MicroRNA-34a Is Induced via p53 during Cisplatin Nephrotoxicity and Contributes to Cell Survival

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MicroRNAs are small noncoding RNAs that are produced endogenously and have emerged as important regulators in pathophysiological conditions such as development and tumorigenesis. Very little is known about the regulation of microRNAs in renal diseases, including acute kidney injury (AKI). In this study, we examined the regulation of microRNA-34a (miR-34a) in experimental models of cisplatin-induced AKI and nephrotoxicity. By Northern blot and real-time polymerase chain reaction analyses, we detected an induction of miR-34a *in vitro* during cisplatin treatment of mouse proximal tubular cells and also *in vivo* during cisplatin nephrotoxicity in C57BL/6 mice. In cultured cells, miR-34a was induced within a few hours. In mice, miR-34a induction was detectable in renal tissues after 1 d of cisplatin treatment and increased to approximately four-fold of control at d 3. During cisplatin treatment, p53 was activated. Inhibition of p53 with pifithrin-α abrogated the induction of miR-34a during cisplatin treatment of proximal tubular cells. *In vivo*, miR-34a induction by cisplatin was abrogated in p53-deficient mice, a result that further confirms a role for p53 in miR-34a induction during cisplatin nephrotoxicity. Functionally, antagonism of miR-34a with specific antisense oligonucleotides increased cell death during cisplatin treatment. Collectively, the results suggest that miR-34a is induced via p53 during cisplatin nephrotoxicity and may play a cytoprotective role for cell survival.

INTRODUCTION

Cisplatin, one of the most potent chemotherapy drugs, is also a well-documented nephrotoxin that induces acute kidney injury (AKI) in cancer patients. In kidneys, cisplatin accumulates in renal tubular cells, resulting in cell injury and death, contributing to the development of AKI (see [1,2] for reviews). Research during the last few years has identified several important signaling pathways that lead to tubular-cell apoptosis during cisplatin nephrotoxicity, including mitogen-activated protein kinase, p53 and others (3–14).

p53 is activated early after cisplatin treatment of renal tubular cells *in vitro* and in animals *in vivo* (10,15–22). Pharmacological and genetic inhibition of p53 attenuates cisplatin-induced apoptosis in cultured tubular cells. An important finding was that cisplatin-induced nephrotoxicity is partially but significantly ameliorated in p53-deficient mice (22). Our recent work has further demonstrated a DNA damage response mediated by ATR-Chk2 in p53 activation and tubular cell apoptosis during cisplatin nephrotoxicity (19). After being activated, p53 can induce the expression of proapoptotic genes, such as *PUMA-α* and *PIDD*, to induce cell injury and death (18,20). On the other hand, p53 may also activate mechanisms for cell survival. For example, p21, a cell-cycle regulator with antiapoptotic activity, is induced during cisplatin nephrotoxicity via both p53-dependent and -independent mechanisms (23). In addition, p53 may contribute to the activation of autophagy, a cytoprotective mechanism for cell survival (24). As a result, the balance between the prodeath and prosurvival factors may determine if a cell can survive or be lethally injured during cisplatin nephrotoxicity.

MicroRNAs are small noncoding RNA molecules of 19–25 nucleotides, which regulate posttranscriptional gene regulation (25–28). MicroRNAs bind to the 3'-untranslated regions of target-gene mRNAs, resulting in stalling of protein synthesis or mRNA degradation. MicroRNAs have emerged as critical regulators of a variety of physiological as well as pathological conditions (25–28). In kidneys, specific microRNAs are highly expressed and play critical roles in signaling of transforming growth factor β during diabetic nephropathy (29–31). Moreover, depletion of microRNAs in podocytes by ablating Dicer, a key enzyme for microRNA production, results in a rapid progression

to end-stage kidney disease, an effect that further demonstrates the involvement of microRNAs in renal development and physiology (32–34). Despite these phenomenal findings, little is known about microRNA regulation in AKI, including injury from cisplatin nephrotoxicity.

In 2007, several studies identified *microRNA-34a* (*miR-34a*) as a key target gene of p53 that is activated during DNA damage, and study results demonstrated that miR-34a might contribute to the ensuing apoptosis (35–40). Because p53 is activated during cisplatin nephrotoxicity and contributes to renal cell injury and death, we investigated miR-34a expression and its role in relevant experimental models. We showed that miR-34a is induced during cisplatin treatment of renal proximal tubular cells *in vitro* and mice *in vivo* and that miR-34a induction under these experimental conditions is p53 dependent. Interestingly, we further found evidence for a protective role of miR-34a against cisplatin-induced apoptosis in renal cells.

