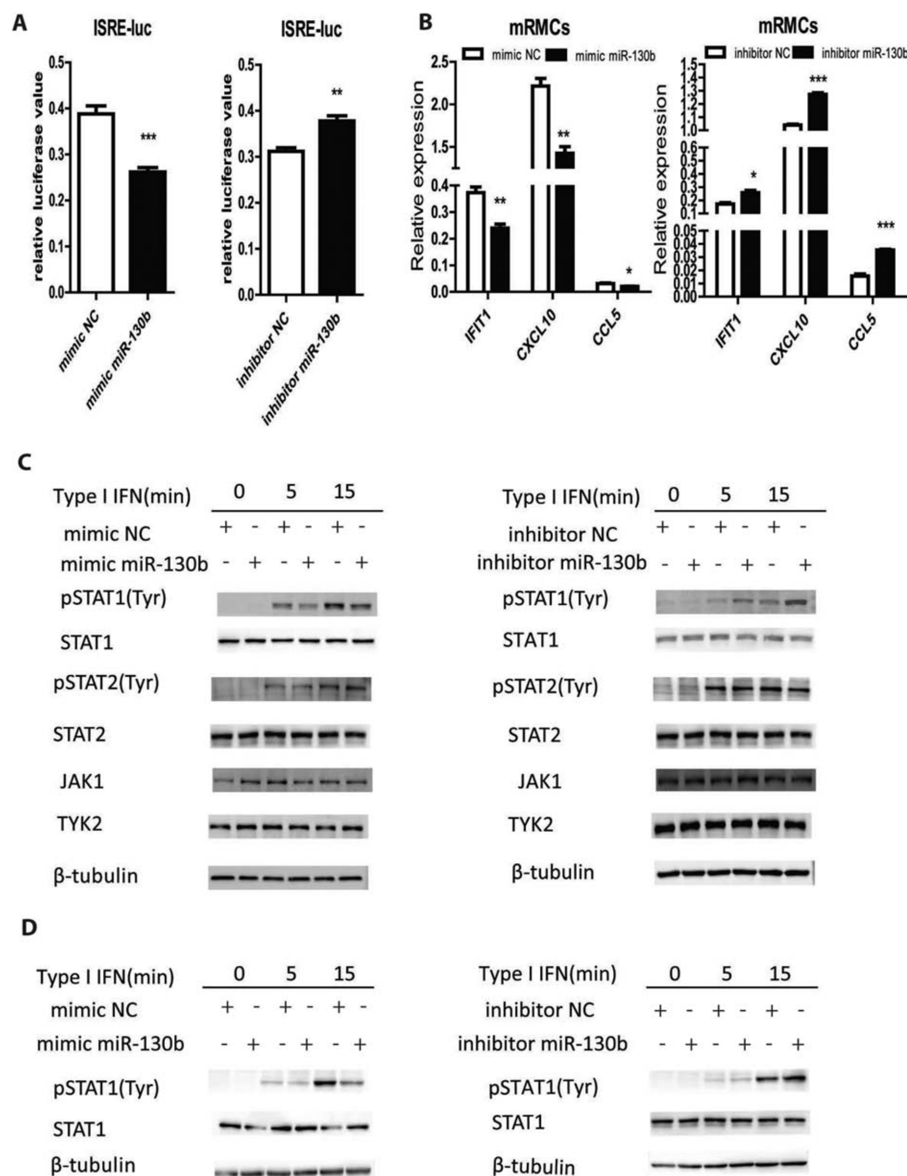

Figure 2. MicroRNA-130b (miR-130b) inhibits the type I interferon (IFN) pathway by reducing tyrosine phosphorylation of STAT-1 in mouse primary renal mesangial cells (mRMCs). **A,** MicroRNA-130b inhibits the activation downstream of type I IFN. SV40 MES 13 cells were cotransfected with IFN-stimulated response element (ISRE)–Luc reporter vector, negative control (NC) mimic or inhibitor, or miR-130b mimic or inhibitor. After 24 hours, cells were incubated with type I IFN for 6 hours and luciferase activity in cell lysates was detected. **B,** MicroRNA-130b negatively regulates IFN-inducible genes in mouse primary renal mesangial cells. Primary renal mesangial cells from 8-week-old C57BL/6 mice (n = 5) were transfected with negative control mimic or inhibitor or with miR-130b mimic or inhibitor. After 24 hours, cells were incubated with type I IFN for 6 hours. Messenger RNA levels of IFN-inducible genes (*IFIT1*, *CXCL10*, and *CCL5*) were quantified. **C** and **D,** Primary renal mesangial cells from 8-week-old C57BL/6 mice (n = 5) (**C**) and human primary renal mesangial cells (**D**) were transfected with negative control mimic or inhibitor or with miR-130b mimic or inhibitor. After 24 hours, cells were stimulated with type I IFN. Expression of STAT-1 phosphorylated at tyrosine, STAT-1, STAT-2 phosphorylated at tyrosine, STAT-2, JAK-1, and Tyk-2 was analyzed by Western blotting. Equal protein loading was confirmed by use of β-tubulin. In **A** and **B**, values are the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$ versus negative control mimic or inhibitor.

tyrosine phosphorylation of STAT-1 in human and (NZB × NZW)F1 mouse primary RMCs (Figure 2D) (see Supplementary Figure 3, http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract), with little effect on tyrosine phosphorylation of STAT-2 and total JAK-1, Tyk-2, and STAT-2 protein levels (not shown). Taken

