MicroRNA-30a Sensitizes Tumor Cells to cis-Platinum via Suppressing Beclin 1-mediated Autophagy

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Autophagy is activated in cancer cells during chemotherapy and often contributes to tumor chemotherapy resistance. In this study, we characterized the role of microRNA-30a (miR-30a) in the coordination of cancer cell apoptosis and autophagy, which determines the sensitivity of cancer cells to chemotherapy. First, the autophagy activity in cancer cells increased after cis-dichloro-diamine platinum (cis-DDP) or Taxol treatment, as indicated by the elevated expression of beclin 1, a key regulator of autophagy, and increased number of LC3-positive autophagosomes. Second, miRNA screening using a TaqMan probe-based quantitative RT-PCR assay identified that miR-30a, a miRNA that targets beclin 1, was significantly reduced in tumor cells by cis-DDP treatment. Forced expression of miR-30a significantly reduced beclin 1 and the autophagy activity of tumor cells induced by cis-DDP. Third, the blockade of tumor cell autophagy activity by miR-30a expression or 3-methyladenine significantly increased tumor cell apoptosis induced by cis-DDP treatment. Finally, an in vivo tumor implantation mouse model clearly showed that elevation of miR-30a in implanted tumor cells by administration of the recombinant lentivirus expressing miR-30a strongly enhanced cis-DDP-induced autophagy of tumor cells. In conclusion, our results demonstrate for the first time that miR-30a can sensitize tumor cells to cis-DDP via reducing autophagy.

Conclusion: miR-30a can sensitize tumor cells to cis-DDP via reducing autophagy.

Significance: We identify a novel approach to improve chemotherapy efficiency.

Cancer is a class of disease characterized by harmful and excessive cellular growth that interferes with the digestive, nervous, and circulatory systems. Cancer is difficult to cure, especially during the later stages of the disease. Although chemotherapy has become more effective in recent years, resistance to anticancer drugs is common in cancer (1). Many types of tumors can develop the ability to resist conventional antineoplastic medicine (2), which is partially attributed to the insensitivity to cell death induced by cytotoxic agents. The avoidance of apoptosis is one of the hallmarks of cancer cells, and the failure of anticancer treatments to induce apoptosis contributes to chemotherapeutic failure and tumor progression. Autophagy, a conserved catabolic process by which cells digest their own organelles (3), is a homeostatic mechanism that regulates the turnover of long-lived or damaged proteins and organelles and buffers the metabolic stress induced under starvation conditions by recycling intracellular constituents (4). Autophagosomes that engulf organelles or damaged cellular components then fuse with lysosomes and mature into autolysosomes. The amino acids and fatty acids generated by autophagic degradation are reused by the tricarboxylic acid cycle to produce ATP. Thus, autophagy may contribute to sustainable cell survival. Although the antitumorigenic roles of autophagy have also been reported (3), accumulating evidence strongly suggests that autophagy is present and activated in cancer cells under certain stress conditions such as starvation, hypoxia, oxidative damage, or chemotherapy treatment and often contributes to tumor chemotherapy resistance and cancer cell survival in response to various stresses (5–10).

MicroRNAs (miRNAs) are a class of noncoding RNAs consisting of processed products approximately 22 nucleotides in length and have recently emerged as a new class of modulators of gene expression at the posttranscriptional level (11–13). miRNAs guide the binding of the RNA-induced silencing complex to complementary sequences in the 3’ untranslated region of target mRNA molecules, resulting in either mRNA degradation or translational inhibition. Although the target genes of

