MicroRNA-10b modulates cisplatin tolerance by targeting p53 directly in lung cancer cells

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Abstract. MicroRNA (miRNA or miR)-10b is an oncogenic miRNA associated with metastasis that is present in various types of tumor, including lung cancer. However, whether miR-10b is involved in different malignant characteristics, such as drug resistance or stemness, remains unclear. Therefore, the present study investigated whether miR-10b is an upstream regulator of p53. Ectopic expression of miR-10b-agomir decreased the expression of p53 and its downstream effectors, such as Bax and p53 upregulated modulator of apoptosis. Two non-canonical sites, including 1,580-1,587 and 2,029-2,035, located in p53 3'-untranslated region (UTR) were affected by the presence of miR-10b. In functional assays, upregulation of the p53 signaling pathway following cisplatin treatment was associated with decreased levels of miR-10b and upregulation of the luciferase activity of wild-type, but not 1,584, 2,032-dual-mutant, p53 3'-UTR. The ectopic expression of miR-10b-agomir attenuated the stability of p53 3'-UTR and the expression of p53 and its downstream effectors induced by cisplatin. By contrast, the knockdown of miR-10b induced the stability of p53 3'-UTR and increased levels of p53 and the sensitivity of A549 cells to cisplatin treatment. Similar results were also observed for Beas 2B cells. In the clinical investigation, p53 exhibited two distinct associations (cocurrent and countercurrent) with miR-10b in patients with lung cancer. Patients with lung cancer with low p53 and high miR-10b levels exhibited the poorest prognosis, while those with high p53 and low miR-10b exhibited the most favorable prognosis. These findings indicate a novel pathway in which cisplatin induces the levels of p53 by increasing mRNA stability via miR-10b, indicating a novel oncogenic role of miR-10b in promoting the malignant characteristics of non-small cell lung carcinoma.

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Abbreviations: miR, microRNA; SCLC, small cell lung cancer; NSCLC, non-small cell lung cancer cell; LUAD, lung adenocarcinoma; UTR, untranslated region; PUMA, p53 upregulated modulator of apoptosis

Key words: p53, miR-10b, lung cancer, drug tolerance, prognosis

Introduction

Lung cancer has been identified as the leading cause of cancer mortality in Taiwan over the past decade. In 2019, the mortality rate of lung cancer was 19.3% and ranked first among total cancer mortality according to the annual report of Ministry of Health and Welfare (https://www.mohw.gov.tw/np-126-2.html). Despite advances in surgery, radiation therapy, chemotherapy and targeted therapy, treatment is largely unsuccessful; the 5-year survival for patients with lung cancer is ~15-20% (1). Lung cancer can be classified into small cell lung cancer (SCLC) and non-SCLC (NSCLC). NSCLC is subdivided into adenocarcinoma, squamous cell carcinoma and large cell carcinoma (2). NSCLC accounts for ~80% of all lung cancer cases (3). More than 75% of patients with NSCLC develop metastasis during the course of the disease, which is responsible for high mortality; for example, in 2020, lung cancer was the leading cause of cancer mortality, with an estimated 1.8 million deaths (18% of total cancer deaths) globally (4-6). Innate or adaptive resistance to drug treatments, including chemo- and targeted therapy, increases the mortality rate of patients with lung cancer (7,8). Different malignant characteristics of cancer cells may be interconnected. For example,

epithelial to mesenchymal transition (EMT), which is a key step for motility, is acquired in cisplatin-resistant lung cancer cells via the Akt/β-catenin/Snail-dependent pathway (9). In addition to lung cancer, similar connections between motility and drug resistance have also been observed in other types of cancer, such as pancreatic and breast cancer (10). These findings highlight potential novel therapeutic strategies to treat lung tumors by simultaneously targeting multiple malignant characteristics.

The tumor suppressor p53 regulates biological responses to DNA damage, hypoxia, nutrient deprivation and abnormal expression of oncogenes (11-13) and serves a key role in tumor suppression via the induction of arrest, senescence and apoptosis, as well as by abrogating angiogenesis (14). The protein, which serves as a transcriptional factor, is maintained at a low level and has a short half-life under normal physiological conditions. Upon activation, several routes are involved in induction of p53 signaling. p53 mRNA is induced via a transcriptional regulator, such as CCAAT-enhancer-binding protein (15). p53 protein is stabilized by phosphorylation and dissociation from MDM2 and p53 mRNA is stabilized by binding of Wig-1 to its 3'-untranslated region (UTR) (16). The activated p53 then translocates to the nucleus and triggers transcription of target genes, such as p21, p53 upregulated modulator of apoptosis (PUMA) and Bax, to arrest the cell cycle or induce cellular apoptosis (17). Abnormality in p53 is detected in ~50% of all human tumors (18). Furthermore, the function of p53 affects the sensitivity of tumor cells to chemotherapeutic and/or radiotherapeutic agents (19). Furthermore, it has also been reported that p53 inhibits the motility of cancer cells by inhibiting the expression of MET or snail family transcriptional repressor 2 (Slug) (20,21). These results indicate that the malfunction of p53 contributes to multiple malignancies in cancer cells. Clinically, p53 has been considered as a therapeutic target to restore its normal expression or abrogate its oncogenic activity. In lung cancer, positive expression and mutation rates of p53 in adenocarcinoma are half those in squamous cells (22). Therefore, an understanding of the mechanisms underlying the pathogenic routes that cause p53 malfunction may facilitate the development of novel treatment strategies for patients with lung cancer.

MicroRNAs (miRNAs or miRs) are a class of small RNA that inhibit gene expression at the post-transcriptional level. Mature miRNAs are composed of 17-25 nucleotides and primarily bind to the complementary sequence of 3'-UTRs, followed by degradation of target mRNA or abrogation of its translation (23). miRNAs regulate more than two-thirds of all cellular processes, including proliferation, motility, differentiation, metabolism, autophagy and apoptosis, and hence the aberrant expression of miRNAs is hypothesized to be involved in the development of various diseases, such as multiple sclerosis, Parkinson's disease, Type II diabetes and cancer (24,25). The design of specific inhibitors of target proteins, such as Ras and Raf inhibitors, for the treatment of certain diseases is difficult and time-consuming (26,27). However, due to the complementary characteristics of nucleotides, the restoration of abnormally expressed miRNAs with either agomir or antagomir represents a promising option for future therapeutic strategies (28). To date, numerous studies have reported that a large number of miRNAs are abnormally expressed in cancer cells, which causes the dysregulation of various genes involved in cancer pathogenesis, particularly metastasis or drug resistance (29,30). For example, miR-10b is an oncomir that is upregulated in breast, pancreatic, lung, esophageal, neck and head, prostate and colorectal cancer, as well as melanoma, hepatocellular carcinoma and glioma (31). The overexpression of miR-10b decreases expression of HOXD10, zinc finger E-box binding homeobox 1, Kruppel-like factor (KLF)4, epithelial (E-)cadherin and TIAM Rac1-associated GEF 1 (Tiam1), which leads to malignant transformation and development of invasive and metastatic properties of inceptive benignant cancer cells (32-36). miR-10b is also involved in drug resistance; for example, miR-10b is involved in tamoxifen resistance via downregulation of histone deacetylase (HDAC)4 in estrogen receptor-positive breast cancer cells (37). In addition, high levels of miR-10b confer 5-fluorouracil resistance to colorectal cancer cells (38). Wu *et al* (39) demonstrated that miR-10b confers cisplatin resistance via targeting peroxisome proliferator-activated receptor (PPAR)γ in esophageal cancer cells. Furthermore, it has also been reported that higher miR-10b levels are associated with resistance to neoadjuvant therapy and lower survival rate in patients with pancreatic ductal adenocarcinoma (40), suggesting an alternative function of miR-10b. In lung cancer, high expression of miR-10b is positively associated with malignancy and poor prognosis (41). It is therefore important to identify lung cancer-associated miRNAs as biomarkers of treatment response or pharmacological targets. Previous reports imply that miR-10b may exhibit a wide range of functions, such as fibrosis, immune regulation, neuron protection and muscle proliferation beyond promoting motility (42-45). In addition, despite evidence of a connection between miR-10b and malignancy of lung cancer, the genes that coordinate or contribute to multiple oncogenic effects of miR-10b in lung cancer cells have not yet been mechanistically validated. The present review investigated p53 as a novel target of miR-10b and its effect on drug tolerance and motility in lung cancer cells.