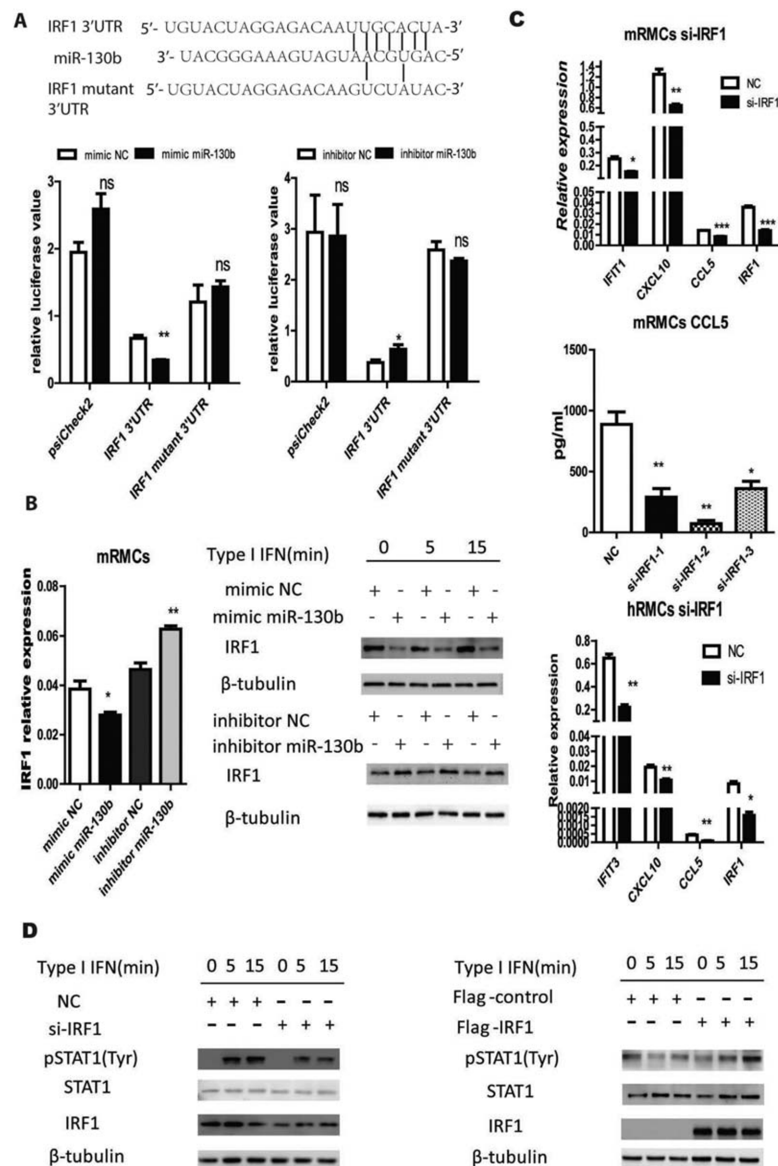

Figure 3. Identification of interferon regulatory factor 1 (IRF-1) as a functional target of microRNA-130b (miR-130b). **A,** Shown are predicted binding sites for miR-130b in the 3′-untranslated region (3′-UTR) of *IRF1*. SV40 MES 13 cells were transfected with the control construct (psiCHECK-2) or with a construct encoding the wild-type *IRF1* 3′-UTR or mutated *IRF1* 3′-UTR (mutant), in addition to negative control (NC) mimic or miR-130b mimic (left) or negative control inhibitor or miR-130b inhibitor (right). After 24 hours, luciferase activity in mesangial cell lysates was detected. **B,** Messenger RNA and protein levels of *IRF1* were reduced by miR-130b in mouse primary renal mesangial cells (mRMCs), as determined by quantitative polymerase chain reaction and Western blotting. **C,** Primary renal mesangial cells from 8-week-old C57BL/6 mice (n = 5) and human primary renal mesangial cells (hRMCs) were transfected with negative control small interfering RNA (siRNA) or with *IRF1* siRNA (si-*IRF1*; 200 n*M*). After 48 hours, cells were incubated with type I interferon (IFN) for 6 hours. Messenger RNA levels of *IRF1* and IFN-inducible genes (*IFIT1*, *CXCL10*, and *CCL5*) were reduced by *IRF1* siRNA. Protein levels of CCL5 in supernatants were repressed by 3 different sequences of *IRF1* siRNA. **D,** Inhibited or promoted IRF-1 protein expression affected levels of tyrosine phosphorylation of STAT-1 in primary renal mesangial cells from 8-week-old C57BL/6 mice (n = 5). In **A–C**, values are the mean ± SEM. In **A**, ∗∗ = $P < 0.01$ versus negative control mimic; ∗ = $P < 0.05$ versus negative control inhibitor. In **B**, ∗ = $P < 0.05$ versus negative control mimic; ∗∗ = $P < 0.01$ versus negative control inhibitor. In **C**, ∗ = $P < 0.05$; ∗∗ = $P < 0.01$; ∗∗∗ = $P < 0.001$ versus negative control siRNA. NS = not significant versus negative control mimic or inhibitor.

together, these data indicate that miR-130b suppresses type I IFN signaling by inhibiting tyrosine phosphorylation of STAT-1.

IRF-1 is a functional target of miR-130b. To further understand how miR-130b regulates type I IFN signaling, we identified potential targets of miR-130b in

