miR-125b Disrupts Mitochondrial Dynamics via Targeting Mitofusin 1 in Cisplatin-Induced Acute Kidney Injury

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Abstract
Background: Mitochondria are dynamic organelles whose structure are maintained by continuous fusion and fission. During acute kidney injury (AKI) progression, mitochondrial fission in renal tubular cells was elevated, characterized by mitochondrial fragmentation. It is tightly associated with mitochondrial dysfunction, which has been proven as a critical mechanism responsible for AKI. However, the initiating factor for the disruption of mitochondrial dynamics in AKI was not well understood. Objectives: To explore the molecular mechanisms of mitochondrial disorders and kidney damage. Methods: We established cisplatin-induced AKI model in C57BL/6 mice and proximal tubular cells, and detected the expression of miR-125b by qPCR. Then we delivered miR-125b antagomir after cisplatin treatment in mice via hydrodynamic-based gene transfer technique. Subsequently, we performed luciferase reporter and immunoblotting assays to prove miR-125b could directly modulate mitofusin1 (MFN1) expression. We also tested the role of miR-125b in mitochondrial and renal injury through immunofluorescent staining, qPCR, and immunoblotting assays. Results: miR-125b levels were induced in cisplatin-challenged mice and cultured tubular cells. Anti-miR-125b could effectively alleviate cisplatin-induced mitochondrial fragmentation and kidney injury both in vitro and in vivo. Furthermore, miR-125b could directly regulate MFN1, which is a key regulator of mitochondrial fusion. Our study indicated that miR-125b is upregulated during cisplatin-induced AKI. Inhibition of miR-125b may suppress mitochondrial and renal damage through upregulating MFN1. This study suggests that miR-125b could be a potential therapeutic target in AKI.

Introduction
Mitochondria are dynamic cellular organelles whose structure is maintained by continuous fusion and fission [1]. Mitochondria are generally elongated and filamentous in physiological conditions, and become fragmented in response to stress stimulation [2, 3]. The abnormal mitochondrial structure is tightly associated with mitochondrial dysfunction and disease pathology [4]. The most crucial function of mitochondria is the production of...
ATP through oxidative phosphorylation [5]. The kidney has a high energy demand due to its active reabsorption of large quantities of solutes. However, the proximal tubular cells (PTCs) have very limited capacity for glycolysis. Instead, they are very rich in mitochondria in order to supply energy via oxidative phosphorylation [5, 6]. Thus, the stability of mitochondria structure and function is critical to the maintenance of normal kidney function.

Mitochondrial dysfunction leads to reduced ATP production and the generation of reactive oxygen species (ROS) which may contribute to the pathological process of acute kidney injury (AKI). AKI is associated with a rapid decline of renal function. It is a worldwide public health problem with increased morbidity and mortality [7]. The causes of AKI mainly include ischemia-reperfusion, sepsis, and various exogenous nephrotoxins [8]. During the development of AKI, mitochondrial fission in tubular cells is elevated, and characterized by mitochondrial fragmentation, which promotes cell apoptosis and renal injury [9]. Recently, more and more studies focus on improving mitochondrial function to prevent PTC injury and restore renal function during AKI. Despite extensive research, the molecular mechanism of mitochondrial abnormalities under the AKI pathological condition remains unclear.

microRNAs are endogenous small, noncoding RNAs of 21–25 nucleotides that play vital roles in gene expression regulation through translational repression and/or mRNA degradation of target genes [10]. In recent years, the relationship between miRNA and mitochondrial damage has received increasing attention. In 2010, Wei et al. [11] established a mouse model with specific ablation of Dicer from renal proximal tubules. These mice were deprived of miRNAs in tubular cells and became resistant to ischemic AKI, demonstrating an important role of miRNAs in AKI [11]. After that, several studies found that lots of miRNAs could participate in the AKI process via regulating mitochondrial morphology and function, such as miR-484 and miR-709 [12, 13]. In this study, we found that miR-125b was significantly upregulated in response to cisplatin treatment both in vitro and in vivo. miR-125b is a highly conserved miRNA which participates in many physiological and pathological processes [14, 15], considering that miR-125b exerted a vital role in mitochondrial metabolism and dynamics regulation in monocyte [16]. We hypothesized that miR-125b could also influence the mitochondrial homeostasis in renal tubular cells via regulating its target genes during AKI.