Materials and methods

Cell lines and culture. Human lung cancer cell lines, including A549, H460, CL1-0 and CL1-5, and non-tumor bronchial cell line Beas 2B were maintained in RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) supplemented with 5% heat-inactivated fetal bovine serum and incubated in a 5% CO_2 incubator at 37˚C.

Reverse transcription-quantitative (RT-q)PCR of miRNA. In each cell line, the expression levels of hsa-miR-10b (hsa-miR-10b-5p, unless otherwise specified, hereafter referred to as miR-10b) were analyzed as previously described (46). Briefly, miRNAs were extracted using miRVANA® miRNA isolation kit (cat. no. AM1560; Thermo Fisher Scientific, Inc.). A total of 5 μl total miRNA was used for RT using a miRNA RT kit (cat. no. 4366596; Thermo Fisher Scientific, Inc.), and 5X miR-10b or RNU6B probe. PCR was performed with TaqMan PCR master mix kit (Thermo Fisher Scientific, Inc.), using a 20X miR-10b or RNU6B probe. Both primers and probes of miR-10b (cat. no. 002218) and RNU6B (cat. no. 001093) were provided by Thermo Fisher Scientific,

Inc. RNU6B served as an internal control. The signals were read using an ABI StepOnePlus. Thermocycling conditions were as follows: Initial denaturation at 95˚C for 10 min; 95˚C for 10 sec followed by 60˚C for 1 min at 40 cycles. Subsequently, the comparative $2^{-\Delta\Delta Cq}$ method (46) was applied to quantify the gene expression levels.

Ectopic expression of miR-10b-agomir or antagomir and pmirGLO-p53 3'-UTR luciferase reporter vector and assay. Human miR-10b-agomir (5'-UACCCUGUAGAACCGAAU UUGUGUU-3') was purchased from Thermo Fisher Scientific, Inc. Both miR-10b antagomir and scramble (sc) RNA were purchased from MDBio, Inc. sc served as miR-10b-agomir control. The initial screening from multiple websites (TargetScan, targetscan.org/vert_72/; miRbase, mirbase.org) did not reveal any conserved binding region of p53 3'-UTR for miR-10b. However, two partially matched regions (1,580-1,587 and 2,029-2,035 from transcription start site) were targeted. The wild-type (WT) 1,035 bps of 3'-UTR of p53 (1,245-2280 from ttranscription start site; forward, GCTAGCCATTCT CCACTTCTTGTTCCC and reverse, GTCGACTAATCCCAG CACTCTGGGAGG), mutant 1,583 (CCAGGGA mutated to CCATTTA) or 2,033 (ACTGGGT mutated to ACTTTTT) or 1,583 + 2,033 dual mutant was cloned into the pmirGLOdual luciferase vector (Promega Corporation). The transfection of 20 nM miR-10b-agomir, sc and antagomir (both designed by MdBio, Inc.) was performed using Oligofectamine and transfection of 1 μg luciferase reporter vector was performed using Lipofectamine® 2000 reagent (both Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C. After 16 h transfection, the transfection medium was replaced with fresh medium and incubated at 37˚C for a further 24-48 h. Luciferase activity of control and transfection cells was normalized with to Renilla luciferase activity according to the protocol of Dual-Luciferase Reporter assay system kit (Promega Corporation) and a luminometer (Mini lumate LB 9506; Titertek-Berthold).

Cell proliferation assay. sc, miR-10b-agomir or antagomir-transfected A549 or Beas 2B cells were seeded in 96-well plates ($5x10^3$ cells/well) overnight at 37˚C followed by treatment with cisplatin for 48 h at 37˚C. In preliminary experiments, 2 μM cisplatin showed a reduction of 20% viability of A549 [lethal dose $(LD)_{25}$] cells while 5 μM cisplatin showed reduction of 60% viability (>LD_{50}). The LD_{25} of cisplatin was used to test the viability of A549 when miR-10b was reduced by antagomir in comparison with untransfected parental cells. In additions, the higher dose of cisplatin (5 μM) was used to verify the adaptive ability of A549 with increased miR-10b expression levels. After 48 h, the medium was removed, washed with 1X PBS and incubated with serum-free medium containing 10 μl MTT stock solution (5 mg/ml) at 37˚C for 1 h, followed by washing with 1X PBS three times. The cells were then exposed to DMSO and the absorbance at 570 nm was measured using a microplate photometer. The absorbance values were normalized to those of sc as relative fold values. Trypan blue exclusion assay was also performed to determine viability. Briefly, the cells were trypsinized and suspended in 1X PBS, then 20 μl cells were and mixed with equal amount of 0.4% trypan blue solution (in PBS) and loaded on a hemacytometer. The cells were then counted under a ZEISS phase-contrast microscope at 40x or 100x magnification, and the blue cells which stained by trypan blue were excluded.

In vitro migration assay. A total of 25,000 cells was added to the upper compartment of a Boyden chamber (48) with 0.5 and 10.0% serum-containing RPMI-1640 medium (Invitrogen; Thermo Fisher Scientific, Inc.) in the bottom well at 37˚C. The upper and bottom chamber was separated by a silicon gasket and a microporous membrane (pore size, 8 μm). After 10 h, cells on the lower surface were fixed with 100% methanol and stained with 1X crystal violet (Sigma-Aldrich; Merck KGaA) for 5 min at room temperature, then washed with ddH_2O. The cells were then examined under a ZEISS phase-contrast microscope at 40x or 100x magnification. Relative motility was expressed as a percentage of cells on compound-treated wells compared with control wells (n>3).

Protein extraction and western blot analysis. Protein extraction and western blot procedures were performed as previously described (49). Briefly, parental, sc- and mir-10b agomir-transfected A549 or Beas 2B cells in the presence or absence of cisplatin were collected and washed three times with ice-cold PBS and lysed in RIPA buffer (25 mM Tris; 150 mM sodium chloride; 1% NP-40; 1% sodium deoxycholate; 0.1% SDS; pH 7.6). The cell lysates were centrifuged at 14,000 x g for 20 min at 4˚C, the supernatant was collected and total protein concentration was determined by the Bradford method. For western blot analysis, equal amounts of proteins (50 μg/lane) were separated by 10-15% SDS-polyacrylamide gel followed by electrophoretic transfer onto a PVDF membrane (EMD Millipore). Following blocking with 5% milk in 1X TBS-Tween for 1 h at room temperature, the membrane was incubated with primary antibodies (all 1:1,000 v/v) overnight at 4˚C. The membrane was then reacted with horseradish peroxidase-conjugated secondary antibody (1:5,000 v/v) for 1 h at room temperature, and the blots were visualized using an ECL-Plus detection kit (PerkinElmer, Inc.). The following antibodies were used: Anti-ATM (G-12; cat. no. sc-377293; Santa Cruz Biotechnology, Inc.), Anti-phosphorylated (phospho)-ATM (ser-1981) (cat. no. sc-47739; Santa Cruz Biotechnology Co., Ltd.), Anti-p53 (DO-1; cat. no. sc-126; Santa Cruz Biotechnology, Inc.), Phospho-p53 (Ser15; cat. no. #9284; Cell Signaling Technology, Inc.), Anti-PTEN (A2B1; cat. no. sc-7974; Santa Cruz Biotechnology, Inc.), PUMAα (B-6; cat. no. sc-377015; Santa Cruz Biotechnology Co., Ltd.), Anti-Bax (B-9; cat. no. sc-7480; Santa Cruz Biotechnology, Inc.), Monoclonal Anti-β-Actin (cat. no. A2228; Sigma-Aldrich; MercK KGaA), Phospho-Akt (Ser473) (D9E; cat no. 9271T; Cell Signaling Technology, Inc.), Anti-Akt1 (B-1; cat. no. sc-5298; Santa Cruz Biotechnology, Inc.), Goat anti-mouse IgG-HRP (cat. no. sc-2005; Santa Cruz Biotechnology, Inc.) and goat anti-rabbit IgG-HRP (cat. no. sc-2004; Santa Cruz Biotechnology, Inc.).

