MicroRNA-146b, a Sensitive Indicator of Mesenchymal Stem Cell Repair of Acute Renal Injury

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ABSTRACT

The role of mesenchymal stem cells (MSCs) in kidney injury repair has been studied widely. However, the underlying molecular mechanism remains unclear. We profiled the altered microRNAs in renal tissues from cisplatin-induced acute kidney injury (AKI) rats treated with or without rat bone marrow MSCs (rMSCs). We observed that microRNA-146b (miR-146b) expression was considerably upregulated in renal tissues from AKI rats compared with that in healthy rats, and the expression decreased following MSC treatment after cisplatin administration. At the early stage of AKI, serum miR-146b levels exhibited a rapid increase that was even faster than that of two conventional renal function indexes: serum creatinine and blood urea nitrogen levels. Furthermore, the serum miR-146b levels in AKI patients were higher than those in healthy people. In vitro exposure to cisplatin also increased miR-146b expression in renal tubular epithelial cells (TECs). miR-146b knockdown protected renal TECs from cisplatin-induced apoptosis and promoted their proliferation. Moreover, ErbB4 was identified as a direct target of miR-146b, and miR-146b inhibition induced ErbB4 expression, resulting in enhanced proliferation of injured renal TECs. In addition, restoration by rMSCs could be controlled through ErbB4 downregulation. In conclusion, elevated miR-146b expression, resulting in enhanced proliferation of injured renal TECs. Over, ErbB4 was identified as a direct target of miR-146b, and miR-146b inhibition induced ErbB4 expression, resulting in enhanced proliferation of injured renal TECs. In addition, restoration by rMSCs could be controlled through ErbB4 downregulation. In conclusion, elevated miR-146b expression contributes to cisplatin-induced AKI, partly through ErbB4 downregulation. Moreover, miR-146b might be an early biomarker for AKI, and miR-146b inhibition could be a novel strategy for AKI treatment.

SIGNIFICANCE

The present study found that microRNA-146b (miR-146b) might be a novel biomarker for acute kidney injury and an indicator for its recovery after treatment with mesenchymal stem cells (MSCs). The results showed that in acute kidney injury induced by cisplatin, miR-146b in serum increased more quickly than did the usual indexes of kidney injury and decreased with restoration of MSCs. In addition, inhibition of miR-146b could ameliorate the apoptosis induced by cisplatin and potentially improve the proliferation by freeing ErbB4 and its downstream proteins.

INTRODUCTION

Acute kidney injury (AKI) is a clinical syndrome characterized by a rapid decrease in renal function that can be assessed through changes in serum creatinine (Cr) and blood urea nitrogen (BUN) levels [1]. Mesenchymal stem cells (MSCs) from various sources can promote tissue repair in disorders such as AKI [2–5]. We previously demonstrated that rat bone marrow MSCs (rMSCs) can participate in the restoration of acute kidney failure in rats [6]. Moreover, human umbilical cord MSCs (hUC-MSCs) can attenuate ischemia/reperfusion-induced acute renal failure and cisplatin-induced AKI or chronic kidney injury [7, 8]. Furthermore, hUC-MSCs modified through hepatocyte growth factor overexpression can improve the amelioration efficiency compared with unmodified hUC-MSCs [9]. We previously suggested that a paracrine mechanism might be mainly responsible for the therapeutic effect of MSCs; however, the involvement of microRNA (miRNA) in this process was not completely characterized.

miRNAs—18–25-nucleotide long, small non-coding RNAs—regulate gene expression and have crucial functions in physiological and pathological conditions through targeting numerous genes [10, 11]. miRNAs are essential for maintaining the development and stability of kidneys [12]. Moreover, many miRNAs participate in the pathogenesis of kidney diseases. Wang et al. reported that miR-200a prevents renal fibrogenesis by repressing transforming growth factor-β2
expression in diabetic nephropathy [13]. Godwin et al. determined the miRNA expression profile of renal ischemia/reperfusion injury and revealed that miR-21 might have a role in preventing the death of tubular epithelial cells (TECs) [14]. However, few studies have determined the alteration of miRNA expression after kidney injury, including cisplatin-induced AKI, and restoration to normal using MSCs. In our study, we identified that miR-146b expression is upregulated after cisplatin administration and returns to normal after rMSC treatment in vivo and in vitro.