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4 The abbreviations used are: miRNA, microRNA; 3-MA, 3-methyladenine; NC, negative control; qRT-PCR, quantitative RT-PCR; LV, lentivirus.
most miRNAs have yet to be elucidated, miRNAs have been implicated in all aspects of cellular processes, including differentiation, proliferation, maturation, and apoptosis. In various types of cancers, many miRNAs are expressed differentially (14) and can serve as either oncogenes or tumor suppressors (15–18). Recently, miRNAs have been linked to cell chemosensitivity in different cancer cells. For example, miR-34a directly affects cell chemosensitivity in Ewing’s sarcoma (19) and breast cancer cells (20). miR-21 plays a role in chemosensitivity in bladder (21) and pancreatic cancer (22), and the expression of miR-140 is associated with chemosensitivity in osteosarcoma tumor xenografts (23). Furthermore, there is increasing evidence that miRNAs play a role in regulating cellular autophagy processes (24–27). Zhu et al. (25) reported that beclin 1, a key autophagy-promoting gene, is a potential target for miR-30a and that miR-30a can negatively regulate beclin 1 expression, resulting in decreased autophagic activity. Brest et al. (26) showed that an miRNA-based alteration in the IRGM gene (encoding a member of the p47 immunity-related GTPase family) regulation can affect the efficacy of autophagy. By studying autophagy induced by ischemia reperfusion, Xiao et al. (27) also identified a critical role of miR-204 in regulating autophagy through the LC3-II protein during ischemia reperfusion. However, the role of miRNAs in autophagy-mediated cancer cell chemotherapy resistance remains largely unknown.

In this study, we utilized cis-dichloride diamine platinum-treated (28, 29) mice and cells as animal and cellular models, respectively, to characterize the chemotherapy-treatment induced reduction of miR-30a in cancer cells. We determined a role for miR-30a in the suppression of beclin 1-mediated autophagy in cancer cells and sensitization of cancer cells to chemotherapy treatment.

**EXPERIMENTAL PROCEDURES**

**Reagents**—The cis-dichloro-diamine platinum, 3-methyladine (3-MA) and paclitaxel (taxol) were purchased from Sigma-Aldrich (St. Louis, MO). All synthetic RNA molecules, including pre-miR-30a molecular, anti-miR-30a antisense oligonucleotide, and scrambled negative control oligonucleotides (miR-NC, pre-nRNA, and anti-nRNA) were purchased from Invitrogen. The antibodies (20) against LC3, beclin 1, and GAPDH were obtained from Santa Cruz Biotechnology (Santa Cruz, California).

**Cell-based Experiments**—Human cancer cells, including HeLa, MCF-7, HepG2, and mouse liver cancer HepS cells were obtained from the China Cell Culture Center (Shanghai, China). Cis-DDP-resistant cancer cells (SGC-7091) and control cells (SGC-7092) were purchased from KenGen biotech (Nanjing, China). Cancer cells were cultured in 6-well plates (35 mm²/well) in DMEM supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 mg/ml streptomycin. To mimic the chemotherapy procedure to tumors, cancer cells were treated with cis-DDP (30 μg/ml) or taxol (0.5 μg/ml) for 6 h. The cells were then harvested for autophagosome measurement, Western blot analysis, and qRT-PCR assay. Cellular transfection with pre-miR-30a or pre-miR-NC was carried out using Lipofectamine 2000 reagent (Invitrogen) under conditions optimized for 80 pmol of RNA per well. A total of 1.0 × 10⁵ cells was seeded into each well for transfection. In some cellular experiments, 10 nm 3-MA was added during cis-DDP treatment to inhibit the autophagy.

**Immunocytochemistry Staining**—Cells were fixed with 4% paraformaldehyde for 10 min on ice, washed, and permeabilized with PBS containing 0.02% Triton X-100, followed by blocking with 2% bovine serum albumin in PBS. Primary LC3 antibodies (Novus Biologicals, Oakville, Ontario, Canada) were applied to the cells at a concentration of 50 μg/ml and incubated overnight at 4 °C. After extensive washes, FITC-conjugated secondary antibodies (Invitrogen) were applied at a concentration of 10 μg/ml and incubated at room temperature for 2 h. Cancer cells treated using a similar procedure in the absence of primary antibody served as a negative control. The images were acquired with a charge-coupled device (CCD)-equipped photomicroscope (Olympus, IX71, Tokyo, Japan). The number of LC3-positive punctures in the cells was counted and analyzed. The number of punctures in every 10 randomly selected cells was used to quantify the autophagosome levels.