In this study, with bioinformatic analysis, we predicted that miR-125b could directly target and regulate the pro-fusion molecule mitofusin1 (MFN1). MFN1 is a key regulator of mitochondrial fusion, which is linked to mitochondrial biogenesis and respiratory functions, impacting cell fate and organism homeostasis [17]. MFN1 maintains a certain abundance of expression under the physiological condition and could be downregulated during the stimulation by stress or injury factors. Modulation of MFN1 levels has been proven to prevent mitochondrial fission and cell apoptosis in various cell context [18, 19]. As so, we speculate whether miR-125b promotes cisplatin-induced mitochondrial dysfunction and kidney injury through inhibiting MFN1 levels. With a series of in vivo and in vitro experiments, we confirmed that MFN1 is a direct target of miR-125b. During cisplatin nephrotoxicity, miR-125b is upregulated and then inhibits MFN1 levels, thereby promoting mitochondrial division and tubular injury.

Materials and Methods

Cell Culture and Treatment

The human renal PTC (HK2) cell line was obtained from the American Type Culture Collection and cultured in DMEM/F-12 medium (Gibco; Thermo Fisher Scientific) supplemented with 10% fetal bovine serum, penicillin (100 U/mL), and streptomycin (100 μg/mL), and maintained at 37°C in 5% CO2 in a humidified incubator. The cells were serum-starved for 12 h before cisplatin treatment. The cells were treated with 20 μM cisplatin for 24 h.

Animal Experiments

We obtained the male C57BL/6 mice (8–10 weeks) from the Model Animal Research Center of Nanjing University (Nanjing, China). The use of animals in this study was approved by the Institutional Animal Care and Use Committee at Jinling Hospital. Male C57BL/6 mice were used to generate the cisplatin-induced AKI model. Cisplatin (20 mg/kg body wt) was given through intraperitoneal injection as described previously [20]. The mice in the control group were injected with normal saline. All the mice were euthanized 72 h after cisplatin administration. Kidneys and blood were collected after the animals were sacrificed. To reduce microRNA levels in the mice kidney, miR-125b antagomir (GenePharma, Shanghai, China) was administered to the mice using a previously described hydrodynamic-based gene transfer technique. Briefly, 40 μg of antagonim in approximately 2.6 mL TransIT-EE Hydrodynamic Delivery Solution (Mirus) was injected into a mice via tail vein in 5–7 s.

Transmission Electron Microscopy and Mitochondrial Fragmentation

Renal cortex tissues were fixed in 3.75% glutaraldehyde and post-fixed in 1% osmium tetroxide. After dehydration in ethanol, the specimens were embedded in epon. Ultrathin sections in 70 nm were stained and examined with Hitachi 7500 transmission electron microscope (Hitachi, Tokyo, Japan). Mitochondria whose length >2 μm were considered filamentous, and those with length...
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Fig. 1. Cisplatin-induced mitochondrial dysregulation and AKI in mice. C57BL/6 mice (male, 8–10 weeks) were given cisplatin (20 mg/kg) or normal saline (control) i.p. on day 0 and sacrificed on day 3. a Representative micrographs of electron microscopy of kidney PTCs from normal saline and cisplatin-treated mice (scale bar = 2 μm). Quantification of the fragmented mitochondria is shown on the right. b qPCR analysis of mtDNA copy number. c Scr levels in each group. d Serum BUN levels in each group. e Representative images of renal PAS staining (scale bar = 50 μm). f Representative images of TUNEL staining. Quantification of TUNEL-positive cells in the kidney (scale bar = 40 μm). g Western blotting analysis of Kim-1, Bax, MFN1, and DRP1 expressions in cisplatin or normal saline–treated mice. h Quantification of g. Student’s t-test was used, *p < 0.05 versus control. Data are shown as mean ± SD (n = 5–6 in each group). AKI, acute kidney injury; i.p., intraperitoneal injection; PTCs, proximal tubular cells; qPCR, quantitative PCR; Scr, serum creatinine; PAS, periodic acid–Schiff; MFN1, mitofusin1.