RMCs by using the target prediction tool (http://www.targetscan.org/vert_71/). Combined with well-known regulators of the type I IFN pathway (31), we noted that IRF-1 is a putative target of miR-130b that contains a binding sequence in its 3′-UTR. Luciferase reporter experiments showed that overexpression of miR-130b markedly repressed luciferase activity of *IRF1* 3′-UTR construct in MES cells, with no effect on the mutant *IRF1* 3′-UTR construct. In contrast, miR-130b inhibitor enhanced reporter activity of the *IRF1* 3′-UTR construct (Figure 3A). To further prove that IRF-1 is a physiologically functional target of miR-130b, we measured IRF-1 expression in mouse RMCs transfected with miR-130b mimic or inhibitor. Consistent with the reporter assays, expression of endogenous IRF-1 was reduced by overexpression of miR-130b at both mRNA and protein levels. Conversely, IRF-1 expression was markedly increased in mouse RMCs transfected with miR-130b inhibitor (Figure 3B). Taken together, these data demonstrated that IRF-1 is a direct target of miR-130b in primary RMCs.

Silencing of IRF-1 impairs the type I IFN signaling pathway in primary RMCs. IRF-1 is reported to positively regulate transcription of type I IFN–inducible genes in some cell types (32); however, its function in primary RMCs has not been investigated. Therefore, to clarify the role of IRF-1 in regulating type I IFN signaling in primary RMCs, we transfected mouse and human primary RMCs with an siRNA directed against *IRF1* or with a control siRNA. Silencing of IRF-1 markedly decreased tyrosine phosphorylation of STAT-1 after type I IFN treatment, with relatively little reduction of the total proteins. Expression and phosphorylation of STAT-2 were not affected (see Supplementary Figure 4A, http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract). Consequently, expression of IFN-inducible genes, including *CCL5*, was reduced in primary RMCs transfected with *IRF1* siRNA (Figure 3C). In contrast, overexpression of IRF-1 enhanced tyrosine phosphorylation of STAT-1 in mouse RMCs after type I IFN treatment (Figure 3D). In addition, knockdown of IRF-1 significantly reduced expression of IFN-inducible genes in primary RMCs of (NZB × NZW)F1 mice (see Supplementary Figures 4B–D). Therefore, we concluded that IRF-1, as a target of miR-130b, positively regulates the type I IFN pathway in mouse and human primary RMCs.