MATERIALS AND METHODS

Cell and Special Reagents

The mouse proximal tubular cell (BUMPT-306) line was originally obtained from W Lieberthal and JH Shwartz at Boston University (41). The cells were cultured in DMEM with 10% fetal bovine serum and 0.6% glutamine. The antibodies used were from the following sources: anti-phospho p53 (Ser-15) antibodies from Cell Signaling Technology (Danvers, MA, USA), anti-p21 antibody from Santa Cruz Biotechnology (Santa Cruz, CA, USA), and anti–puma-α antibody from J Yu at the University of Pittsburgh. Cisplatin and pifithrin-α were purchased from Sigma (St. Louis, MO, USA). Locked-nucleic-acid (LNA) oligonucleotides were purchased from Exiqon, Vedbaek, Denmark.

Cisplatin Treatment of BUMPT Cells

Confluent BUMPT proximal tubular cells were treated with 40 μmol/L cis-

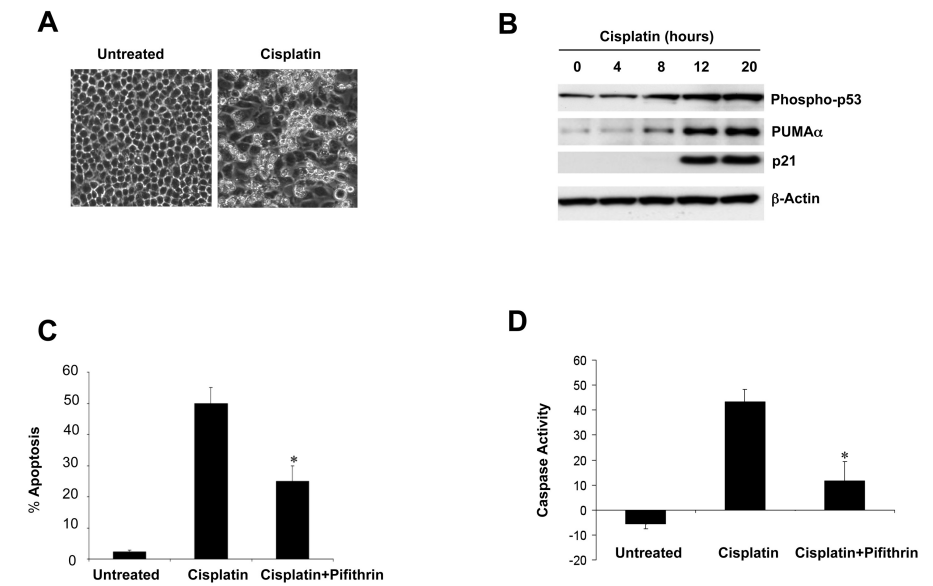**Figure 1.** Cisplatin-induced apoptosis in BUMPT cells. (A) BUMPT cells were untreated or treated with 40 μmol/L cisplatin for 20 h. Cell morphology was recorded by phase-contrast microscopy (10×). (B) BUMPT cells were treated with 40 μmol/L cisplatin for the indicated time. Whole-cell lysates were collected for immunoblot analysis of Phospho-p53 (Ser-15), Puma-α, p21, and β-actin (protein loading and transferring control). (C) BUMPT cells were untreated or treated with 40 μmol/L cisplatin for 24 h in the absence or presence of 20 μmol/L pifithrin-α. Percentage of apoptosis was determined by counting the cells with typical apoptotic morphology. (D) BUMPT cells were treated with 40 μmol/L cisplatin for 20 h in the absence or presence of 20 μmol/L pifithrin-α. Cell lysates were collected to measure caspase activity by an enzymatic assay as described in Methods. Data in C, D: mean ± SD, n = 3; *, significantly different from the cisplatin group.

platin. After incubation for indicated time periods, the cells were morphologically examined for apoptosis or lysed to collect lysate for caspase assay or immunoblot analysis.

Mouse Model of Cisplatin Nephrotoxicity

Wild-type and p53-deficient C57BL/6 mice (male, 8–10 wks old) were intraperitoneally injected with one dose (30 mg/kg body weight) of cisplatin as described previously (22,42). The control animals were injected with saline. To monitor renal injury, blood urea nitrogen levels were determined using the assay kit from Biotron Diagnostics (Hemet, CA, USA). Whole kidney tissues were collected for immunoblot analysis or RNA extraction. All animal experiments were performed according to a protocol approved by the institu-

tional animal care and use committee of the Charlie Norwood VA Medical Center.

Morphological Examination of Apoptosis and Measurement of Caspase Activity

Cells were stained with 10 μg/mL Hoechst 33342 to examine cellular and nuclear morphology by phase contrast and fluorescence microscopy as described previously (18,19). Typical apoptotic morphology was characterized by cellular shrinkage, formation of apoptotic bodies and nuclear condensation and fragmentation. Caspase activity was measured with an enzymatic assay by using the fluorogenic peptide DEVD.AFC, a substrate of executioner caspases including caspase-3, -6 and -7. Briefly, cells were lysed with 1% triton X-100 and the lysate was added to a

caspase reaction mixture containing 50 μmol/L DEVD.AFC for 1 h of incubation. The fluorescence signal of AFC liberated by caspase activity was measured at excitation (360 nm) and emission (530 nm). Caspase activity was then calculated by using a standard curve of free AFC.