Meta-analysis. The OncoLnc database (oncolnc.org/) (50) was used to analyze the association between expression levels of miR-10b and p53 in the clinic. Expression levels of miR-10b or p53 in tumor tissue from 488 patients with lung adenocarcinoma (LUAD) were analyzed using SPSS (v21; IBM Corp.) to calculate the Pearson's correlation (2-tailed) between these two

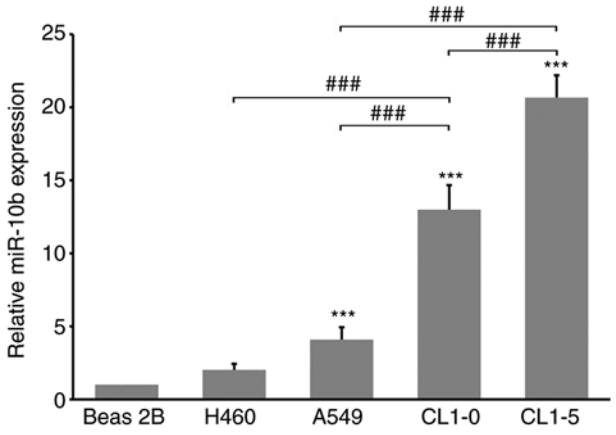

Figure 1. Expression levels of miR-10b in normal and tumorous lung cells. miRs were extracted from tumorous lung or bronchial cells and subjected to reverse transcription-quantitative PCR with miR-10b primers. The expression levels of miR-10b from each cell line were normalized to that of Beas 2B. ***P<0.001 vs. Beas 2B. ###P<0.001. miR, microRNA.

genes. Patients were subdivided into high and low expression of miR-10b or p53 based on the median value. The patients who exhibited the same expression trends of miR-10b and p53 (high-high or low-low) were defined as cocurrent, while inverse trends (low-high or high-low) between miR-10b and p53 were defined as countercurrent. In addition, the survival probability was estimated by the Kaplan-Meier plot method using SPSS (v21; IBM Corp.) followed by Tarone-Ware test. P<0.05 was considered to indicate a statistically significant difference.

Statistical analysis. Data are presented as the mean ± SD (n≥3) and were analyzed using GraphPad Prism software version 8.0 (GraphPad Software, Inc.) (51). One-way or two-way ANOVA followed by Tukey's post hoc test was used to compare differences between multiple groups. P<0.05 was considered to indicate a statistically significant difference.

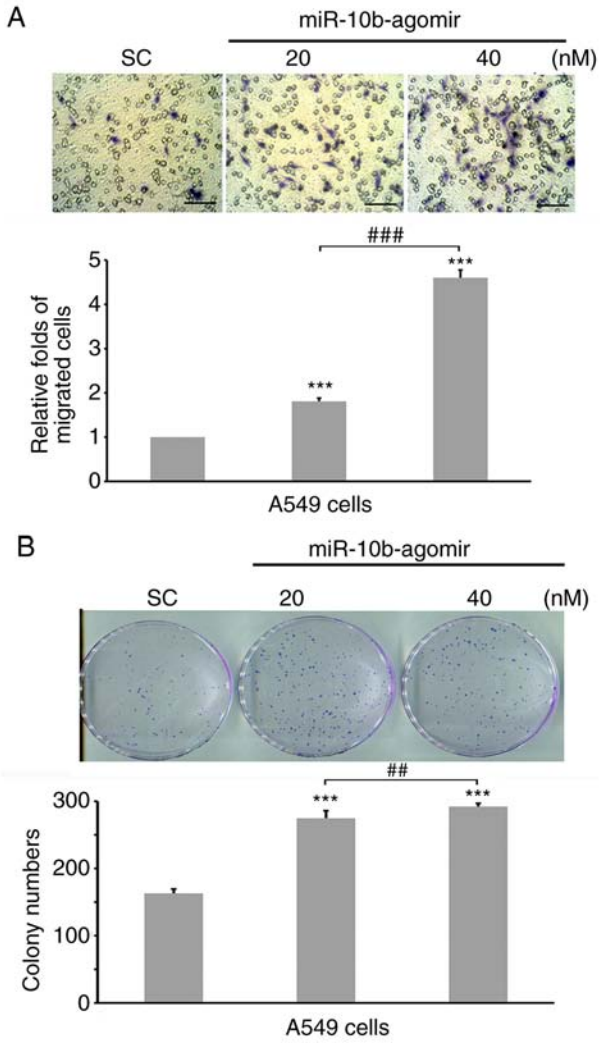

Figure 2. Overexpression of miR-10b increases motility and viability of non-small cell lung cancer cells. Parental or miR-10b-transfected A549 cells were subjected to (A) migration assay in a Boyden chamber or (B) colony formation assay. The migrated cells were counted and normalized to the parent A549 cells. The colonies were then counted. Scale bar, 25 μm. ***P<0.001 vs. sc. ##P<0.01, ###P<0.001. miR, microRNA; sc, scramble.

Results

miR-10b exhibits higher expression in highly invasive lung cancer cells. In order to characterize the role of miR-10b in lung cancer cells the expression levels of miR-10b in different lung cancer cells and non-tumor bronchial Beas 2B cells were analyzed. miR-10b exhibited the highest levels in CL1-5 cells; these levels were ~1.5-times greater than in CL1-0, 5-times greater than in A549 and 10-times greater than in Beas 2B cells. CL1-5 cells are known to exhibit high migration/invasion ability and the present results are consistent with those of previous reports concerning the association between miR-10b expression levels and cellular motility (Fig. 1) (33,52,53). CL cells harbor an oncogenic-mutant p53 (21), these results imply a potential association between p53 and miR-10b in lung cancer cells. In order to confirm whether miR-10b is also involved in the motility of lung cancer cells, 20 or 40 nM miR-10b-agomir were transfected into A549 cells, followed by invasion assay. Our preliminary experiments demonstrated that 20 and 40 nM miR-10b-agomir inhibited p53 expression in a dose-dependent manner (data not shown), thus these dosages were selected for subsequent experiments. Expression of endogenous miR-10b in untransfected and sc transfections showed no difference (Fig. S1), thus, in the following experiments, sc was applied as control. Increasing miR-10b levels significantly induced invasion of both types of cell in a dose-dependent manner (Fig. 2A and B). These results showed that miR-10b regulated the motility of lung cancer cells, as has been demonstrated in other types of cancer (34,54). Protein expression levels were analyzed following treatment with miR-10b. miR-10b-agomir decreased expression levels of E-cadherin (Fig. 3), in accordance with past reports (35,55). Levels of p53 and its downstream target genes, PUMA and Bax (56), were also decreased in the presence of miR-10b-agomir (Fig. 3). These results suggested that, in addition to motility-associated signaling, miR-10b is involved in the regulation of p53 pathways.

p53 is a direct target of miR-10b. In order to address whether miR-10b directly regulates expression of p53, luciferase reporters carrying the 3'-UTR of p53 genes (1,000 bps) were