MicroRNA-130b correlates negatively with IRF-1 expression in kidney biopsy samples from LN patients. To better understand the roles of miR-130b in the development of human LN, we analyzed the expression of miR-130b, its target IRF-1, and type I IFN–inducible genes in kidney biopsy samples from LN patients and control patients. Levels of *IRF1* and type I IFN–inducible genes were significantly increased in the kidneys of LN patients compared with those in control patients (Figures 4A and B). Moreover, we noted that the expression of *IRF1* showed a markedly negative correlation with miR-130b levels in kidneys of LN patients (Figure 4C). Therefore, these results indicate that miR-130b might be involved in the development of LN by regulating *IRF1* expression.

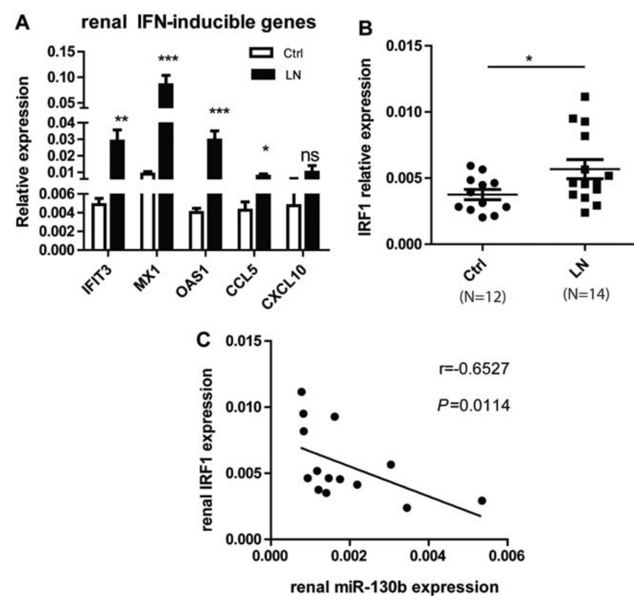

Figure 4. Expression of mRNA for interferon regulatory factor 1 (IRF-1) and interferon (IFN)–inducible genes in kidney biopsy samples from patients with lupus nephritis (LN). **A** and **B,** Expression of mRNA for IFN-inducible genes (**A**) and *IRF1* (**B**) was elevated in kidney biopsy samples from LN patients compared with that in kidney biopsy samples from control patients (Ctrl). **C,** Expression of renal microRNA-130b (miR-130b) correlated negatively with levels of *IRF1* in LN patients. In **A**, values are the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$; $*** = P < 0.001$. In **B**, symbols represent individual subjects; bars show the mean ± SEM. $* = P < 0.05$. NS = not significant.

Suppression of IFN*α*-accelerated LN development by miR-130b. To investigate the potential utility of miR-130b agomir in preventing or treating LN, miR-130b agomir and control agomir were administered to IFNα5 adenovirus–treated (NZB × NZW)F1 mice via tail vein injection. In the prevention study, renal miR-130b expression was significantly increased in mice treated with miR-130b agomir 7 weeks after IFNα5 adenovirus treatment, compared with control agomir–treated mice (Figure 5A). IFNα5 adenovirus treatment exacerbated proteinuria in (NZB × NZW)F1 mice; however, miR-130b agomir reduced the level of urinary