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were measured using the Dual-Luciferase Report Assay System. 24 h later, cell lysates were prepared and luciferase activities were measured.

We transfected the constructs combined with miR-125b mimic or nc mimic into 293T using GP-transfect-Mate (GenePharm). 48 h after transfection, we performed luciferase reporter assay according to the manufacturer’s instructions. Luciferase activities were normalized with the Renilla luciferase activities. We quantified the luciferase activities with a luminometer (Promega).

Serum Biochemistry

We centrifuged the whole blood of mice at 1,000 g for 15 min to separate serum. We measured the serum creatinine (Scr) and urea nitrogen (BUN) from mice by using the fully automatic bio analysis machine (Raytoy, China).

We performed Western blotting to detect the expression levels of mitochondrial fission and fusion-related proteins, such as Kim1, Bax, DRP1, and b-actin. We extracted total protein from the kidney cortex and 293T cells by using TRIzol Reagent (Invitrogen) according to the manufacturer’s instructions. The protein concentration was determined by using the BCA protein assay kit (Pierce). We incubated the proteins with Antifade mounting medium for further examination using confocal fluorescent microscopy.

Results

Cisplatin Treatment Caused Mitochondrial Fragmentation in vitro

In order to generate the cisplatin-induced AKI model, male C57BL/6 mice were intraperitoneally injected cisplatin at a dose of 20 mg/kg. The mice were sacrificed on day 3, and kidney tissue were collected for subsequent analysis. We observed the morphology of mitochondria in mouse kidneys cortices with transmission electron microscopy. EM micrographs showed that compared with the normal saline group, many PTCs fragmented their mitochondria into small, round suborganelles. Quantitative morphometric analysis confirmed that the PTCs with fragmented mitochondria were increased in cisplatin-treated mice (Fig. 1a). At the molecular level, cisplatin insult affected the level of the main proteins, which regulates mitochondria fission and fusion. Downregulation of MFN1 and upregulation of DRP1 were detected by Western blotting (Fig. 1g, h). Furthermore, mtDNA copy number was declined in response to cisplatin treatment (Fig. 1b), suggesting less of mitochondria. After cisplatin injection, the levels of BUN and SCR were increased on day 3 (Fig. 1c, d).

Statistical Analysis

The data were presented as the mean ± SD. Analyses were performed with GraphPad Prism software (GraphPad Software Inc., La Jolla, CA, USA). The normality test of the data was performed by the Shapiro-Wilk test. Differences between groups were compared using the Student’s t-test or ANOVA method, and post hoc analyses were analyzed using the Bonferroni correction or Dunnnett correction. p < 0.05 was considered statistically significant.
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Cisplatin treatment upregulated miR-125b levels both in vivo and in vitro. We next assessed apoptotic cells in kidneys using TUNEL staining. As shown in Figure 1f, TUNEL-positive cells were increased in the cisplatin group. Accordingly, cisplatin treated also induced the expression of the proapoptotic protein Bax (Fig. 1g, h). Taken together, these data demonstrated that cisplatin could induce AKI and mitochondrial damage in vivo.