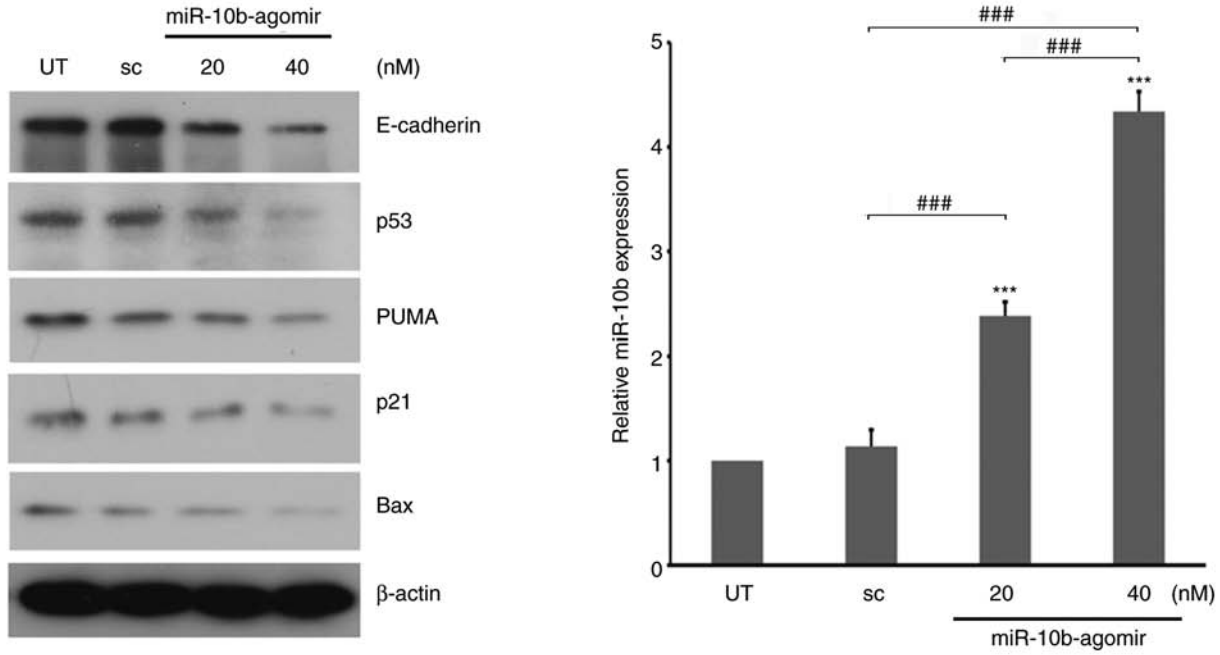

Figure 3. miR-10b attenuates expression of p53 signaling molecules. The parental or miR-10b-agomir-transfected A549 cells were subjected to western blotting or quantitative PCR to reveal the expression levels of p53 signaling molecules or miR-10b, respectively. ***P<0.001 vs. UT. ###P<0.001. miR, microRNA; UT, untransfected; sc, scramble; E-cadherin, epithelial cadherin; PUMA, p53 upregulated modulator of apoptosis.

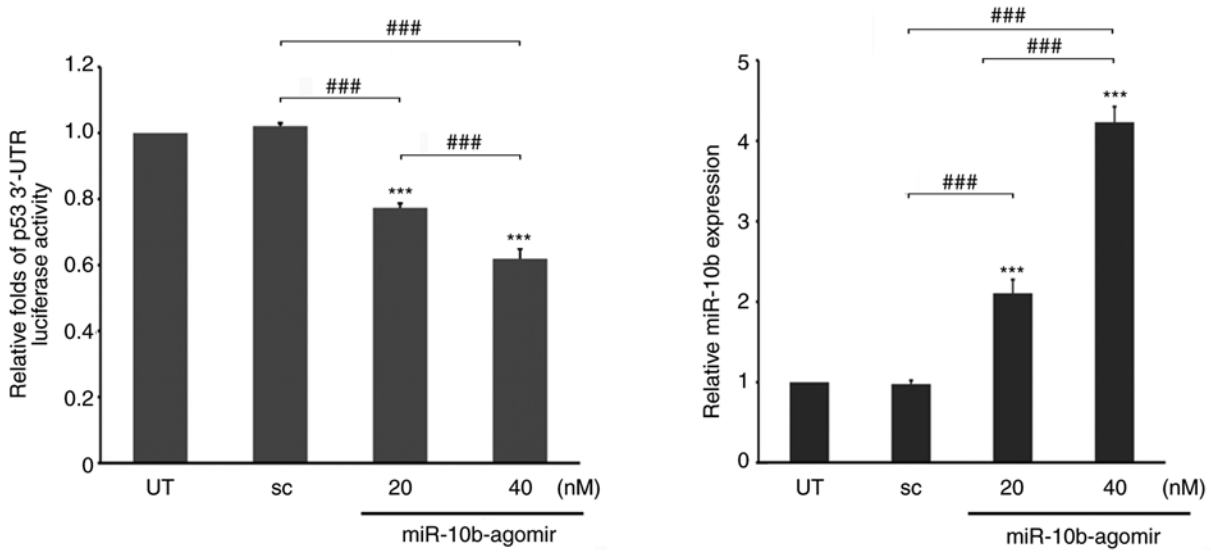

Figure 4. miR-10b-agomir decreases the stability of p53 3'-UTR. p53 3'-UTR luciferase reporter-transfected A549 cells were transfected with different doses of miR-10b-agomir, followed by luciferase assay. The luciferase activity of parent or miR-10b transfected cells was normalized to that of the parental cells. ***P<0.001 vs. UT; ###P<0.001. miR, microRNA; UTR, untranslated region; UT, untransfected; sc, scramble.

treated with different doses of miR-10b-agomir in A549 cells. These results revealed that the ectopic expression of miR-10b significantly decreased the stability of p53 3'-UTR in a dose-dependent manner (Fig. 4), indicating that miR-10b may directly regulate p53 expression by binding to its 3'-UTR region. Therefore, the binding sites of p53 3'-UTR for miR-10b were mapped. The initial screening from multiple websites (TargetScan; targetscan.org/vert_72/; miRbase, mirbase.org) did not reveal any conserved binding region of p53 3'-UTR for miR-10b. However, two partially matched regions (1,580-1,587 and 2,029-2,035 from transcription start site) were identified. It was next determined whether these regions were targeted by miR-10b. Two candidate regions were identified that resembled the miR-10b-targeting sequence of p53 3'-UTR and the corresponding mutations are depicted in Fig. 5A. WT and single and double mutants of p53 3'-UTR luciferase were either transfected alone or co-transfected with miR-10b into A549 cells. These results indicated that either 1,583 or 2,533 single mutant moderately recovered the p53 3'-UTR stability, which was decreased by miR-10b-agomir. However, simultaneous mutation of these two sites significantly attenuated miR-10b-mediated instability of p53 3'-UTR (Fig. 5B), which

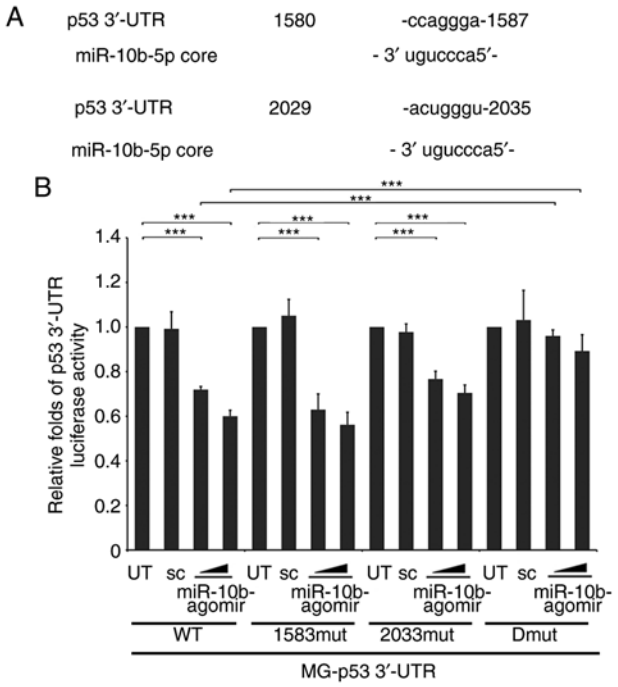

Figure 5. Identification of target sites of miR-10b in p53 3'-UTR. (A) Alignment between the miR-10b targeting stem site and two consensus sites of p53 3'-UTR (1,580-1,587 and 2,029-2,035). Bold text indicates pairing miR-10b-core and complementary target mRNA sequence. (B) WT, 1,583 or 2,023 mut or 1,583-2,023 Dmut pMGL-p53 3'-UTR luciferase reporter were co-transfected with different doses of miR-10b-agomir into A549 cells, followed by analysis of luciferase activity. The luciferase activity was then normalized to UT. ***P<0.001. miR, mircoRNA; UTR, untranslated region; WT, wild-type; mut, mutant; sc, scramble; Dmut, dual mutant.