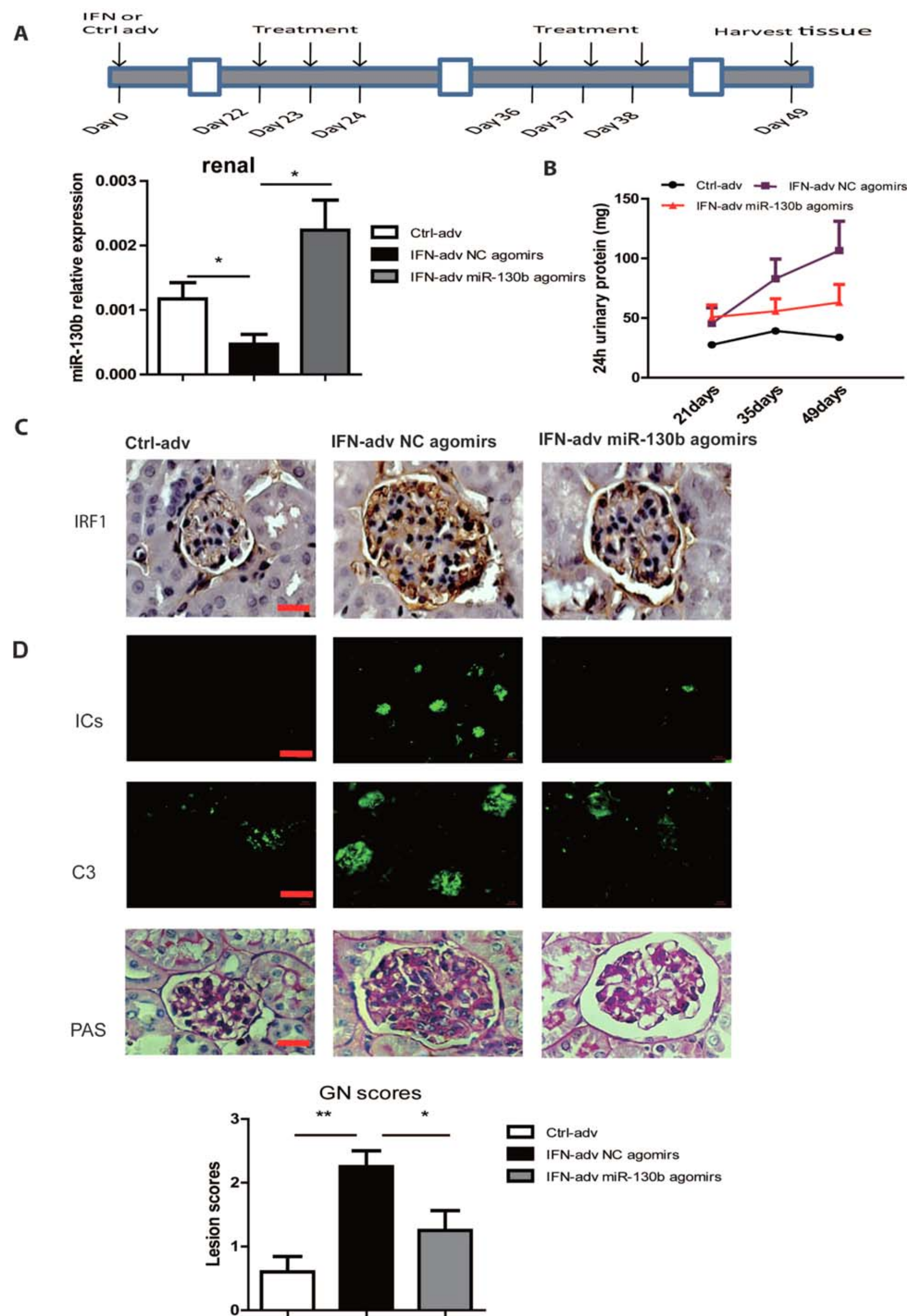

Figure 5. Efficacy of microRNA-130b (miR-130b) overexpression in preventing interferon-α (IFNα)–accelerated lupus nephritis (LN). **A,** Design of the prevention study. Quantitative polymerase chain reaction was used to determine miR-130b expression in renal tissues from control adenovirus (Ctrl-adv)–treated (NZB × NZW)F1 mice or from IFNα-expressing adenovirus (IFN-adv)–treated (NZB × NZW)F1 mice injected intravenously with negative control (NC) agomir or miR-130b agomir. **B,** Proteinuria, as quantified by measuring 24-hour urinary protein. **C,** Histologic analysis of interferon regulatory factor 1 (IRF-1) protein expression in mice. Bar = 25 μm. **D,** Deposition of immune complexes (ICs) and C3, as analyzed by immunofluorescence. Bars = 50 μm and 25 μm, respectively. Periodic acid–Schiff (PAS)–stained kidney sections were analyzed for renal lesion scores, which showed that miR-130b agomir suppressed IFNα-accelerated LN in mice. Bar = 25 μm. Data are representative of 2 independent experiments (n = 5–8 mice per group). In **A**, **B**, and **D** (bottom), values are the mean ± SEM. $* = P < 0.05$; $** = P < 0.01$. GN = glomerulonephritis.