miR-146b is strongly expressed in tumor tissues, such as papillary thyroid carcinoma, prostate cancer, liver cancer, and renal cell carcinoma [15–18]. Previous miRNA analysis results suggested that miR-146b expression is upregulated in patients undergoing hemodialysis [19]. miR-146b expression is associated with the prognosis of papillary thyroid carcinoma and the malignancy of hepatocellular carcinoma [15, 17]. miR-146b-5p can inhibit the migration and invasion of cancerous tissue by acting on MMP16 and epidermal growth factor receptor (EGFR) in glioma [20, 21]. In addition to cancer, miR-146b has a crucial role in epithelial cells and inflammation. miR-146b alleviates colitis in mice by improving epithelial barrier function and activating the nuclear factor-κB pathway [22]. Cheng et al. demonstrated that miR-146 inhibits endothelial inflammatory activation by negatively regulating proinflammatory pathways [23]. Because inflammation is a major part of AKI, miR-146b might function as a critical factor in AKI development.

In the present study, we identified miR-146b as a crucial target in AKI treatment using rMSCs both in vivo and in vitro. We observed that the expression of miR-146b was strongly upregulated in both animals and human patients with AKI. miR-146b knockdown ameliorated cisplatin-induced apoptosis and prompted TEC proliferation through upregulation of ErbB4 expression. Our findings facilitate further understanding of the role of miRNA in MSC-mediated AKI repair and provide a novel biomarker for AKI diagnosis and therapy.

**Isolation and Characterization of rMSCs**

One-month-old Sprague-Dawley rats were immersed in 75% ethanol for 5 minutes after anesthetization and decapitated to obtain the bilateral lower limb femurs without the attached muscles or adipose tissues. All procedures were performed under aseptic conditions. After douching with phosphate-buffered saline (PBS), the medulla ossium was collected and centrifuged at 800 rpm. The sediment was suspended in 4 ml of low-glucose Dulbecco’s modified Eagle’s medium (LG-DMEM) containing 10% fetal bovine serum, penicillin, and streptomycin and cultured at 37°C with 5% CO2. The expression of typical surface markers in passage 3 rMSCs was analyzed through flow cytometry.

**Histology and Immunohistochemical Staining**

Kidneys embedded in paraffin were cut into 4-μm-thick slices and stained using the standard hematoxylin and eosin staining protocol. Through immunohistochemistry, we observed proliferating cell nuclear antigen (PCNA) expression using a specific rabbit polyclonal antibody (BioWorld, New York, NY, http://www.bioworld.com).
Terminal Deoxynucleotidyl Transferase dUTP Nick-End Labeling Assay

A terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL) assay was performed according to the manufacturer’s instructions (Wuhan Boster Biological Engineering Co., Ltd., Wuhan, China, http://www.boster.com.cn). The TUNEL-Fluorescein Isothiocyanate (FITC) Apoptosis Detection Kit (Vazyme Biotech Co., Jiangsu, China, http://www.vazyme.com), which stains the nucleus blue (Hoechst stain) and 3’-OH green (FITC stain), was used in accordance with its protocol.

Cell Counting and Colony Formation

Cells were transfected with the miRNA control or inhibitor for 6 hours and transferred to complete HG-DMEM. After 48 hours, the cells were collected and counted. In total, $5 \times 10^3$ cells were replated in 24-well plates (Corning, Corning, NY, http://www.corning.com) and 3.5-cm cell culture dishes (Corning). Cell counting was performed using procedures described previously [25]. The cells in the 3.5-cm cell culture dishes were cultured until day 7, immobilized using 4% paraformaldehyde, and stained with crystal violet.