**Real-time qRT-PCR**—Total RNA was extracted from cell and tissue samples using TRIzol (Invitrogen) according to the manufacturer’s protocol. The RNA concentrations were determined using a biophotometer (Eppendorf), and reverse transcription was performed using Avian Myeloblastosis Virus (AMV) reverse transcriptase (TaKaRa) with 1 μg of starting RNA per sample. The TaqMan miRNA probe-based qRT-PCR assay was performed as described previously (30). Real-time PCR was performed using a TaqMan PCR kit on an Applied Biosystems 7500 sequence detection system. All reactions, including the no template controls, were run in triplicate. After the reactions, the Ct values were determined using fixed-threshold settings. To calculate the absolute expression levels of miR-30, a series of concentrations of synthetic miR-30a were reverse-transcribed, amplified, and calculated to serve as the standard curve.

**Recombinant Lentivirus-expressing miR-30a (LV-miR-30a)**—The recombinant adenovirus-expressing miR-30 was generated by PCR and obtained from GenePharma (Shanghai, China). miRNA precursor sequences were amplified using the following primers: the forward primer for miR-30a, 5-GACG-GTACCTGGTGGAAGAACAACTTCG-3, and the reverse primer for miR-30a, 5-CAGAAGCTTCCATCAAACCTTCAAC-3. The fragments were cloned into a shuttle vector that included enhanced GFP (eGFP) driven by the ubiquitously expressed elongation factor 1 α (EF1α) promoter (31). Lentivirus-eGFP was used as the control construct. The lentivirus titer in plaque-forming units was determined by a plaque formation assay following infection of HEK293 cells. The titers of the purified virus used for mouse experiments were 1.0 × 1.0 × 10¹¹/ml, and all virus preparations were stored at −80 °C until use.

**Apoptosis Assay**—Cell apoptosis was determined using an annexin V-FITC apoptosis detection kit (BD Biosciences). Briefly, 2.0 × 10⁶ cells were resuspended in 0.5 ml of binding buffer and incubated with annexin V-FITC and propidium iodide for 10 min in the dark at room temperature. A FACScan flow cytometer (BD Biosciences) equipped with a FITC signal detector FL1 (excitation 488 nm, green) and a phycoerythrin emission signal detector FL3 (excitation 585 nm, red) was used.
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to analyze cellular apoptosis (32, 33). The results were calculated using the CellQuest™ Pro software (BD Biosciences) and expressed as the percentage of apoptotic cells from the total cells.

**Western Blot Analysis**—Cancer cells were lysed in radioimmunoprecipitation assay buffer, and the protein concentration was determined using a BCA kit (Thermo Fisher Scientific, Billerica, MA). Equal amounts (60 μg) of cell lysates were resolved by SDS-PAGE and transferred to PVDF membranes (Amer sham Biosciences). The PVDF membranes were incubated in 5% nonfat milk dissolved in TBS containing 0.1% Tween 20 for 1 h and then immunoblotted with antibodies against LC3 or beclin 1. The bound antibodies were detected by enzyme-linked chemiluminescence (Pierce). Quantification of protein bands was performed using ImageJ software. Equal protein loading was monitored by blotting the same samples with an antibody against GAPDH.

**Animal Experiments**—Animal maintenance and experimental procedures were carried out in accordance with the National Institutes of Health Guidelines for Use of Experimental Animals and approved by the Medicine Animal Care Committee of Nanjing University (Nanjing, China). Approximately 10⁶ HepS cancer cells were subcutaneously transplanted into 6-week-old BALB/c mice, as described previously (31), with a minor modification. Two weeks later, the mice that carried solid tumors (~6 mm in size) were divided into six groups with six to eight mice in each group. The mouse groups were administered various reagents every other day by tail vein injection. Group 1 (control) received 200 μl of saline alone. Group 2 received 200 μl of saline containing 300 μg/ml cis-DDP. Groups 3 and 4 received 200 μl of saline containing 300 μg/ml cis-DDP and ~10¹¹ titer/ml lentivirus-expressing miR-30a (LV-miR-30a) (group 3) or miR-NC (LV-miR-NC) (group 4). Groups 5 and 6 received ~10¹¹ titer/ml LV-miR-30a or LV-miR-NC, respectively. Only all mice were sacrificed 2 weeks later, and the tumors were dissected out. After measuring the sizes, the tumors were processed and submitted to Western blotting and qRT-PCR analysis. In a separate set of experiments, tumor implanted mice were treated with or without 20, 30, and 60 μg/ml cis-DDP in the presence or absence of LV-miR-30a or LV-miR-NC every other day. After 10 days, the mice were sacrificed, and the tumors sizes were measured. The animal experiment was repeated three times.