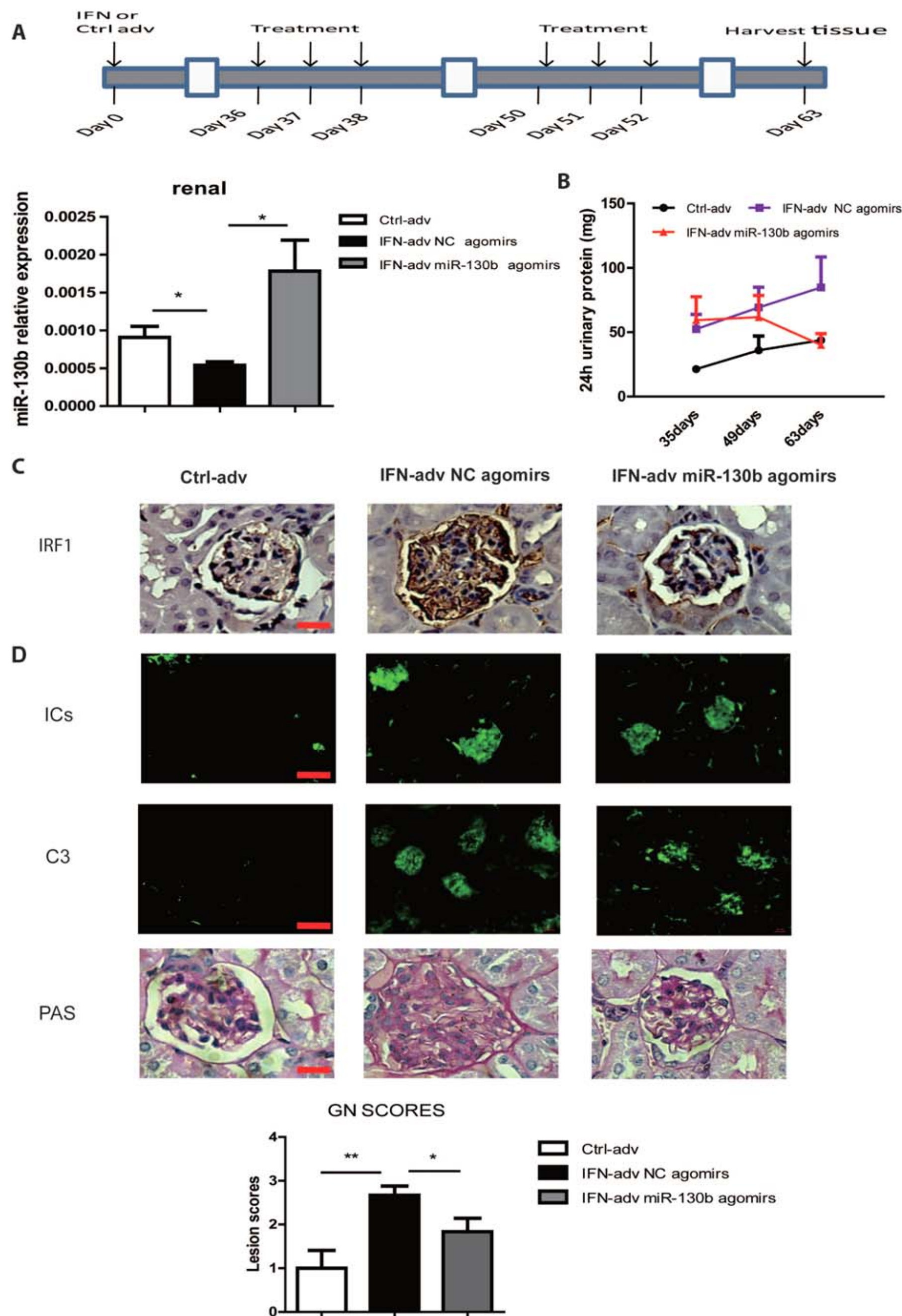

Figure 6. Efficacy of miR-130b overexpression in treating IFNα-accelerated LN. **A,** Design of the late-stage treatment study. Quantitative polymerase chain reaction was used to determine miR-130b expression in renal tissues from control adenovirus–treated (NZB $\times$ NZW)F1 mice or from IFNα-expressing adenovirus–treated (NZB $\times$ NZW)F1 mice injected intravenously with negative control agomir or miR-130b agomir. **B,** Proteinuria, as quantified by measuring 24-hour urinary protein. **C,** Histologic analysis of IRF-1 protein expression in mice. **D,** Deposition of immune complexes and C3, as analyzed by immunofluorescence. PAS-stained kidney sections were analyzed for renal lesion scores, which showed that miR-130b agomir ameliorated IFNα-accelerated LN in mice. Bar = 25 μm. Data are representative of 2 independent experiments (n = 5–6 mice per group). In **C** and **D** bars = 25 μm. In **A**, **B**, and **D** (bottom), values are the mean $\pm$ SEM. $* = P < 0.05$; $** = P < 0.01$. See Figure 5 for definitions.

protein (Figure 5B). Consistent with the in vitro data, in vivo overexpression of miR-130b inhibited aberrant IRF-1 expression induced in glomeruli by IFNα5 adenovirus, as assayed by immunohistochemical staining (Figure 5C). Moreover, the deposition of immune complexes and C3 in glomeruli after IFNα5 adenovirus treatment was markedly reduced in miR-130b agomir–treated mice compared with that in control agomir–treated mice. Finally, histologic examination revealed that IFNα5 adenovirus–treated (NZB $\times$ NZW)F1 mice developed severe glomerulonephritis. Notably, when their renal lesion scores were analyzed, miR-130b agomir–treated mice had normal glomeruli and no significant mesangial hypercellularity or glomerular enlargement (Figure 5D).