Fig. 2. Cisplatin treatment upregulated miR-125b levels both in vivo and in vitro. a qRT-PCR analysis of miR-125b in cisplatin-treated mouse kidney on day3. Student’s t-test was used, *p < 0.05 versus control (normal saline). Data are shown as mean ± SD (n = 5–6 per group). b qPCR analysis of miR-125b in renal tubular cells exposed to cisplatin insult at various dosages (0–20 μM). One-way ANOVA was used, and post hoc analyses were analyzed using the Dunnett T correction. *p < 0.05 versus 0 μM. Data are presented as the mean ± SD of 3 independent experiments. c qPCR analysis of miR-125b in cisplatin-exposed HK2 for different time points (0, 6, 12, and 24 h). Differences between the groups at time point were analyzed by repeated measures one-way ANOVA, and post hoc analyses were analyzed using the Dunnnett T correction. *p < 0.05 versus 0 h. Data are presented as the mean ± SD of 3 independent experiments. qPCR, quantitative PCR.

Cisplatin Upregulated miR-125b Levels Both in vivo and in vitro

In our previous work, we found that plasma miR-125b levels were upregulated in nephrotic syndrome patients relative to healthy controls, which indicated the critical role of miR-125b in nephropathy [22], considering the vital role of miR-125b in mitochondrial metabolism and dynamics [23]. We questioned whether miR-125b was involved in cisplatin-induced nephropathy. First of all, qPCR was performed to evaluate the miR-125b expression in the cisplatin-induced AKI model. The results showed the upregulation of miR-125b in the renal cortex of cisplatin injection (Fig. 2a). Next, cultured tubular epithelial cells were given different doses of cisplatin (0, 1, 5, 10, and 20 μM) for 24 h, or treated with 20 μM cisplatin in various time points (0 h, 6 h, 12 h, and 24 h), and then miR-125b expressions were detected (Fig. 2b, c). Our results found that cisplatin could induce overexpression of miR-125b in a dose- and time-dependent manner.

Anti-miR-125b Alleviated Kidney Injury and Mitochondrial Dysfunction in Cisplatin-Induced AKI Mice

To determine the role of miR-125b in vivo, we tested the effect of miR-125b inhibition on the cisplatin-induced AKI model. miR-125b antagonist or nc was administered to mice using the TransIT in vivo gene delivery system (Mirus) [24] on the second day after cisplatin injection (Fig. 3a). As expected, cisplatin-induced mir-125b upregulation was abrogated after anti-miR-125b transfer (Fig. 3b). To evaluate the influence of anti-miR-125b on mitochondrial morphology, we observed the ultrastructure of mitochondria using transmission electron microscope. The increased mitochondrial division in cisplatin-induced AKI mice was attenuated after miR-125b antagomir administration (Fig. 3c). In line with the alterations in mitochondrial morphology, the elevated levels of DRP1 in cisplatin-treated mice were decreased in the anti-miR-125b-administrated group. In addition, cisplatin-induced MNF1 decrease was rescued through miR-125b inhibition (Fig. 3j, k). Moreover, qPCR analysis showed that cisplatin-downregulated mtDNA copy number was restored by miR-125b antagomir (Fig. 3d). Notably, in addition to improve mitochondrial fragmentation, inhibition of miR-125b also relieved cisplatin-induced damage to kidney function. As shown in Figure 3e and f, both Scr and BUN increased by cisplatin treatment were reduced after miR-125b an-
CS7BL/6 mice, male, 8–10 week old

Normal saline or cisplatin injection
miR-125b antagomir or nc (hydrodynamic injection)

Scr, mg/dL
BUN, mg/dL
mtDNA/18s

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Consistently, miR-125b antagomir ameliorated the histological damage in renal tubules (Fig. 3g) as well as Kim1 levels (Fig. 3j, k). Cisplatin-induced apoptosis was also decreased by miR-125b inhibition, as shown by the TUNEL assay (Fig. 3h, i) and Bax expression (Fig. 3j, k). Collectively, these results supported that anti-miR-125b could effectively alleviate mitochondrial fragmentation and tubular injury in cisplatin-induced nephritis.