Analysis of mir-34a by Real-Time Polymerase Chain Reaction

Total RNA was extracted using the mirVana kit (Ambion, Austin, TX, USA). For real-time polymerase chain reaction (PCR), 40 ng of total RNA was reverse-transcribed into cDNA by using the miRNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA). Real-time PCR was carried out using the Taqman miRNA assay kit (Applied Biosystems), which included sequence-specific primers for cDNA synthesis and Taqman probes for real-time PCR. Quantification was done using ΔCt values.

Northern Blot Analysis of miR-34a

For Northern blot analysis, total RNA was extracted by using the mirVana kit (Ambion) and run on 15% acrylamide-bisacrylamide (19:1) gel containing 7 mol/L urea in Tris-borate-EDTA buffer. The RNA was then transferred on to Hybond membrane (Amersham, Uppsala, Sweden) at 200 mA for 1 h, followed by ultraviolet crosslinking. The membrane was dried at 80°C for 1 h, followed by prehybridization for 1 h in Ultra-Hyb-Oligo hybridization buffer (Ambion). The radiolabeled probe (complementary of the mature microRNA sequence) was added to the prehybridization buffer and incubated overnight at 37°C and then washed in 2 × standard saline citrate (Ambion) buffer (0.1% sodium dodecyl sulfate [SDS]) and exposed to X-ray film at –70°C.

Transfection of LNA Oligonucleotides

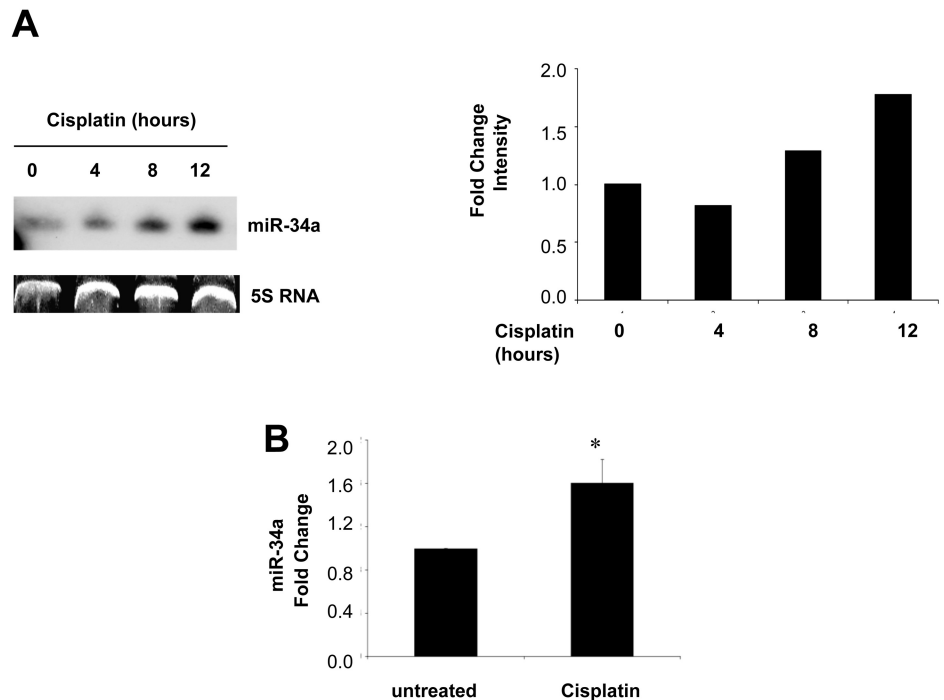BUMPT cells were plated at 50%–60% confluence. On the next day, the cells were transfected with 100 nmol/L of

A

B

Figure 2. MicroRNA-34a induction during cisplatin treatment of BUMPT cells. (A) BUMPT cells were treated with 40 μmol/L cisplatin for indicated time periods. Total RNA enriched in microRNAs was then isolated for Northern blot analysis using a radiolabeled probe against mature miR-34a. 5S RNA was shown as a sample loading control. Left panel, Northern blots; right panel, densitometry analysis. For densitometry, the miR-34a signal of each condition was normalized with its 5S-RNA signal and then compared with that of the control (0 cisplatin), which was arbitrarily set as one. (B) BUMPT cells were untreated or treated with 40 μmol/L cisplatin for 12 h. Total RNA enriched in microRNAs was then isolated for real-time PCR analysis by using specific primers for mature miR-34a. As an endogenous control, snoRNA-202 was also analyzed in all samples for normalization. Data: mean ± SD, n = 3; *, significantly different from the untreated group.

LNA oligonucleotides by use of Lipofectamine 2000 reagent. After an additional 1 d of culture, the cells were used for treatment.