Cell Transfection

The 3’ untranslated region (3’UTR) of ErbB4 was obtained and ligated into the pmirGLO dual-luciferase miRNA target expression vector (Promega, Madison, WI, http://www.promega.com). The miRNA inhibitor and negative control (NC) were synthesized and purified (Bioneer, Daejeon, South Korea, http://www.bioneer.com). Transfection was performed with the X-tremeGENE small interfering RNA (siRNA) transfection reagent (Roche Life Sciences, Roche Diagnostics, Indianapolis, IN, http://www.lifescience.roche.com).
for NRK52E cells and Lipofectamine 2000 (Thermo Fisher) for 293T cells. The cells were transfected with the miRNA inhibitor and its NC at 200 nM and with the mimic and its NC at 25 nM. Luciferase activity was measured using the Dual-Glo luciferase assay system (GloMax 20/20; Promega). Furthermore, 100 nM ErbB4 siRNA and its NC were applied in the knockdown experiment.

Western Blot Analysis

Western blot analysis of the samples was performed as described previously [24]. We used the following primary antibodies: anti-glyceraldehyde-3-phosphate dehydrogenase, anti-Bcl-2-associated X (Bax), anti-Bcl-2, anti-PCNA, anti-phosphorylated (p)-stat5, anti-total (t)-stat5 (BioWorld), anti-ErbB4, anti-p-raf1/2, anti-t-raf1/2, anti-p-mek, anti-t-mek (Signalway Antibody, College Park, MD, http://www.sabbitech.com), anti-p-erk, anti-t-erk (Cell Signaling Technology, Danvers, MA, http://www.cellsignal.com), and anti-c-myc (Proteintech Group, Rosemont, IL, http://www.ptglab.com).

Statistical Analysis

Data are expressed as the mean ± SD. Statistically significant differences between groups were assessed by analysis of variance (ANOVA) with two-way classification or ANOVA with the Student-Newman-Keuls multicomparison test or the unpaired t test. p < .05 was considered statistically significant.

RESULTS

RMSCs Restore Injury From Cisplatin-Induced AKI

We previously established a cisplatin-induced AKI model [8, 24]. To verify the efficiency of rMSCs in repairing AKI, we first isolated rMSCs from Sprague-Dawley rats and cultured them for three passages (supplemental online Fig. 1A). We then characterized the immunotype of the used rMSCs through flow cytometry. The results of fluorescence-activated cell sorting showed that rMSCs were positive for CD29, CD44, and CD90 but negative for CD45 (supplemental online Fig. 1B). Next, we confirmed that the rMSCs could be induced to differentiate into osteogenic and adipogenic lineages (supplemental online Fig. 1C–1F). We tracked the homing of the injected rMSCs using live animal imaging and observed that CM-Dil-labeled rMSCs could localize at the injury site after injection into the AKI rats (supplemental online Fig. 2A, 2B). Serum Cr and BUN levels increased 2 days after cisplatin treatment and remained at higher levels until 5 days after treatment. However, transplantation with rMSCs notably reduced the serum Cr and BUN levels in the AKI rats (supplemental online Fig. 2C, 2D). Western blotting (supplemental online Fig. 2E) and hematoxylin and eosin staining showed that rMSC administration alleviated the inflammatory reaction in the renal tissues (supplemental online Fig. 2F). The results of TUNEL assay and immunohistochemical staining (supplemental online Fig. 2F) showed that rMSC treatment enhanced PCNA expression and effectively ameliorated cisplatin-induced apoptosis.