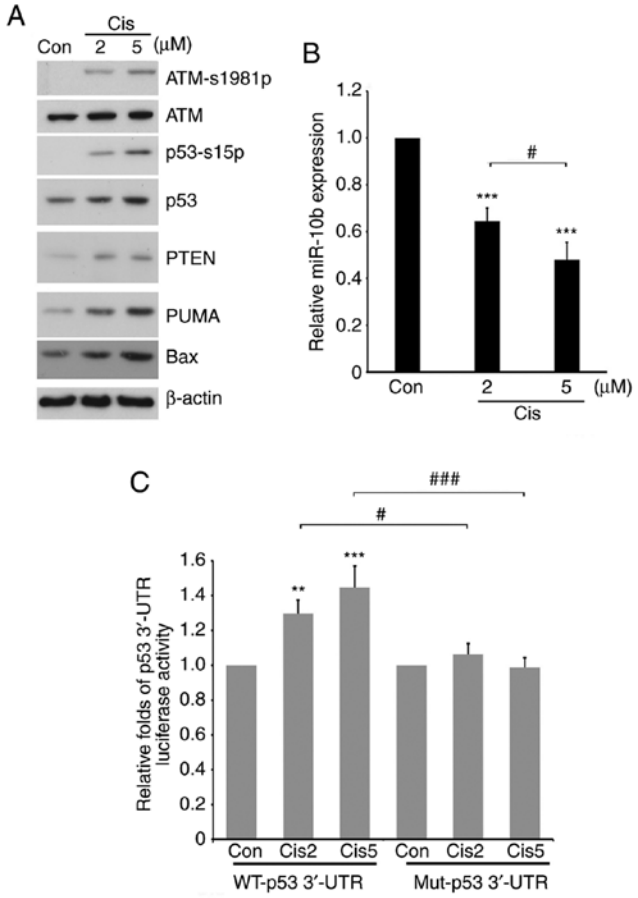

Figure 6. Cis decreases levels of miR-10b while inducing p53 3'-UTR stability and expression of p53 signaling molecules. pMGL-WT or -dual mut p53 3'-UTR luciferase reporter-transfected A549 cells were treated with different doses of cis for 48 h. The cells were then subjected to (A) western blotting to analyze the expression of p53 and apoptosis-associated molecules or (B) reverse transcription-quantitative PCR to reveal the levels of miR-10b. The expression levels of miR-10b were normalized to the Con. (C) Luciferase activity assay; values were normalized to the Con. **P<0.01, ***P<0.001 vs. Con; #P<0.05; ###P<0.001. Cis, cisplatin; miR, microRNA; UTR, untranslated region; WT, wild-type; mut, mutant; Con, control; PUMA, p53 upregulated modulator of apoptosis.

indicated that p53 was a target gene that was directly regulated by miR-10b. Furthermore, these results also suggest the possibility that miR-10b binds to its target gene via a consensus motif.

miR-10b is involved in upregulation of p53 via 3'-UTR stability following treatment with cisplatin. In order to investigate whether miR-10b is involved in the regulation of p53 expression under physiological conditions, different doses of cisplatin, a chemotherapeutic drug for patients with lung cancer and an inducer of p53 expression (57), was administered into A549 cells in the presence or absence of transfection with WT or double-mutant p53 3'-UTR luciferase vector for 24 h. The results suggested that the addition of cisplatin induced phosphorylation of ATM, an upstream regulator of p53, and increased expression of p53 and its downstream effectors, such as PUMA and Bax (Fig. 6A). Cisplatin decreased the expression of miR-10b in a dose-dependent manner (Fig. 6B), suggesting an inverse association between p53 and miR-10b in the presence of cisplatin. Furthermore, the stability of WT, but not double-mutant p53 3'-UTR was increased when treated with cisplatin (Fig. 6C), which suggested that miR-10b directly participated in the upregulation of p53 following treatment with cisplatin. These results indicate a novel mechanism underlying regulation of p53 expression levels following chemotherapy in NCSLC.

The expression level of p53 is involved in the response to both chemotherapeutic drug treatments (58). Therefore, to characterize the role of miR-10b in regulating the cell response to cisplatin, A549-p53 3'-UTR luciferase transfectants were transfected with miR-10b-agomir, followed by treatment with cisplatin. Cisplatin significantly decreased expression of endogenous miR-10b while inducing the stability of p53 3'-UTR (Fig. 7A and B). By contrast, in cells with ectopically expressed miR-10b-agomir, the stability of p53 3'-UTR was low even in the presence of cisplatin. Furthermore, western blotting revealed that p53 and its downstream effectors, such as Bax, PUMA and PTEN, were decreased by miR-10b-agomir (Fig. 7C). On the other hand, treatment with cisplatin increased levels of p53 signaling molecules; ectopic expression of miR-10b-agomir attenuated such upregulation, which activated Akt (Fig. 7C) and increased the viability of lung cancer cells (Fig. 7D). By contrast, knockdown of miR-10b by antagomir increased p53 3'-UTR stability and expression of p53 and its downstream effectors (Fig. 8A and B); furthermore, the decrease in miR-10b sensitized A549 cells to cisplatin treatment even at a lower dose (Fig. 8C). Similar results were also observed in non-tumorous bronchial Beas 2B cells (Fig. 9), suggesting a role of elevated miR-10b in

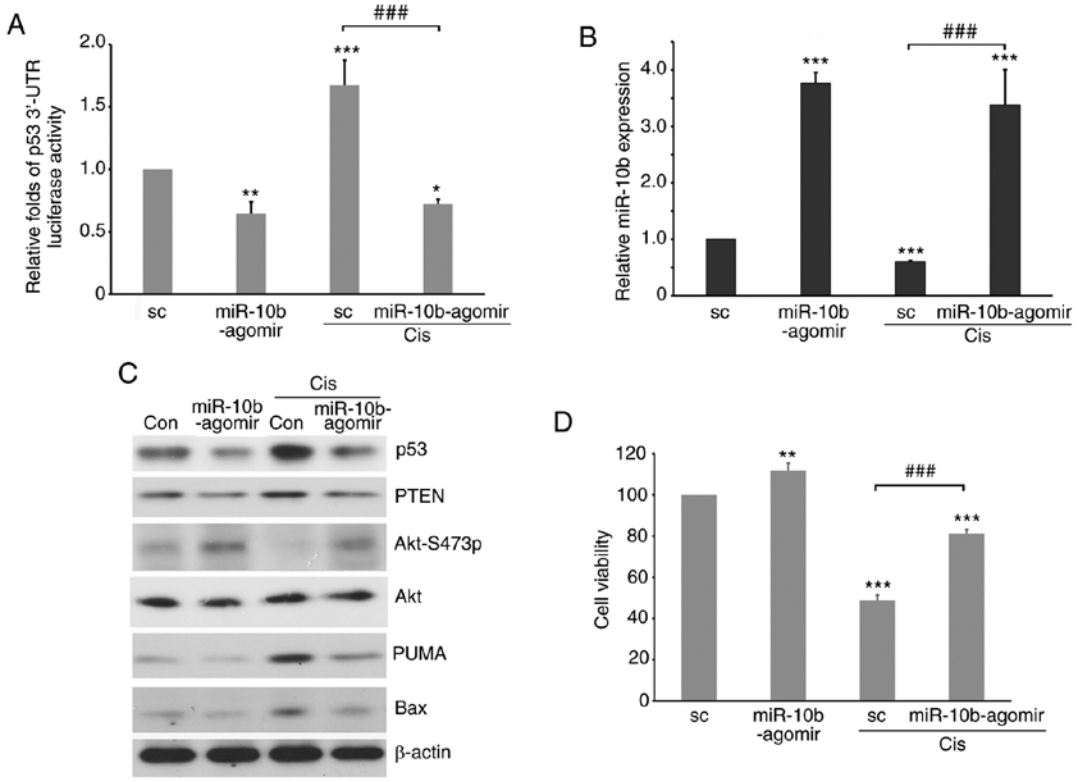

Figure 7. Ectopic expression of miR-10b-agomir decreases the stability of p53 3'-UTR and levels of p53 signaling molecules induced by cis. MG-p53 3'-UTR luciferase reporter-transfected A549 cells were transfected with miR-10b-agomir, followed by treatment with cis for 48 h, and subjected to (A) luciferase reporter assay to analyze p53 3'-UTR stability, (B) reverse transcription-quantitative PCR to analyze the expression levels of miR-10b, (C) western blotting to detect expression levels of p53 and apoptosis-associated molecules or (D) trypan blue exclusion assay to analyze cell viability. *P<0.05, **P<0.01, ***P<0.001 vs. sc; $^{###}$P<0.001. miR, microRNA; UTR, untranslated region; sc, scramble; PUMA, p53 upregulated modulator of apoptosis.