**Statistical Analysis**—The qRT-PCR assays were performed in triplicate, and each experiment was repeated three times. The data were presented as the means ± S.E. of three or more independent experiments, and the differences were considered statistically significant at p < 0.05 by Student’s t test.

**RESULTS**

cis-DDP or Taxol Treatment Increases Autophagy Activity but Decreases miR-30a Level in Cancer Cells—As an anticancer reagent, cis-DDP has been widely used in clinical chemotherapy of tumor patients. In this study, we treated tumor cells with 30 μg/ml cis-DDP. As shown in Fig. 1, A and B, and supplemental Fig. 1, the autophagy activity of three different cancer cells, indicated by anti-LC3 antibody-labeled autophagosomes (Fig. 1A and supplemental Fig. 1, arrowheads) was significantly enhanced by cis-DDP treatment. There was about a 5-fold increase in the number of autophagosomes in the cis-DDP-treated HeLa, MCF-7, and HepG2 cancer cells compared with non-treated cells (Fig. 1B). Autophagosomes were positively labeled with an antibody against microtubule-associated protein-1 light chain-3 (LC3), a protein related to autophagosome formation. Western blot analysis confirmed that there was a higher level of LC3-II in cis-DDP-treated cells compared with non-treated cells (Fig. 1C). The ratio of LC3-II versus LC3-I was increased from 0.3–0.5 range in the non-treated cells to 1.8–3.1 in the cis-DDP-treated cells (Fig. 1D). The autophagy-related protein beclin 1 (34) was also increased greatly after cis-DDP treatment (Fig. 1, C and E). The ratio of beclin 1 to GAPDH in HeLa, MCF-7, and HepG2 cells was increased about 3.0-, 2.0- and 2.5-fold in cis-DDP-treated cells compared with non-treated control cells, respectively.

Using a TaqMan probe-based qRT-PCR assay, we determined the expression levels of a panel of miRNAs that have been reported to be associated with cellular autophagy or apoptosis, particularly miR-30a (25). As shown in Fig. 1F, among the 22 miRNAs tested, miR-30a had the largest reduction in HeLa cells following cis-DPP treatment. In addition, a similar reduction of miR-30a was observed in MCF-7 and HepG2 cells after cis-DPP treatment (supplemental Fig. 2). Because miR-30a is able to target beclin 1 (25), our results suggest that the elevation of beclin 1 in cis-DDP-treated cancer cells might be due to the down-regulation of miR-30a by cis-DDP treatment.

To show that activation of cancer cell autophagy and reduction of miR-30a is not limited to cis-DDP treatment, we employed taxol (35), another widely used chemotherapeutic agent, to treat three cancer cell lines. As shown in Fig. 2 and supplemental Fig. 3, taxol treatment strongly increased the autophagosome number (Fig. 2A and supplemental 3A), the ratio of LC3-II/LC3-I (Fig. 2C, supplemental Fig. 3B) and the level of beclin 1 (Fig. 2D) but reduced the level of miR-30a (Fig. 2B) in HeLa, MCF-7, and HepG2 cells. Taken together, these results demonstrate that chemotherapy of tumor cells by cis-DDP or taxol will increase cancer cell autophagy activity but decrease miR-30a level in cancer cells.