miR-125b Directly Modulates MFN1 Expression

To explore the mechanism underlying the effects of miR-125b on cisplatin-induced kidney injury, we searched the putative targets for miR-125b through TargetScan, the miRNA target analyzing database, and predicted MFN1 as a miR-125b target (Fig. 4a). MFN1 plays a vital role in mitochondrial fusion, and we also observed MFN1 down-regulation in kidneys of cisplatin-treated AKI mice. Therefore, we wondered whether miR-125b participates in cisplatin-induced AKI through directly inhibiting MFN1. To assess whether miR-125b directly targets the 3′-UTR of MFN1, we performed luciferase reporter assays using reporters carrying either the wild-type (WT) or mutant MFN1 3′-UTR (Fig. 4a). WT or mutant reporters and miR-125b mimic or nc mimic were co-transfected in 293T cells, and then luciferase activity was detected. The results showed that the activity of WT luciferase was significantly decreased by miR-125b-mimic compared with nc-mimic. However, mutation of the predicted seed region completely abolished the effects of miR-125b-mimic on reporter gene expression (Fig. 4b).

**Fig. 3.** miR-125b antagomir attenuates cisplatin-induced mitochondrial dysfunction and kidney injury. **a** Schematic diagram of the experimental design. Mice were given cisplatin or normal saline through i.p. on day 0. On the second day (day 1), we delivered the miR-125b antagomir and control vector (nc) through tail vein of mice via a previously described hydrodynamic-based gene-transfer technique. All the mice were sacrificed on day 3. **b** qPCR showing that miR-125b antagomir inhibited miR-125b expression effectively. **c** Representative micrographs of electron microscopy. Quantification of the fragmented mitochondria is shown on the right (scale bar = 2 μm). **d** qPCR analysis of mtDNA copy number. **e** Scr levels in each group. **f** Serum BUN levels in each group. **g** Representative images of PAS staining in different groups (scale bar = 50 μm). **h** Representative images of TUNEL staining (scale bar = 40 μm). **i** Quantification of TUNEL-positive cells in the kidney. **j** Immunoblotting analysis of the expression of Kim-1, Bax, MFN1, and DRP1. **k** Quantification of J. Two-way ANOVA was used, *p < 0.05 versus anti-NC + control, #p < 0.05 versus anti-NC + cisplatin. Data are shown as mean ± SD (n = 6–7 per group). i.p., intraperitoneal injection; qPCR, quantitative PCR; Scr, serum creatinine; PAS, periodic acid-Schiff; MFN1, mitofusin1.

**Fig. 4.** MFN1 is a direct target of miR-125b. **a** The predicted miR-125b binding site in the MFN1 3′UTR (Targetscan) and the mutations made in the seed-recognizing sites to create a MUT MFN1 3′-UTR for the luciferase assay. Two-way ANOVA was used, *p < 0.05 versus nc mimic. **b** The luciferase assay. Two-way ANOVA was used, *p < 0.05 versus nc mimic. **c** Immunoblotting of the MFN1 expression in nc or miR-125b mimic transfected tubular cells. **d** Quantification of C, Student’s t-test was used, *p < 0.05 versus nc mimic. All data are presented as the mean ± SD of 3 independent experiments. MFN1, mitofusin1; MUT, mutant.
Cisplatin miRNA inhibitor

(a) miTOX/Hoechst3342 Merge

(b) Cisplatin miRNA inhibitor

(c) % cells with mitochondrial fragmentation

(d) Relative protein levels of Kim1

(e) Relative protein levels of Bax

(f) Relative protein levels of DRP1

(g) Relative protein levels of MFN1

(h) MitoSOX fluorescence intensity (fold over control)

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In addition, Western blotting showed that the expression of MFN1 was decreased after miR-125b mimic transfection (Fig. 4c, d). All these results indicated that miR-125b could directly target the 3′-UTR of MFN1.