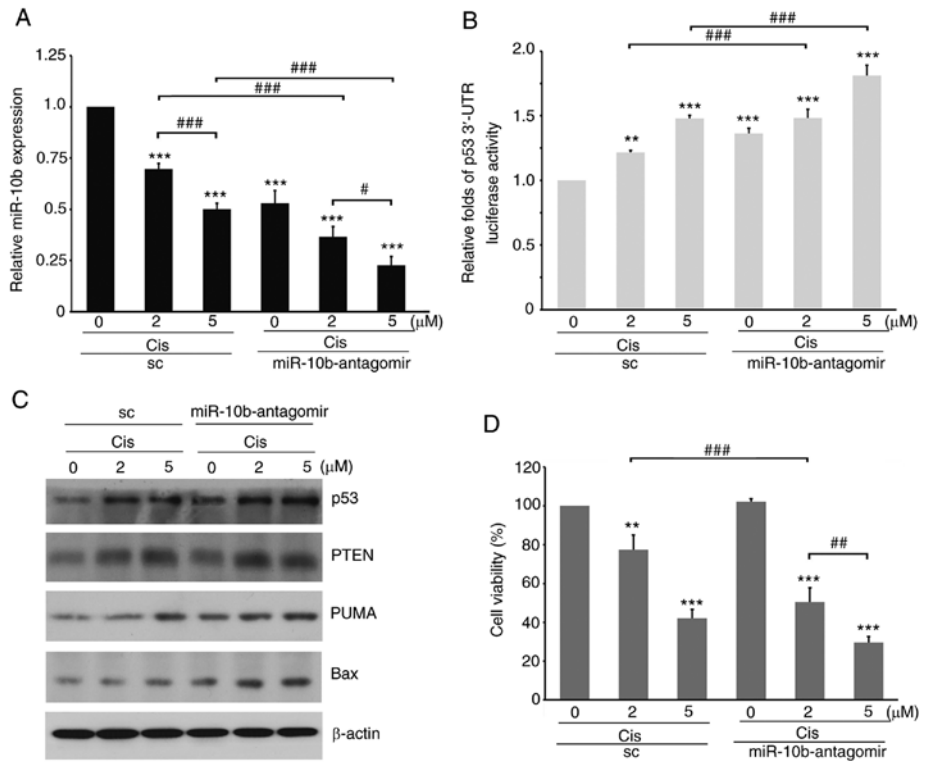

Figure 8. Attenuation of miR-10b increases expression of p53 signaling molecules and sensitivity to cis treatment. Untransfected MG-p53 3'-UTR luciferase reporter-transfected A549 cells or cells transfected with miR-10b-antagomir were treated with cis for 48 h and then subjected to (A) luciferase reporter assay to analyze p53 3'-UTR stability, (B) reverse transcription-quantitative PCR to analyze expression of miR-10b, (C) western blotting to determine the expression of p53 and apoptosis-associated molecules or (D) trypan blue exclusion assay to analyze cell viability. **P<0.01, ***P<0.001 vs. sc-cis 0; $^{##}$P 0.01; $^{###}$P<0.001. miR, microRNA; UTR, untranslated region; sc, scramble; cis, cisplatin; PUMA, p53 upregulated modulator of apoptosis.

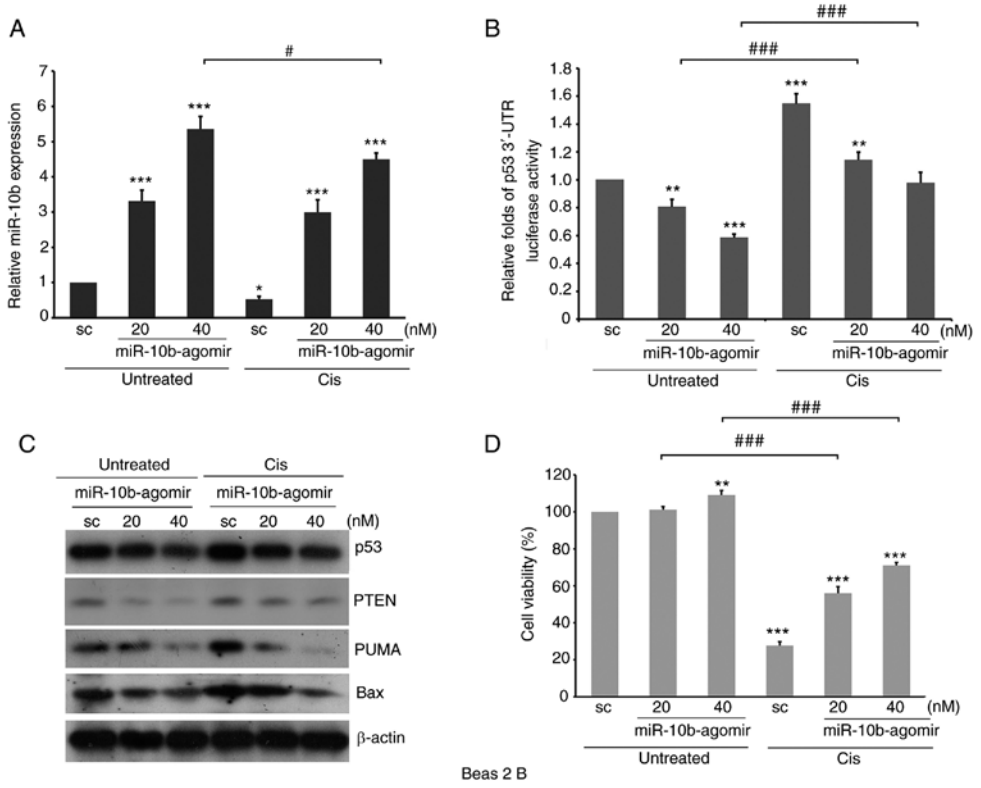

Figure 9. miR-10b is involved in cell death and expression of p53 signaling modules induced by cis in Beas 2B cells. (A) MG-p53 3'-UTR luciferase reporter-transfected Beas 2B cells were transfected with miR-10b-agomir, followed by treatment with cis for 48 h, and then subjected to luciferase reporter assay to analyze p53 3'-UTR stability. (B) Reverse transcription-quantitative PCR analyzed expression of miR-10b. (C) Western blotting identified expression of p53 and apoptosis-associated molecules. (D) Trypan blue exclusion assay analyzed cell viability. *P<0.05, **P<0.01, ***P<0.001 vs. untreated-sc; $^{\#}$P<0.05; $^{\#\#\#}$P<0.001. miR, microRNA; cis, cisplatin; UTR, untranslated region; sc, scramble; PUMA, p53 upregulated modulator of apoptosis.