Immunoblot Analysis

We followed a standard protocol of immunoblot analysis. Briefly, equal amounts of protein samples were loaded and resolved on SDS–polyacrylamide gel electrophoresis gels. The proteins were then transferred onto a polyvinylidene-difluoride membrane, which was blocked with 5% bovine serum albumin and incubated in primary and secondary antibodies. The antigens on the blots were revealed by use of the ECL chemiluminescence kit from Pierce (Rockford, IL, USA).

RESULTS

Cisplatin-Induced Apoptosis and p53 Activation in BUMPT Cells

In our previous studies we examined cisplatin-induced nephrotoxicity mainly using an immortalized rat kidney proximal tubular cell line and C57BL/6 mice (10,16,18,19,22,42). To facilitate microRNA research, we further characterized cisplatin-induced injury in BUMPT cells, a mouse kidney proximal tubular cell line established by Lieberthal, Schwartz and colleagues at Boston University (Sinha *et al.* [41]). Dose-response experiments suggested that 40 μmol/L cisplatin for 24 h induced about 50% apoptosis in BUMPT cells. These cells showed a typical morphology of apoptosis, including a

shrunken configuration and formation of apoptotic bodies (Figure 1A). The cells also had condensed and fragmented nuclei as revealed by Hoechest33342 staining (not shown). To detect p53 activation during cisplatin treatment of BUMPT cells, we collected whole-cell lysate at different time points for immunoblot analysis. As shown in Figure 1B, cisplatin induced p53 (ser-15) phosphorylation in a time-dependent manner. Consistently, cisplatin induced the expression of PUMA-α and p21, two transcriptional targets of p53, suggesting a robust p53 activation response during cisplatin incubation of BUMPT cells (Figure 1B). Functionally, inhibition of p53 by pifithrin-α partially but significantly ameliorated cisplatin-induced apoptosis in BUMPT cells. The apoptosis was suppressed by pifithrin-α from 50% to about 25% (Figure 1C). Pifithrin-α also attenuated cisplatin-induced caspase activation in BUMPT cells (Figure 1D). Together, these analyses further confirmed a role for p53 in cisplatin-induced apoptosis in BUMPT cells.

Induction of miR-34a during Cisplatin Treatment of BUMPT Cells

Little is known about microRNA regulation in AKI, including injury from cisplatin nephrotoxicity. Recent studies have demonstrated p53-dependent upregulation of microRNAs in the miR-34 family (35–39). Because p53 is activated during cisplatin treatment of renal cells and tissues, we reasoned that miR-34 might be induced under the conditions of cisplatin nephrotoxicity. To test this possibility, we initially examined miR-34a expression during cisplatin treatment of BUMPT cells. BUMPT cells were incubated with 40 μmol/L cisplatin for 0–12 h to collect microRNA-enriched RNA using the mirVana kit from Ambion. The samples were initially analyzed by Northern blot analysis using a radiolabeled miR-34a–specific probe. As shown in Figure 2A, miR-34a was indeed upregulated during cisplatin treatment of BUMPT cells. The miR-34a induction was cisplatin-treatment time dependent,
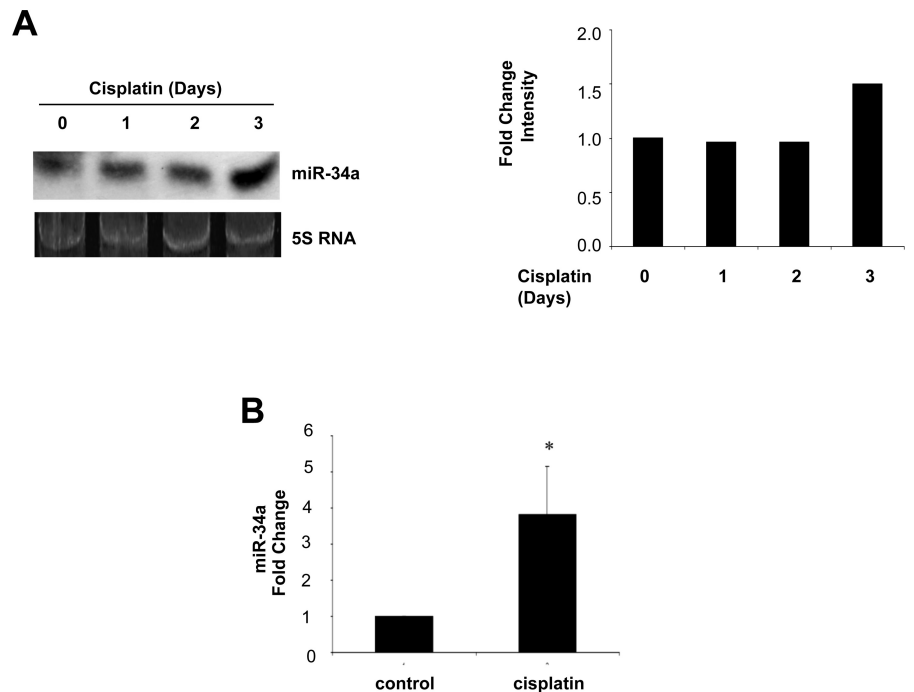
Figure 3. MicroRNA-34a induction during cisplatin treatment of C57BL/6 mice. Male C57BL/6 mice (age 8–10 wks) were injected with 30 mg/kg body weight cisplatin. Kidneys were collected at d 1, 2 and 3 after cisplatin treatment for the isolation of total RNA. (A) Northen blot analysis of miR-34a expression in renal tissues. Total RNA (10 μg) from renal tissues was subjected to Northern blot analysis using a specific miR-34a probe. 5S RNA was recorded as a sample loading control. Left panel, Northern blots; right panel, densitometry analysis. For densitometry, the miR-34a signal of each condition was normalized with its 5S-RNA signal and then compared with that of the control (0 cisplatin), which was arbitrarily set as one. (B) Real-time PCR analysis of miR-34a. Total RNA was isolated from sham control mice and mice with 3 d of cisplatin treatment for real-time PCR using miR-34a–specific primers. snoRNA-202 was also analyzed in all samples as an endogenous control for normalization. Data: mean ± SD, n = 3; *, significantly different from the control group.