Next, to test the therapeutic potential of increasing miR-130b expression in the late stage of IFNα-accelerated LN, we treated mice with IFNα5 adenovirus for 5 weeks before treating them with miR-130b agomir (Figure 6A). During this period, the animals displayed high proteinuria, high serum levels of autoantibodies and immune complexes, and high C3 deposition in kidneys. We then treated mice with miR-130b agomir and monitored them for another 4 weeks. Mice treated with miR-130b agomir showed reduced proteinuria, down-regulated IRF-1 expression, and attenuated proliferative glomerulonephritis, although miR-130b agomir had little effect on deposition of immune complexes or C3 in renal tissues. In contrast, mice treated with control agomir displayed progressive proteinuria and kidney injury (Figures 6B–D). These data implied that up-regulation of miR-130b ameliorates renal injury in the late stage of LN. Therefore, combining all of the data described above, we have demonstrated the therapeutic efficacy of promoting miR-130b expression with miR-130b agomir for treatment of IFNα-accelerated LN.

DISCUSSION

The miRNA profiles reported to date have predominantly focused on immune cells in various autoimmune diseases. Thus, we analyzed the expression of microRNAs in kidney biopsy samples from LN patients by using microRNA array analysis. We found that miR-130b expression was significantly reduced in the kidneys of LN patients as compared with control patients. Furthermore, levels of miR-130b were progressively down-regulated during the process of murine LN. MicroRNA-130b, an miRNA discovered in recent years, is closely associated with multiple diseases such as cancers (33). Recently, miR-130b expression was reported to be reduced in the serum of patients with diabetic nephropathy and in the kidneys of diabetic mice. MicroRNA-130b ameliorates renal fibrosis by regulating expression of transforming growth factor β receptor type I and other profibrotic factors (34,35). Importantly, Wang et al showed that abnormal expression of serum miR-130b exacerbates renal damage in LN patients by affecting epithelial-to-mesenchymal transition in renal tubular cells (29). In the present study, we found that expression of miR-130b is inhibited in RMCs of IFNα-treated mice and that miR-130b levels are further regulated by type I IFN in RMCs (see Supplementary Figure 5, http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract).