**Forced Expression of miR-30a Enhances cis-DDP-induced Cancer Cell Apoptosis via Repressing Beclin 1-mediated Cancer Cell Autophagy**—To further test the role of miR-30a in inducing beclin 1-mediated autophagy of cis-DDP-treated HeLa cells, we transfected cells with pre-miR-30a or a random control oligonucleotide (pre-miR-NC) prior to cis-DDP treatment. As shown in supplemental Fig. 4, transfection with pre-miR-30a increased the miR-30a level ~30-fold, and elevation of miR-30a reduced beclin 1 expression. Forced expression of miR-30a in HeLa cells also strongly depressed the cis-DDP-induced autophagy (Fig. 3, A and B). Western blot analysis further showed that the expression of the ratio of LC3-II/LC3-I (Fig. 3, C and D) and autophagy-related beclin 1 (Fig. 3, C and E) both decreased. Employing FITC-conjugated annexin V to label cell surface inverted phosphatidylserine on apoptotic cells, we found that forced expression of miR-30a in HeLa cells significantly enhanced cellular apoptosis induced by cis-DDP treatment. As shown in Fig. 3, F and G, flow cytometry analysis showed that after overexpression of miR-30a, the apoptotic rate...
of cis-DDP-treated HeLa cells increased from 24% to 44%. Serving as a control, overexpression of miR-NC did not change the autophagic activity and apoptotic rate of HeLa cells induced by cis-DDP treatment. Furthermore, by switching cis-DDP to taxol, we observed a similar inhibitory effect of miR-30a overexpression on taxol-induced autophagy activity in MCF-7 and HepG2 cells (supplemental Fig. 5, A–C). Through suppressing cancer cell autophagy activity, overexpression of miR-30a also increased cancer cell apoptosis under taxol treatment (supplemental 5D). These results show that miR-30a can inhibit beclin 1-mediated cancer cell autophagy, which results in an increased apoptosis of cancer cells under the treatment with chemotherapeutic agents such as cis-DDP and taxol.

miR-30a Enhances the Sensitivity of Cancer Cells to cis-DDP Treatment—Because elevation of miR-30a levels in HeLa cells can increase cis-DDP-induced cellular apoptosis, we further analyzed whether miR-30a can enhance the sensitivity of cancer cells to cis-DDP. In this experiment, HeLa cells transfected with pre-miR-30a or pre-miR-NC were treated with various concentrations of cis-DDP. As shown in Fig. 4, A and B, miR-30a significantly increased the efficiency of cis-DDP in causing cellular apoptosis. In the control group or HeLa cells transfected with pre-miR-NC, 20 μg/ml cis-DDP was required to produce a significant cellular apoptosis. However, in cells transfected with pre-miR-30a, only 5 μg/ml cis-DDP was required to efficiently cause HeLa cell apoptosis. At the same concentration of cis-DDP, the cellular apoptotic rate was significantly higher in the miR-30a-overexpressed HeLa cells than in the control cells transfected with pre-miR-NC.

Because miR-30a targets beclin 1-mediated autophagy (25) and cancer cells can utilize autophagy pathway to survive the killing by chemotherapeutic agents, the reason that miR-30a can sensitize tumor cells to cis-DDP or Taxol may be its inhibition on cancer cell autophagy. To further clarify the relationship between cancer cell autophagy and apoptosis both induced by chemotherapeutic agents, we employed a chemical blocker of autophagy, 3-MA (36), as a control. As shown in Fig. 4C, 3-MA effectively improved the sensitivity of HeLa cells to cis-
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DDP at 10 μg/ml. Furthermore, forced expression miR-30a in a cis-DDP resistant cancer cell line, SGC-7091, also enhanced the cell apoptosis caused by cis-DDP (Fig. 4D). As shown, compared with the control SGC-7092 cells, which are sensitive to cis-DDP treatment, SGC-7091 cells showed no apparent apoptosis when the concentration of cis-DDP was increased to 80 μg/ml. However, overexpression of miR-30a in SGC-7091 cells significantly increased the sensitivity of SGC-709 cells response to cis-DDP treatment. In a similar fashion, 3-MA treatment also strongly increased the apoptosis of SGC-7091 cells, even at low concentration of cis-DDP (Fig. 4E). Taken together, these results indicate that miR-30a can enhance the sensitivity of cancer cells to cis-DDP treatment through inhibiting cancer cell autophagy activity.