becoming noticeable at 8 h and increasing thereafter to a marked level at 12 h. The results were verified by densitometry analysis of the blots (Figure 2A, right panel). To have a more quantitative measurement, we performed real-time PCR analysis. As shown in Figure 2B, the expression of miR-34a increased about 1.6-fold after 12 h of cisplatin treatment.

miR-34a Induction during Cisplatin Nephrotoxicity *In Vivo*

To examine miR-34a expression during cisplatin nephrotoxicity *in vivo*, we used a mouse model of cisplatin nephrotoxicity. In this model, a single injection of 30 mg/kg cisplatin induced AKI in C57BL/6 mice within 2–3 d (22,42,43). In

our experiments, blood urea nitrogen was increased from the control level of 45 mg/mL to 194 mg/dL at d 3 of cisplatin treatment. We collected renal tissues on d 0, 1, 2 and 3 after cisplatin injection. MicroRNA-enriched RNA samples were then isolated from the kidney tissues for Northern blot and real-time PCR analyses. Northern blot analysis showed that miR-34a was induced in renal tissues following cisplatin treatment, starting from d 1 and becoming evident at d 3 (Figure 3A). In real-time PCR analysis, miR-34a expression was increased to about four-fold of control at d 3 of cisplatin treatment (Figure 3B). Together, the results demonstrated miR-34a induction under *in vitro* and *in vivo* ex-

perimental conditions of cisplatin nephrotoxicity.

p53-Dependence of miR-34a Induction during Cisplatin Nephrotoxicity

On the basis of recent findings (35–39), we hypothesized that miR-34a induction during cisplatin nephrotoxicity was mediated by p53. To test this possibility, we initially examined the effects of pifithrin-α on cisplatin-induced miR-34a expression in BUMPT cells. BUMPT cells were treated for 12 h with cisplatin in the presence or absence of 20 μmol/L pifithrin-α. Total RNA samples were collected for real-time PCR analysis. As shown in Figure 4A, cisplatin induced miR-34a to approximately 1.6-fold of control, which was suppressed to the control level by pifithrin-α. We confirmed that pifithrin-α could suppress cisplatin-induced p53 phosphorylation or activation in BUMPT cells (Figure 4B, lane 6 versus lane 4). To further establish the role of p53 in cisplatin-induced miR-34a expression, we used p53-deficient mice. Wild-type and p53-deficient mice were injected with 30 mg/kg cisplatin and renal tissues were collected at d 0 and d 3. The tissues were then extracted for RNAs for real-time PCR analysis of miR-34a. As shown in Figure 4C, miR-34a expression in wild-type mice increased to 2.8-fold of controls after cisplatin treatment. In sharp contrast, the expression of miR-34a in p53-deficient mice was about 0.7-fold of wild-type control tissues (Figure 4C). These results suggest that miR-34a induction during cisplatin nephrotoxicity is largely p53 dependent.