Identification of miR-146b in Cisplatin-Induced AKI Rats Using Microarray Analysis

We collected renal tissues from the AKI rats treated with and without rMSCs for 5 days and performed a gene microarray analysis to profile the altered miRNAs. As shown in Figure 1A, rMSC treatment caused significant changes in 44 miRNAs (p < .05). A pie chart clarified the results of the gene microarray: 36 miRNAs were upregulated and 8 were downregulated (Fig. 1B). Detailed data are shown in supplemental online Table 1. On the basis of many reported studies, we focused on four miRNAs and selected miR-146b for its stable changes in different baths (supplemental online Fig. 3). Through quantitative RT-PCR analysis, we further
verified the increase of miR-146b in the kidneys from AKI rats by comparing it with that from the sham control rats. We also confirmed the inhibition of miR-146b expression in kidneys from rMSC-treated rats (Fig. 1C). A low-dose cisplatin-induced experiment in vitro showed that miR-146b expression in NRK52E cells increased 24 hours after treatment and was maintained until 42 hours (Fig. 1D). Moreover, we collected serum from patients who had experienced blood loss leading to AKI, patients with chronic kidney disease (CKD) with acute exacerbation, and healthy controls and detected the expression of hs-miR-146b-5p, the human homolog of rno-miR-146b, using TaqMan-based real-time RT-PCR analysis. We observed that the hs-miR-146b-5p levels in the patients with renal disease were remarkably higher than those in the healthy controls (Fig. 1E). Because miR-146b inhibition was apparent after rMSC treatment, we selected it as the target for our next study. We identified miR-146b as a candidate microRNA, the expression of which altered in response to the development of cisplatin-induced AKI.

miR-146b Is a Sensitive Indicator of AKI
Considering the outcome of the clinical specimens, we next investigated the time course of miR-146b expression in the serum and kidney tissues of AKI rats. To investigate the sensitivity, we used a dose of cisplatin lower than that used in the model but that could still induce evident apoptosis in the rat kidney (Fig. 2A, 2B). We observed an increase in serum Cr and BUN levels in AKI rats 3–4 days after cisplatin treatment (Fig. 2C, 2D). Furthermore, the serum miR-146b levels in the AKI rats increased 1 day after cisplatin treatment (Fig. 2E). The miR-146b levels in the renal tissue of AKI rats began increasing 4 days after treatment and gradually decreased 5 days after treatment (Fig. 2F). Moreover, the experiments in vitro showed that the miR-146b levels increased rapidly in both NRK52E and HK-2 cells cultured in media without serum (supplemental online Fig. 4). In summary, these data suggest that miR-146b is a sensitive indicator of renal injury.

Figure 3. RMSCs alleviate cisplatin-induced renal injury and reduce miR-146b expression in vitro. (A): The number of PCNA-positive cells was higher in the rMSC group than in the PBS group. Original magnification ×200. (B): The number of TUNEL-positive cells increased in the PBS group but decreased in the rMSC group. Original magnification ×200. (C): Ratio of positive cells in each group from (A) and (B). (D): The results of Western blot analysis showed that the PCNA level was lower in the PBS group than in the rMSC group, indicating that rMSCs could promote the proliferation of injured NRK52E cells. In contrast, the ratio of Bax to Bcl-2 increased in the PBS group and decreased in the rMSC group, demonstrating that rMSCs reversed the apoptosis caused by cisplatin. (E): miR-146b expression in NRK52E cells from different groups. The cells in the rMSC group were cocultured with rMSCs in Transwell plates after pretreatment with cisplatin for 6 hours; normal NRK52E cells were used as controls. Analysis of variance was performed with the Student-Newman-Keuls multicomparison test. ***p < .001; ****p < .0001. Abbreviations: Bax, Bcl-2-associated X; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; miR-146b, microRNA-146b; rMSC, rat bone marrow mesenchymal stem cell; PBS, phosphate-buffered saline; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.
miR-146b Is Downregulated After Coculturing RMSCs With TECs