**The Protective Effect of Anti-miR-125b in Cisplatin-Treated Renal Tubular Cells**

To further verify the renal protection of anti-miR-125b, we compared the effects of miR-125b inhibitor or nc inhibitor transfected cells combined with cisplatin treatment. The MitoTracker assay showed that miR-125b inhibitor could suppress mitochondrial fragmentation during cisplatin treatment, verifying the protective effect of anti-miR-125b (Fig. 5a). Consistently, as shown in Fig. 5b, c, the increased mitochondrial ROS production and the decreased mtDNA copy number induced by cisplatin were reversed after miR-125b inhibition. Furthermore, cisplatin-induced decrease of MFN1 was reversed by anti-miR-125b (Fig. 5d, g). Cisplatin-induced upregulation of DRP1 was downregulated after miR-125b inhibitor transfection (Fig. 5d, h). We also evaluated the apoptosis and injury in anti-miR-125b-transfected renal tubular cells. As shown in Figure 5d and f, cisplatin-upregulated Kim1 and Bax levels were inhibited by anti-miR-125b. Our results demonstrated that anti-miR-125b exerts its mitochondrial and renal protection role in cisplatin-challenged HK2 cells.

**Discussion**

Mitochondria are an highly dynamic organelle that change their shape constantly through fission and fusion, which is defined as mitochondrial dynamics [25]. This balance is broken and progress toward division during the AKI pathological process [9]. And the excess of fission may result in mitochondrial fragmentation, which is tightly coordinated with the functional abnormalities [26]. The disruption of the mitochondrial structure also leads to a series of danger signals such as releases of cytochrome c, a trigger of apoptosis, as well as mtDNA [3].

Furthermore, ROS released by damaged mitochondria play a vital role in the oxidative stress process during AKI. It is worth noting that mitochondria dysfunction appear earlier than clinical manifestations of kidney injury in AKI [27, 28]. All these evidence strongly suggested that mitochondria might be a compelling therapeutic target in AKI treatment.

To further explore the mechanism of mitochondrial injury, we established the AKI animal model via a single dose injection of cisplatin (20 mg/kg). After 3 days, the mice showed renal pathological changes, renal function decline, and increased mitochondrial division. Besides mitochondrial structure abnormality, the excessive of ROS production as well as the decrease of mtDNA copy number were also evoked by cisplatin in renal tubular cells, which were consistent with other literature [29]. Based on this injury model, we revealed the critical role of miR-125b in mitochondrial dynamic and kidney injury regulation in this study. In our previous work, we had found that miR-125b levels were upregulated in plasma of focal segmental glomerulosclerosis (FSGS) patients relative to healthy controls [22]. Another study discovered that miR-125b levels in the kidney cortex were significantly upregulated in different types of kidney diseases by deep sequencing [30]. miR-125b was a multifaceted molecule, which can act as a cancer promoter or a cancer repressor depending on the cell context [31]. It plays crucial roles in various pathological and physiological processes like cell differentiation, proliferation, and apoptosis [32]. Zhang and colleagues [33] identified that miR-125b could promote apoptosis through regulating Mcl-1, Bcl-w, and IL-6R in hepatocellular carcinoma. Furthermore, it has been reported that miR-125b has the ability to regulate the mitochondria dynamic and function in monocyte [16]. Recently, growing evidence indicated that miRNA could play an important role in mitochondrial structure and function. It has been reported that mir-668 inhibited MTP18 to preserve mitochondrial dynamics and the viability of renal tubular cells in ischemic AKI [13]. miR-499 was proven to be able to prevent cardiomyocyte apoptosis by targeting DRP1 activation and consequent mi-
tochondrial division [34]. These reports illustrated that targeting mitochondrial damage has become a potential strategy for disease therapy.

Based on these clues, we speculated whether miR-125b was involved in cisplatin nephritis through mitochondria regulation. Our results showed that miR-125b levels were increased after cisplatin administration both in vitro and in vivo. Furthermore, inhibit miR-125b expression with miR-125b antagonir injection could alleviate the mitochondrial dysfunction as well as kidney injury. These results indicated that miR-125b plays a critical role in cisplatin-induced mitochondrial and renal damage. Considering its short sequence, miR-125b is convenient for administration and therefore has the potential to become an effective target for the AKI treatment.