Blockade of miR-34a Increases Cell Death during Cisplatin Treatment of BUMPT Cells

The rapid induction of miR-34a shown in our experimental models of cisplatin nephrotoxicity suggested that this microRNA might participate in renal cell injury and death. To test this possibility, we used a LNA-modified antisense oligonucleotide of miR-34a (anti–miR-34a). As a control, an LNA-modified oligonucleotide
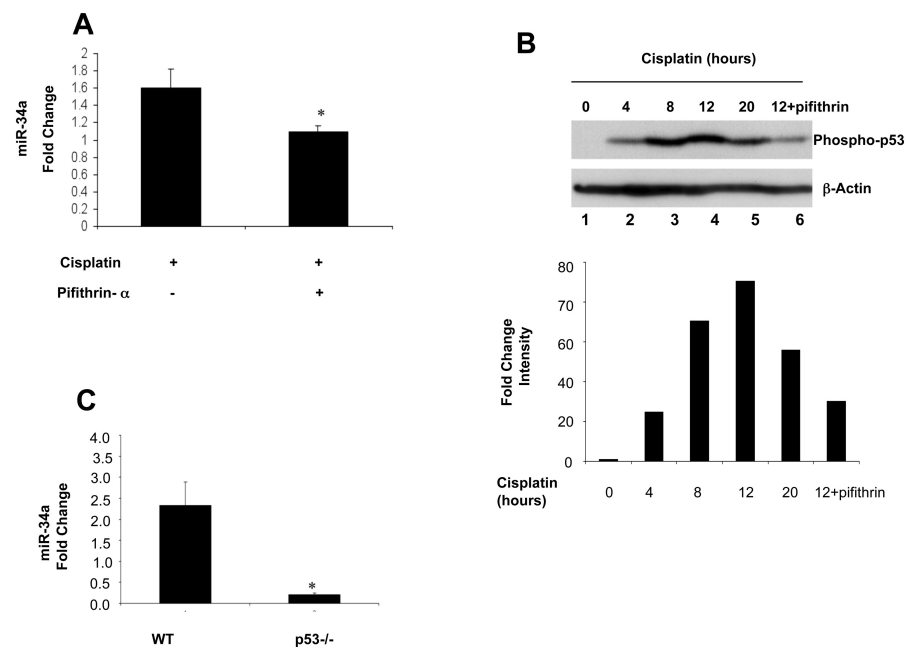

Figure 4. MicroRNA-34a induction during cisplatin nephrotoxicity is p53 dependent. (A) Effects of pifithrin-α on cisplatin-induced miR-34a expression in BUMPT cells. BUMPT cells were treated with 40 μmol/L cisplatin for 12 h in the presence or absence of 20 μmol/L pifithrin-α. Total RNA was isolated for real-time PCR analysis of miR-34a using specific primers. (B) Effects of pifithrin-α on cisplatin-induced p53 activation. BUMPT cells were treated with 40 μmol/L cisplatin for 0–20 h in the presence or absence of 20 μmol/L pifithrin-α. Whole cell lysates were collected for immunoblot analysis of phospho-p53 (ser-15) and β-actin. Upper panel, immunoblots; lower panel, densitometry analysis. For densitometry, the phosph-p53 signal of each condition was normalized with its β-actin signal and then compared with that of the control (0 cisplatin), which was arbitrarily set as one. (C) miR-34a induction during cisplatin treatment of wild-type and p53-deficient mice. Male wild-type and p53-deficient mice (age 8–10 wks) were injected with 30 mg/kg body weight cisplatin. Kidneys were collected at d 3 of cisplatin injection to isolate total RNA for real-time PCR analysis of miR-34a. (A,C) The miR-34a signals were normalized with that of control samples, which were arbitrarily set as one. Data are expressed as mean ± SD, n = 3; *, significantly different from the cisplatin and wild-type groups.

of scrambled sequence was used (scrambled). We first verified the effects of anti-miR-34a on the expression of miR-34a during cisplatin treatment. As shown in Figure 5A, cisplatin induced miR-34a in scrambled sequence-transfected cells, but not in anti–miR-34a–transfected cells. Interestingly, transfection of anti-miR-34a increased apoptosis during cisplatin treatment of BUMPT cells. Morphological evaluation revealed that cisplatin induced 43% apoptosis in scrambled sequence-transfected cells, but 53% in anti–miR-34a–transfected cells (Figure 5B). Consistently, caspase activation during cisplatin treatment was also higher in