**Intravenous Injection of Recombinant Lentivirus Expressing miR-30a Enhances the Efficiency of cis-DDP in Tumor Killing**—Next, we tested whether delivery of miR-30a can increase the efficiency of cis-DDP in killing implanted tumors in BALB/c mice. In this experiment, 6-week-old male mice were implanted with ~10^6 HepS tumor cells. After 14 days post-implantation, mice were treated with cis-DDP at a concentration of 60 mg/kg body weight. At the same time, mice were intravenously injected with or without 10^11 titer/ml lentivirus expressing miR-30a (LV-miR-30a) or nonspecific control miR-NC (LV-miR-NC). As shown in Fig. 5, A and B, treatment with 60 mg/kg body weight of cis-DDP alone effectively reduced the size of the implanted tumors compared with non-treated control mice. However, injection with LV-miR-30a strongly increased the efficiency of cis-DDP treatment and further decreased the tumor size. A TaqMan probe-based qRT-PCR assay confirmed that the injection of LV-miR-30a significantly increased the levels of miR-30a in tumors even in the presence of cis-DDP treatment (Fig. 5C). Biochemical analysis further showed that LV-miR-30a injection reduced the cis-DDP-induced autophagy activity of the implanted tumor cells, as indicated by the LC3-II/LC3-I ratio and beclin 1 expression (Fig. 5, D–F).

An enhanced effect of forced miR-30a expression on the anti-tumor activity of cis-DDP was also further tested using a lower concentration of cis-DDP. As shown in Fig. 5, G–J, LV-miR-30a injection significantly increased the sensitivity of the implanted tumor cells in mice to cis-DDP treatment and enhanced the efficiency of cis-DDP in tumor suppression.

**DISCUSSION**

Autophagy, an intracellular self-defense mechanism, serves as an important pathway for cells not only to prevent the toxic accumulation of damaged or unnecessary components but also to recycle the degraded components to sustain metabolic homeostasis. Heightened autophagy has been widely found in cancer cells faced with metabolic and therapeutic stress and contributes to the chemotherapy resistance of various types of tumors (37). Blocking cancer cell autophagy is emerging as a novel approach to enhance the efficiency of chemotherapy in cancer (38, 39). Here, we demonstrated that treatment with cis-DDP or taxol caused an activation of autophagy in several cancer cells, and this enhanced autophagy was involved in regulating tumor cell resistance to cis-DDP or Taxol. Our results from both in vitro and in vivo experiments clearly showed that inhibition of beclin 1-mediated tumor cell autophagy by elevation of miR-30a significantly promoted cis-DDP-induced tumor cell apoptosis.

As a clinically relevant anticancer drug, cis-DDP is widely used for the treatment of many types of cancers. Although the cytotoxicity of cis-DDP to cancer cells is not completely understood, the major mechanism underlying its antitumor activity has been ascribed to the induction of DNA damage via inducing loop structures and condensation of DNA molecules by platinum compounds (29, 40). Previous studies indicated that, like many other antitumor reagents, cis-DDP treatment causes tumor cell resistance to cis-DDP (37, 41). Although there are many factors that may contribute to the cis-platinum-resistance of tumor cells, increased tumor cell autophagy activity by cis-DDP treatment plays a key role in this process (37). Our study confirmed that cis-DDP or taxol treatment increased tumor cell autophagy, as indicated by the elevation of autophagy-related proteins beclin 1 and LC3-II, and further demonstrated that the possible mechanism underlying cis-DDP-mediated autophagic activity enhancement was the reduction of miR-30a by cis-DDP. As shown by the results derived from both a mouse model (Fig. 5) and cell culture experiments (Figs. 2–4), forced expression of miR-30a decreased the expression level of its target gene beclin 1 (25) and beclin 1-mediated autophagy, which in turn promoted cis-DDP-induced tumor cell apoptosis.