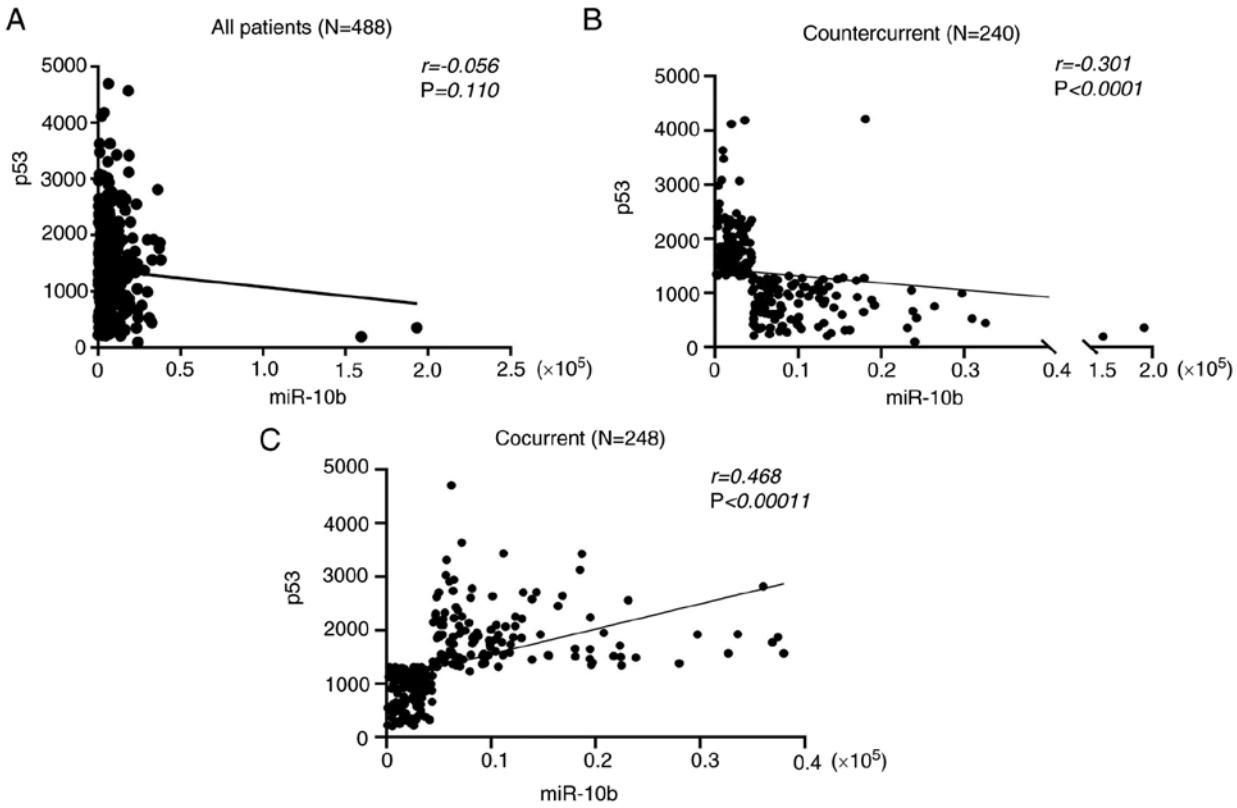

Figure 10. Association between expression levels of p53 and miR-10b in patients with lung cancer. The correlations between miR-10b and the p53 expression pattern in The Cancer Genome Atlas lung adenocarcinoma cohort was analyzed in (A) all patients and patients with (B) opposite (countercurrent) or (C) similar (cocurrent) trends between miR-10b and p53 levels. Expression correlation was statistically analyzed by Spearman's rho. miR, microRNA.

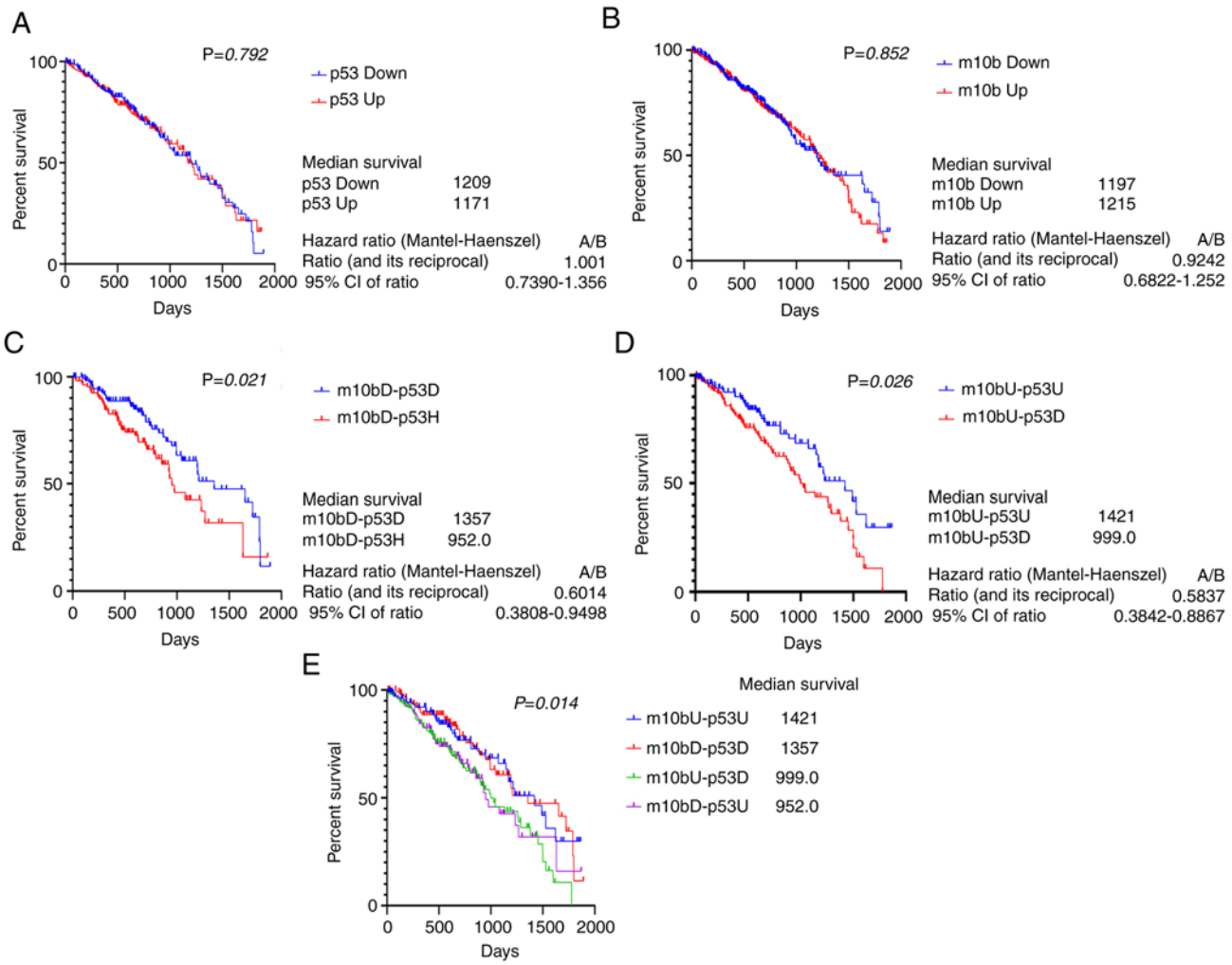

Figure 11. Distinct associations between miR-10b and p53 levels predict different prognosis of patients with lung cancer. Kaplan-Meier plot analysis demonstrated overall survival of lung adenocarcinoma cases of (A) p53 or (B) miR-10b-alone, (C) low miR-10b/low p53 vs. low miR-10b/high p53 and (D) high miR-10b/low p53 vs. high miR-10b/high p53. (E) Comparison of overall survival curves between different combinations of miR-10b/p53 expression of patients with lung adenocarcinoma. miR, microRNA.

overcoming the therapeutic effects of cisplatin. These results indicated that, in addition to serving as a canonical pathway in the regulation of cell motility, miR-10b may also participate in regulating the response to toxicity of chemotherapeutic drugs, such as cisplatin, by directly affecting the stability of p53 3'-UTR in tumorous and non-tumorous cells.