To determine the effect of rMSCs in vitro, we exposed NRK52E cells to cisplatin. Cisplatin treatment led to a decrease in the number of PCNA-positive cells. Nevertheless, rMSC treatment increased the number of PCNA-positive cells (Fig. 3A). Moreover, the TUNEL assay revealed that the cisplatin group had more apoptosis cells than did the control group and rMSC treatment rescued NRK52E cells from cisplatin-induced apoptosis (Fig. 3B). PCNA- and TUNEL-positive cells were counted in 10 consecutive fields (Fig. 3C). The expression of PCNA, a proliferation-related protein, decreased in the cisplatin group but was restored in the rMSC group. In contrast, the ratio of Bax to Bcl-2 increased in the cisplatin group but decreased in the rMSC group (Fig. 3D). miR-146b expression in NRK52E cells was inconsistent with that in vivo: it increased after cisplatin administrated and decreased after coculturing with rMSCs (Fig. 3E). We also demonstrated that rMSCs cocultured with injured NRK52E cells led to miR-146b upregulation in the rMSCs (supplemental online Fig. 5). In summary, cisplatin can induce miR-146b upregulation, which is reversed by rMSC intervention.

miR-146b Regulates the Survival and Proliferation of NRK52E Cells

To explore the potential role of miR-146b in cisplatin-induced AKI, we synthesized a specific inhibitor for knocking down endogenous miR-146b in NRK52E cells. We verified the efficiency of the inhibitor in NRK52E cells using real-time RT-PCR, with a scramble fragment (NC) as the control (Fig. 4A). The transfected cells were collected 48 hours after transfection, replated, and cultured continually. The cells in the inhibitor group grew faster than did those in the control group (Fig. 4B), which was consistent with the results of the colony formation assay (Fig. 4C). In addition, transfection with the miR-146b inhibitor after 48 hours increased the PCNA positivity of the NRK52E cells (Fig. 4D). The number of apoptotic cells in the inhibitor group was considerably less than that in the NC group, as suggested by the TUNEL-FITC staining (Fig. 4E). PCNA- and TUNEL-FITC-positive cells were counted in 10 consecutive fields

Figure 4. MicroRNA (miR)-146b inhibition increases cell proliferation and inhibits apoptosis. (A): Real-time reverse transcription polymerase chain reaction analysis showed that miR-146b was suppressed in NRK52E cells transfected with the inhibitor. NRK52E cells treated with the NC were used as controls. Analysis of variance (ANOVA) was conducted with the t test. *** p < .001. (B): Time-course NRK52E cell counting was performed in two groups (in triplicate). Data were analyzed using two-way ANOVA followed by the Bonferroni t test. ***, p < .001. (C): Representative images of cell colonies in groups. (D): The number of PCNA-positive cells was higher in the inhibitor group than in the NC Group. Original magnification ×200. (E): Representative images of the TUNEL-FITC assay. The ratio of green to blue was higher in the NC group than in the other groups. Original magnification ×200. (F): Ratio of positive cells in each group from (D) and (E) ***, p < .01. Abbreviations: N.C., negative control; PCNA, proliferating cell nuclear antigen; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling; TUNEL-FITC, terminal deoxynucleotidyl transferase dUTP nick-end labeling-fluorescein isothiocyanate.
miR-146b as a New Indicator of AKI MSC Treatment