Serving as a key modulator of cellular autophagy, beclin 1 has been reported to function as both a negative and positive regulator of mammalian cell growth and tumorigenesis. In some human cancers, including breast, ovarian and esophageal cancers (42–44), beclin 1 protein levels were decreased compared with normal counterparts. However, increased expression of
beclin 1 has been reported in colorectal cancers, gastric cancers, and intrahepatic cholangiocellular carcinomas (10, 45). These discrepancies suggest that beclin 1 has different expression patterns in different tissues. In this study, we found that beclin 1 protein expression was markedly increased in HeLa, MCF-7 and HepG2 cells treated with cis-DDP, suggesting beclin 1 functions as an inducer of autophagy and a positive regulator of tumor cell survival and growth.

Besides miR-30a, our results showed that a significant reduction expression of miR-107, miR-143, and miR-145 after DDP treatment. However, bioinformatics analysis and literature survey show no direct involvement of these miRNAs in autophagy activity of cancer cells. The roles of these miRNAs in modulating the sensitivity of cancer cells to cis-DDP treatment need further study.

It has been reported that HeLa and other cervical cancer cells usually are human papillomavirus-positive and that human papillomavirus oncogene E6/E7 causes a rapid degradation of p53 protein, which plays a critical role in cervical carcinogenesis and chemosensitivity (46, 47). It may argue that the effect of miR-30a on enhancing HeLa cell chemosensitivity is due to abnormal p53 functions in HeLa cells. However, in this study, we also used MCF-7 and HepG2 cells that carry a normal expressed p53 protein (48, 49). The level of miR-30a in all these cancer cell lines is reduced after cis-DDP or taxol treatment, and forced expression of miR-30a inhibits beclin-1-dependent autophagy and improves the chemosensitivity of cancer cells. The results suggest that the chemotherapeutic treatment-induced autophagy significantly contributes to the insensitivity of cancer cells to che-

**FIGURE 3.** Forced expression of miR-30a in HeLa cells decreased cis-DDP-induced autophagy activity but enhanced the cellular apoptotic rate. *A*, immunostaining of cis-DDP-induced autophagosomes in HeLa cells transfected with pre-miR-30a or pre-miR-NC. The autophagosomes are indicated by arrowheads. **B**, quantitative analysis of autophagosomes in HeLa cells. **C–E**, Western blot analysis of LC3-I, LC3-II and beclin 1 in HeLa cells. HeLa cells were transfected with pre-miR-30a or pre-miR-NC and treated with or without cis-DDP. Note that both cis-DDP-induced beclin 1 and LC3-II were blocked by miR-30a overexpression. **F–G**, the apoptotic rate of HeLa cells was detected by FITC-annexin V labeling and flow cytometric analysis. HeLa cells were transfected with pre-miR-30a or pre-miR-NC and treated with or without cis-DDP. Note that overexpression of miR-30a significantly enhanced the apoptotic rate of HeLa cells treated with cis-DDP. The data are presented as the mean ± S.E. for three independent experiments. *, p < 0.05; **, p < 0.01.
motherapeutic agents and that p53 function may be not directly involved in this response.

Although the mechanism underlying the reduction of miR-30a by cis-DDP treatment remains unknown, this study demonstrates for the first time that enhancement of miR-30a expression in tumor cells can block beclin 1-mediated autophagy and sensitizes tumor cells to antitumor reagents such as cis-DDP. Our animal model study further implicates that lentivirus-delivered miR-30a through intravenous injection may have the potential to become a novel approach...
to enhance the efficiency of chemotherapy during cancer treatment.

In conclusion, our study shows that cis-DDP treatment induces an activation of autophagy that promotes tumor cell survival and chemoresistance. Inhibition of autophagy by the elevation of miR-30a expression induces the reduction of tumor cell growth and increases cellular apoptosis during cis-DDP treatment. Down-regulation of beclin 1 by miR-30a sensitizes cultured HeLa cells and mouse-implanted HepS tumor cells to cytotoxic cis-DDP and helps enhancing the therapeutic effect of these DNA damage drugs in tumor treatment.

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