Next, we further explore the mechanism of miR-125b-regulated mitochondrial and renal injury. Through the bioinformatics database searching, we found that miR-125b is predicted to directly target and regulate MFN1. MFN1 is a large GTPase that belongs to mitofusin protein family. Mitofusins are key components involved in the mitochondrial fusion, which include MFN1 and MFN2. Lots of evidence illustrated that MFN1 plays a critical role in mitochondrial morphology and cell injury. It has been shown that MFN1 is decreased in response to injury factors and then leads to the mitochondrial membrane permeabilization, which contributes to mitochondrial fission and apoptosis [18, 19, 35]. However, MFN2 has also been reported as a modulator of mitochondrial function through affecting its dynamics [36, 37]. It is noteworthy that MFN1 regulated mitochondrial docking and fusion more efficiently than MFN2, due to its higher GTPase activity [38]. Besides, MFN1 instead of MFN2 is required to regulate Opa1-driven mitochondrial fusion [39]. Considering that MFN1 instead of MFN2 has been predicted as a target of miR-125b, we performed luciferase reporter gene experiment and Western blotting, and confirmed that miR-125b could indeed suppress MFN1 levels. Combined with our previous observation that MFN1 levels were repressed in response to cisplatin treatment, miR-125b inhibition can alleviate cisplatin-induced AKI process. Our data indicated that miR-125b could regulate mitochondrial division and AKI pathological process via inhibiting MFN1 expressions. In this study, we demonstrated that during cisplatin-induced AKI, miR-125b is upregulated and directly inhibited MFN1 expressions, which attributed to excessive mitochondrial fission and resulted in tubular cell apoptosis. Furthermore, we illustrated the applicability of miR-125b as a therapeutic target in cisplatin nephritis.

Statement of Ethics

The experiments conducted in this study received the approval of the Animal Care and Use Committee of Jinling Hospital, Jiangsu, China (2016NZGKJ-043).

Conflict of Interest Statement

Zhihong Liu is the Editor-in-Chief of the journal Kidney Diseases. The other authors have no conflicts of interest to declare.

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Author Contributions

Y.Z. and Z.L. designed the research; Y.Z., Y.L., M.Z., S.L., and X.Z. performed the experiments; Y.Z. and Y.L. analyzed the data and drafted the manuscript; Z.L. reviewed the literature and revised the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article.

References

1. Chacinska A, Koehler CM, Milenkovic D, Lithgow T, Pfanner N. Importing mitochondrial proteins: machineries and mechanisms. Cell. 2009 Aug;138(4):628–44.
2. Brooks C, Wei Q, Cho SG, Dong Z. Regulation of mitochondrial dynamics in acute kidney injury in cell culture and rodent models. J Clin Invest. 2009 May;119(5):1275–85.
3. Scorrano L, Ashiya M, Buttle K, Weiler S, Oakes SA, Mannella CA, et al. A distinct pathway remodels mitochondrial cristae and mobilizes cytochrome c during apoptosis. Dev Cell. 2002 Jan;2(1):55–67.
4. Whitley BN, Engelhart EA, Hoppins S. Mitochondrial dynamics and their potential as a therapeutic target. Mitochondrion. 2019 Nov;49:269–83.
5. Emma F, Montini G, Parikh SM, Salviati L. Mitochondrial dysfunction in inherited renal disease and acute kidney injury. Nat Rev Nephrol. 2016 May;12(5):267–80.
6. Lan R, Geng H, Singha PK, Saikumar P, Bottinger EP, Weinberg JM, et al. Mitochondrial pathology and glycolytic shift during proximal tubule atrophy after ischemic AKI. J Am Soc Nephrol. 2016;27(11):3356–67.
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