Type I IFN is known to be a key cytokine in the pathogenesis of LN. The expression of IFN-inducible genes is elevated in SLE patients, and their levels correlate with disease activity and severity (36,37). The pathogenic role of type I IFN in the development of LN is also suggested by the finding that administration of exogenous IFNα to lupus-prone mice accelerates the progression and severity of the disease (9,28,38,39). However, the regulatory mechanisms of the type I IFN pathway in the kidney remain unclear.

Our study is the first to show that miR-130b correlates inversely with activation of the type I IFN pathway in kidneys from LN patients and that it represses this pathway in primary RMCs, implying a significant role for miR-130b in kidney injury. Our study further demonstrates that miR-130b represses the type I IFN pathway in RMCs by targeting IRF-1, independently of its effect on IRF-3 and IRF-7 expression or phosphorylation (see Supplementary Figure 6, http://onlinelibrary.wiley.com/doi/10.1002/art.39725/abstract). As a positive regulator of the IFN pathway, IRF-1 mediates both response to type I IFN and the induction of IFN. In the present study, we have shown that *IRF1* expression correlates negatively with miR-130b levels in kidneys from LN patients. Consistent with miR-130b overexpression data, knockdown of IRF-1 inhibits tyrosine phosphorylation of STAT-1 with no alteration of STAT-1 total protein levels, leading to down-regulated expression of IFN-inducible genes.

Interestingly, findings of a recent study imply that IRF-1 is a critical mediator of inflammation in SLE patients through its interaction with histone-modifying enzymes (40). Meanwhile, ChIP-Seq data imply that the 2 key members of the retinoic acid–inducible gene 1 (RIG-1)–like receptor signaling pathway, RIG-1 and melanoma differentiation–associated protein 5, are both IRF-1 targets and are significantly up-regulated in SLE (40). Su et al reported that IRF-1 can regulate RIG-1 expression induced by type I IFN (41), and Jiang et al showed that RIG-1 induction promotes tyrosine phosphorylation of

STAT-1 independently of alterations in STAT-1 protein levels (42). Although we cannot exclude the possibility that miR-130b might negatively modulate type I IFN signaling through other molecular mechanisms, the noncanonical IRF-1/RIG-1/STAT-1 activation pathway we observed here might account for the regulatory role of miR-130b in type I IFN signaling. Intriguingly, it has been reported that deletion of IRF-1 could alleviate renal injury in mice (43). Similarly, our data showed that IRF-1 expression is highly induced in glomeruli of mice with IFNα-accelerated LN, while levels of IRF-1 are significantly decreased after treatment with miR-130b agomir. These findings suggest that miR-130b plays a pivotal role in progression of LN by targeting IRF-1.

Targeted biologic therapies offer more specific and effective treatment of LN with fewer unwanted side effects. For example, CTLA-4Ig has been used as a therapeutic agent for LN by blocking both T cell and B cell costimulatory interactions (44,45). Recent studies suggest that modulation of miRNA expression by administration of oligonucleotides to mice could control disease progression (46,47). In the present study, our data demonstrated for the first time that overexpression of miR-130b in vivo through miR-130b agomir ameliorated kidney lesions in IFNα-treated (NZB $\times$ NZW)F1 mice, exerting preventive and even therapeutic effects. This implies that miR-130b, as a key regulator of LN, has potential therapeutic application in LN disease and particularly provides a novel treatment entry point for patients with late-stage LN.

Although these results encourage the clinical development of miR-130b, some problems still need to be studied further. Because immune cells are known to be critical for the pathogenesis of LN (48–51), we cannot exclude the possibility that miR-130b also affects immune cells. Therefore, to more fully understand the effects on LN of different renal levels of miR-130b, we could deliver miR-130b specifically into kidney tissues in vivo, particularly into RMCs, using materials biology techniques or immunoliposomes (52).

In conclusion, the present study indicates that miR-130b plays a crucial role in the pathogenesis of LN by targeting IRF-1 and thus down-regulating the type I IFN pathway. Our data exemplify the applicability of miRNA therapeutics in an LN disease model. Specifically, in vivo administration of miR-130b agomir can block LN development at an early stage of the disease and, more importantly, we can also apply this novel intervention to efficiently ameliorate renal damage at the late stage of LN.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Drs. S. Zhou and Shen had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

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