ErbB4 Is a Potential Target of miR-146b During AKI

We identified the potential targets of miRNA using the TargetScan program (Whitehead Institute for Biomedical Research, Cambridge, MA, http://www.wi.mit.edu). On the basis of recent research, we studied ErbB4 because of its critical role in the ErbB4 pathway of renal epithelial cell proliferation (Fig. 5A). To demonstrate the direct regulation of ErbB4 by miR-146b, the 3'-UTR of ErbB4 mRNA was cloned and constructed into the luciferase reporter vector. The results of dual-luciferase reporter assays indicated that miR-146b overexpression reduced luciferase activity in 293T cells transfected with a wild-type (WT) reporter vector. In contrast, this reduction was not evident in cells with a mutant (MU) reporter vector. Furthermore, miR-146b knockdown promoted luciferase activity in the WT group but had no significant influence on the MU group (Fig. 5B). miR-146b knockdown restored ErbB4 protein expression in NRK52E cells (Fig. 5C). Moreover, we detected the expression of the downstream proteins of ErbB4 pathway after transfection with specific siRNAs. p-mek, p-erk1/2, c-myc, and p-stat5 expression exhibited an apparent decrease after siRNA2 transfection, which was accompanied by a decrease in ErbB4 expression. (Fig. 5D). Real-time reverse transcription polymerase chain reaction analysis of ErbB4 mRNA expression in NRK52E cells after transfection with the NC, siRNA1, or siRNA2. (E) Western blot analysis of the downstream proteins of the ErbB4 pathway after transfection with specific siRNAs. p-mek, p-erk1/2, c-myc, and p-stat5 expression exhibited a significant decrease after siRNA2 transfection, which was accompanied by a decrease in ErbB4 expression. (F) A schematic diagram of miR-146b participation in acute kidney injury. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; miR-146b, microRNA-146b; MU, mutant 3' untranslated region sequence; N.C., negative control; p, phosphorylated; PCNA, proliferating cell nuclear antigen; siRNA, small interfering RNA; t, total; WT, wild type 3' untranslated region sequence complementary to the seed sequence of miRNA.
target ErbB4 to decrease the survival of cisplatin-injured NRK52E cells.

**Downregulation of ErbB4 Inhibits Cell Survival**

We knocked down the ErbB4 in NRK52E cells after cisplatin-induced injury and cocultured these cells with rMSCs. The injured cells transfected with the NC exhibited no restoration. The number of positive cells increased in injured NRK52E cells transfected with ErbB4 siRNA and then cocultured with rMSCs. Original magnification ×200. (B): Representative graphs of the TUNEL assay. The number of positive cells increased in injured NRK52E cells transfected with siRNA compared with those transfected with the NC. Original magnification ×200. (C): Ratio of positive cells in each group from (A) and (B). (D): Western blot analysis results showed that erk and c-myc were downregulated in restored NRK52E cells transfected with siRNA compared with those transfected with the NC. ***, p < .001. Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; N.C., negative control; PCNA, proliferating cell nuclear antigen; rMSCs, rat bone marrow mesenchymal stem cells; siRNA, small interfering RNA; TUNEL, terminal deoxynucleotidyl transferase dUTP nick-end labeling.

**DISCUSSION**

The potential of MSCs in renal injury repair has been investigated widely [26, 27]. Recent studies have demonstrated that miRNAs are critically involved in the development of kidney diseases [13–16]. We determined the miRNA profile in the renal tissues of AKI rats treated with rMSCs and observed that the expression of 44 miRNAs significantly changed in these renal tissues after rMSC treatment. We focused on miR-92b, -146b, -150*, and miR-455 first (supplemental online Fig. 3). We also verified stable alteration in miR-146b using the AKI rat model and cell culture model through real-time RT-PCR analyses and observed that miR-146b expression gradually increased in the serum and renal tissues of AKI rats but decreased after rMSC administration. For the first time, we report that miR-146b-regulated suppression of ErbB4 is a potential causal mechanism in cisplatin-induced AKI and that the inhibition of miR-146b is a potential mechanism by which bone marrow MSCs alleviate cisplatin-induced AKI.

Our findings have demonstrated that miR-146b is a potential noninvasive biomarker for AKI. Previous studies have indicated that miRNA expression correlates with the pathophysiological changes of AKI [28]. Moreover, miR-146b has been proposed as an indicator for papillary thyroid carcinoma and prostate cancer [15, 17]. In addition, miR-146b expression correlated with the degree of the malignancy of lung cancer and is upregulated in CKD [29]. Furthermore, a miRNA highly homologous to miR-146b is
associated with the development of chronic renal inflammation [19]. According to previous findings, miR-146b expression is also elevated in AKI with prevalent tubular injuries, including disorder and renal tubular necrosis. Moreover, we observed that compared with the traditional indexes for renal function such as serum Cr and BUN levels, serum miR-146b levels changed rapidly even after low-dose cisplatin treatment and remained stable at a high level. Thus, miR-146b responded rapidly to the injuries in AKI rats. To demonstrate the clinical significance of miR-146b in the diagnosis of kidney injuries, we examined the expression of hs-miR-146b-5p, the human homologous molecules of miR-146b, in the serum of patients (mainly those with AKI and CKD) and healthy controls. The results of the TaqMan-based real-time PCR analyses showed that the serum miR-146b levels were significantly higher in the patients than were those in the healthy controls, suggesting that miR-146b has great potential to be developed as a reliable indicator of kidney injury.