anti–miR-34a–transfected cells (Figure 5C). We further determined the effects of anti–miR-34a on long-term survival of BUMPT cells following cisplatin treatment. To this end, the cells were incubated with cisplatin for 24 h, followed by recovery in fresh medium for another 24 h. Whole-cell lysate was then collected for protein determination. As shown in Figure 5D, significantly lower amounts of proteins were recovered in the anti–miR-34a–transfected cells than the scrambled sequence-transfected cells (Figure 5D), suggesting that fewer cells survived cisplatin injury in the anti–miR-34a–transfected group. Thus, blockade of
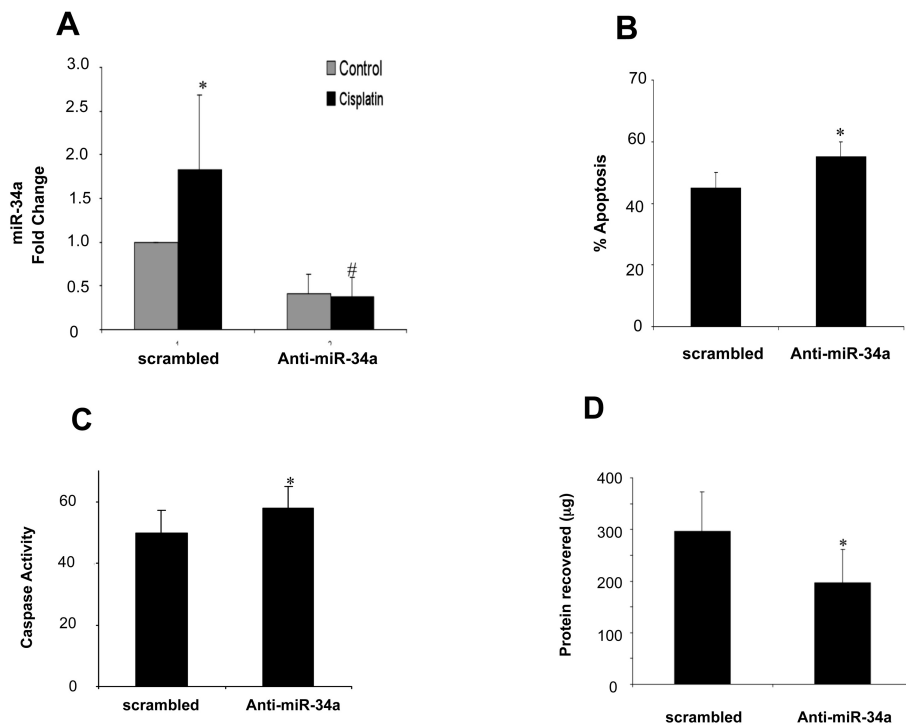
A

B

C

D

Figure 5. Blockade of miR-34a increased cisplatin-induced apoptosis in BUMPT cells. BUMPT cells were transfected with 100 nmol/L LNA-modified antisense oligonucleotide of miR-34a (anti–miR-34a) or LNA-modified oligonucleotide of scrambled sequence (scrambled). The cells were then left untreated or treated for 20 h with 40 μmol/L cisplatin. (A) Effects of anti–miR-34a on miR-34a induction during cisplatin treatment. Total RNA was isolated for real-time PCR analysis of miR-34a using specific primers. (B) Effects of anti–miR-34a on cisplatin-induced apoptosis. Percentage of apoptotic cells was determined by morphologi-cal methods. (C) Effects of anti–miR-34a on cisplatin-induced caspase activation. Cell lysate was collected for an enzymatic assay of caspase activity. (D) Effects of anti–miR-34a on cell survival following cisplatin treatment. After cisplatin treatment for 24 h, the cells were incubated in fresh medium for 24 h of recovery. Whole cell lysate was then collected to determine the recovered protein to indicate cell survival. Data: mean ± SD, n = 3; *, sig-nificantly different from control and scrambled groups.

miR-34a increased BUMPT cell injury and death during cisplatin treatment, suggesting that miR-34a may play a cyto-protective role under these experimental conditions.

DISCUSSION

MicroRNAs have emerged as impor-tant regulators of various pathophysio-logical conditions (25–28). By repressing gene expression, microRNAs regulate processes of cellular activity and homeo-stasis, including growth, proliferation, survival and death (25–28). However, very little is known about the role and regulation of microRNAs in the develop-ment of renal diseases (29,44). The results of this study, in which we used *in vitro* cell culture as well as *in vivo* mouse mod-els, demonstrated early miR-34a induc-tion during cisplatin-induced nephrotox-icity. The induction of miR-34a was shown to be p53 dependent. Interest-ingly, our results also indicate that miR-34a induced during cisplatin nephrotoxi-city may be a cytoprotective mechanism for cell survival.

A robust induction of miR-34a has been reported recently in response to DNA damage or genotoxic stress (35–39). Importantly, the results of these studies indicated that miR-34a is downstream of p53 and plays an important role in p53-mediated apoptosis and cell-cycle arrest. In experimental models of cisplatin nephrotoxicity, our recent work has re-vealed a rapid DNA damage response, which is centered on ATR and Chk2 (19). The activation of ATR and Chk2 leads to the phosphorylation and activation of p53, which induces the expression of genes that promote cell death by apopto-sis (19). Such genes include *PUMA-α* and *PIDD* (18,20). Another interesting finding was that p53 might also induce the ex-pression of genes that promote cell sur-vival. For example, p21, a cell-cycle regu-lator with demonstrated antiapoptotic activity, is induced during cisplatin nephrotoxicity via both p53-dependent and -independent mechanisms (23). Our current results have demonstrated miR-34a induction during cisplatin nephro-toxicity. Notably, cisplatin induced miR-34a in both cultured proximal tubular (BUMPT) cells and renal tissues in C57BL/6 mice (see Figures 2 and 3). To our knowledge, this evidence is among the first reported for microRNA regula-tion in an experimental model of AKI. In this study, we observed miR-34a induc-tion during cisplatin injury, and it was not clear whether and when miR-34a ex-pression would return to the basal level. However, it is likely that the microRNA expression profile during renal recovery or regeneration is different from that of the injury phase.