RMSCs ameliorated cisplatin-induced AKI in vivo and in vitro. In addition, miR-146b expression was decreased in AKI kidneys with RMSCs administered, identical to the findings with NRK52E cells. We further demonstrated that miR-146b participated in the regulation of cell apoptosis and proliferation using a loss-of-function strategy. We observed that miR-146b knockdown could rescue cells from cisplatin-induced apoptosis and prompt the proliferation of the surviving cells. Moreover, miR-146b expression in RMSCs co-cultured with the injured NRK52E cells was increased (supplemental online Fig. 5); however, the mechanism of this increase remains unclear. Future studies are warranted to identify the molecules in RMSCs that inhibit miR-146b expression.

Using TargetScan (Whitehead Institute for Biomedical Research), a microRNA target prediction program, ErbB4, Siah2, iRACK1, and other candidate molecules were found to be candidates. As reported previously, miR-146a was verified to target ErbB4 directly [30, 31]. ErbB4, also known as human epidermal growth receptor 4 (HER4), is a member of the EGFR family [32]. ErbB4 has an indicative role in kidney development. In vivo, ErbB4 was expressed in the developing tubules of nephron [33]. Veikkolainen et al. demonstrated that ErbB4 participates in the proliferation and polarization of renal epithelial cells and in the formation of ducts during kidney development [34]. In addition, a recent study reported that ErbB4 knockout accelerated the progression of polycystic kidney disease [35]. We have confirmed the direct regulation of ErbB4 by miR-146b through dual-luciferase reporter assays. Western blot analysis showed that miR-146b inhibition induced ErbB4 upregulation. Downstream adaptors of ErbB4 transduce signals to ERK1/2(p44/p42 MAPK), JNK, AKT, and STAT5 and activate transcription factors for promoting proliferation and migration [36–38]. We synthesized two siRNAs for ErbB4, and siRNA2 reduced ErbB4 expression efficiently. Immunoblotting indicated that siRNA2 inhibited the STAT5 and raf/mek/erk/c-myc pathways. Therefore, miR-146b induction during AKI accelerated cell apoptosis and restrained proliferation by targeting ErbB4. In contrast, rMSC treatment reduced miR-146b expression and restored ErbB4 expression, thus repairing the injured renal tissues.

**CONCLUSION**

The results of the present investigation suggest that MSCs could reduce miR-146b expression in cisplatin-induced AKI. miR-146b inhibits cell proliferation and prompts cell apoptosis through ErbB4 in cisplatin-induced AKI. Our findings not only provide insights into the involvement of miRNA in MSC-mediated AKI repair, but also indicate a novel biomarker for AKI diagnosis and therapy.

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**AUTHOR CONTRIBUTIONS**

Y. Zhu: conception and design, collection and/or assembly of data, data analysis and interpretation, manuscript writing; J.Y. and Y. Zhou: collection and/or assembly of data, data analysis and interpretation; L.Y., Y.T., W.L., J.Z., and H.G.: collection and/or assembly of data; Z.S.: administrative support, data analysis and interpretation; H.J. and J.X.: data analysis and interpretation; B.Z.: data analysis and interpretation, manuscript writing; M.W., Y.Y., and F.M.: administrative support; X.Z.: conception and design, manuscript writing; W.X. and H.Q.: conception and design, financial support, final approval of manuscript.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

W.X. and H.Q. are uncompensated patent holders. The other authors indicated no potential conflicts of interest.

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