We have further provided evidence that miR-34a induction by cisplatin is mediated by p53. We showed that in BUMPT cells, miR-34a induction by cisplatin was attenuated by pifithrin-α, a pharmacological inhibitor of p53 (Fig-ure 4A). Furthermore, miR-34a induction during cisplatin nephrotoxicity was ab-rogated in p53-deficient mice (Figure 4C). In the *in vivo* model, 30 mg/kg cisplatin was used to induce kidney injury in C57/BL6 mice. This dose was also used by this and other laboratories in previ-ous studies (22,42,43,45). It is notewor-thy that cisplatin at 30 mg/kg induced apoptosis mainly in proximal and distal tubules in kidneys, and not in collecting

tubules or glomeruli; some necrosis was induced and mainly localized in proximal tubules (42). Thus cisplatin at this dose does not induce widespread tissue damage, and the model is suitable for the study of cisplatin nephrotoxicity. These observations support the conclusion that *miR-34a* is a transcriptional target gene of p53. In fact, the promoter region of the *miR-34a* gene contains p53-binding sites (38). Thus, data suggest that binding of p53 to these sites may activate the transcription of miR-34a during cisplatin-induced injury in renal cells and tissues.

When *miR-34a* was first identified as a target gene of p53, it was shown to mediate the antiproliferative and proapoptotic effects of p53 (35–39). For example, Raver-Shapira *et al*. showed that antagonism of miR-34a could block etoposide-induced apoptosis in U2OS cells (37). In human colon cancer cells, Tazawa *et al*. showed that expression of miR-34a led to complete suppression of cell proliferation and induced senescence-like phenotypes (39). However, our current results do not support a proapoptotic role for miR-34a in cisplatin-induced injury in BUMPT cells. We showed that blockade of miR-34a with LNA-modified antisense oligonucleotides increased apoptosis during cisplatin treatment of BUMPT cells (Figures 5B, C). In addition, blockade of miR-34a decreased long-term survival of the cells following cisplatin treatment (Figure 5D). Of note, in this experiment we measured the amount of total cell proteins recovered, which may not be an accurate indication of cell survival. However, the results were supported measurement results for cell death or apoptosis (Figure 5B, C). Together, the results suggest that miR-34a induction during cisplatin treatment of BUMPT cells is not proapoptotic, but instead is cytoprotective. This observation was unexpected. However, a very recent study by Kato *et al*. demonstrated in *Caenorhabditis elegans* that loss-of-function mutation of *miR-34a* sensitized somatic cells to radiation injury, suggesting a cytoprotective role for miR-34a in these

cells (46). In addition, in mammalian cell lines with different levels of miR-34a, there seemed to be an inverse correlation between miR-34a expression and cellular radiosensitivity, that is, cells with low miR-34a were more sensitive to irradiation-induced injury and death (46). Together, these results and those from our current study support a cytoprotective role for miR-34a under certain experimental conditions. It is currently unclear why miR-34a has been found to be proapoptotic in some studies, but anti-apoptotic in others. We speculate that miR-34a has multiple target genes, and the role played by miR-34a depends on which genes are under its regulation or suppression under various experimental conditions.

MicroRNAs regulate diverse cellular processes by repressing target gene expression. However, target identification is one of the main challenges in understanding the functional role of specific microRNAs. A microRNA can have multiple targets and the target genes of a microRNA can change under different experimental conditions. Several target genes have been suggested for miR-34a, but most of the targets are related to cell-cycle regulation. For example, cyclin E, CDK4, CDK6 and CDC25C were shown to be repressed by miR-34a, resulting in cell-cycle arrest at G1 and G2 phases (35–39). In terms of the regulation of cell death, Bommer *et al*. showed that Bcl-2 is a direct target of miR-34a (40). However, regulation of *Bcl-2*, a well-established antiapoptotic gene, cannot explain the cytoprotective role of miR-34a observed in our study, because repression of Bcl-2 should sensitize the cells to cisplatin injury. In our study, expression of either miR-34a or anti–miR-34a did not change the expression of Bcl-2 in BUMPT cells (data not shown), ruling out *Bcl-2* as a target gene of miR-34a in these cells. We speculate that miR-34a induced during cisplatin nephrotoxicity may regulate or repress a proapoptotic gene(s), leading to death resistance and cell survival. Future studies should identify such gene(s) for a

better understanding of the cytoprotective role of miR-34a.

In conclusion, this study has demonstrated the first evidence for microRNA regulation in an experimental model of AKI. miR-34a is induced via p53 during cisplatin nephrotoxicity and may play a cytoprotective role for cell survival.

DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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