Countercurrent of miR-10b-p53 levels are positively correlated with prognosis in patients with lung cancer. In order to understand the clinical relevance of the association between miR-10b, p53 and the prognosis of patients with lung cancer, Affymetrix microarray data of a lung adenocarcinoma cancer cohort was analyzed using the Oncolnc database [data originally derived from The Cancer Genome Atlas (TCGA) database]. In the preliminary analysis, the expression levels of miR-10b and p53 showed no correlation in 488 patients with LUAD (r=-0.056, Fig. 10A); however, in a differentiated classification, the expression of miR-10b and p53 could be categorized into two groups: Cocurrent (same expression trend between miR-10b and p53) and countercurrent (inverse expression trend between miR-10b and p53). The expression association between miR-10b and p53 in the countercurrent group indicated a moderate negative correlation (r=-0.301, Fig. 10B); the cocurrent group of miR-10b and p53 indicated a moderate positive correlation (r=0.486, Fig. 10C). The results showed that both the expression trends between miR-10b and p53 were significant, suggesting that patients with LUAD exhibited two distinct expression associations between miR-10b and p53. Correlation between the miR-10b-p53 expression patterns and the prognosis of patients with LUAD were analyzed. Kaplan-Meier plot analysis indicated that neither miR-10b nor p53 expression-alone was correlated with prognosis (Fig. 11A and B). By contrast, patients with low miR-10b/high p53 status displayed lower survival rate than those with low miR-10b/low p53 (median overall survival, 952 vs. 1,357 days, Fig. 11C) and high miR-10b/low p53 status was associated with lower survival rate than high miR-10b/high p53 (median survival, 999 vs. 1,421 days, Fig. 11D). In addition, the survival rate of patients with high miR-10b/low p53 was lower than for those with high miR-10b-alone (median survival, 999 vs. 1,215 days; Fig. 11A and D). These results indicated that the prognosis of patients with LUAD with countercurrent expression of miR-10b and p53 was worse than those with cocurrent expression of miR-10b and p53; low miR-10b/high

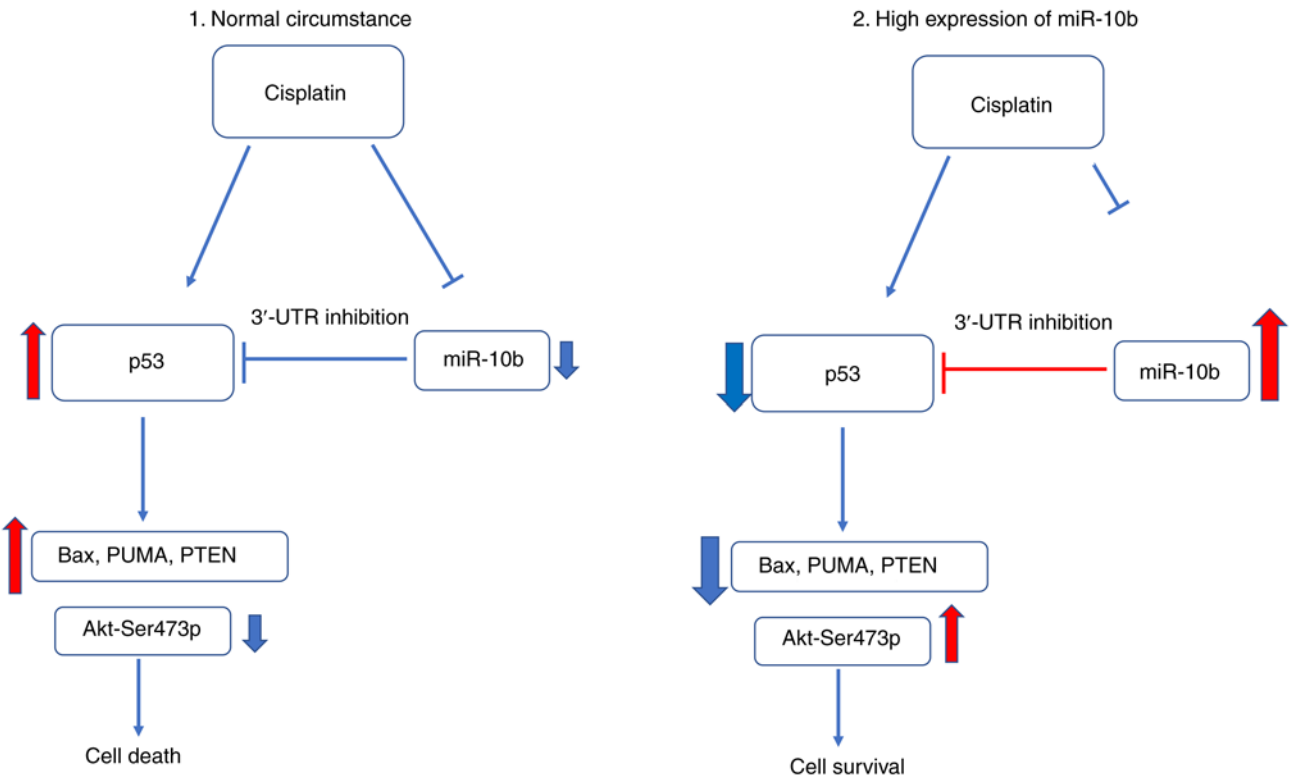

Figure 12. Schematic representation of functional interaction between miR-10b and p53 in regulating cisplatin tolerance in lung cancer cells. miR, microRNA; UTR, untranslated region; PUMA, p53 upregulated modulator of apoptosis.

p53 and high miR-10b/low p53 were associated a similarly poor outcome (Fig. 11E).

Discussion

The miRNA miR-10b has been identified as a driver for motility and a metastasis-associated oncomir in breast cancer (33). The metastasis-promoting characteristics have been confirmed in different types of cancer, such as melanoma, hepatocellular carcinoma, glioma and acute myeloid leukemia, as well as head and neck, endometrial, colorectal and lung cancer (59). Several target genes of miR-10b are associated with EMT or motility, such as E-cadherin, HOXD10, HOXB3, KLF4 and itchy E3 ubiquitin protein ligase (32,35,54,60,61). Furthermore, miR-10b is involved in TGF-β-mediated EMT in breast tumor and hyaluronan-induced migration/invasion in head and neck tumor cells (62,63). In addition, past reports indicated alternative roles of miR-10b in regulating cisplatin resistance via targeting PPARγ or tamoxifen resistance via downregulation of HDAC4 (37,39). Chen *et al* (60) demonstrated that miR-10b simultaneously induces proliferation and invasion via the inhibition of HOXB3 in endometrial cancer cells. These results suggest a wide role of miR-10b in promoting malignancy in various types of cancer cell. In agreement with these results, the present study demonstrated that fluctuations of endogenous miR-10b via agomir or antagomir significantly affect the motility of A549, CL1-0 and CL1-5 cells (data not shown). Furthermore, p53, a tumor suppressor with multiple functions, was identified as a target gene of miR-10b. The present results demonstrated the existence of two non-classical binding sites of p53 3'-UTR (1,580-1,587 and 2,029-2,035) for miR-10b and their regulation in p53 mRNA stability, which extends knowledge of potential target genes and roles of miR-10b in regulating physiological processes, such as drug response and cellular growth (64,65). Lu *et al* (66) reported that mir-10 targets p53 in colorectal cancer cells. The region 2,029-2,035 of p53 3'-UTR was identified as a miR-10b regulatory element; this is consistent with the present findings, which also identified another sequence located in the region 1,580-1,587. Furthermore, Lu *et al* revealed induction of stability of mutant p53 3'-UTR in the presence of miR-10b. However, the present results showed that ectopic expression of miR-10b decreased the stability of p53 3'-UTR with a single mutation of either region 1,580-1,587 or 2,029-2,035 (data not shown). This difference may be attributed to differences in the cell models used. In addition, Sun *et al* (67) reported that miR-10b regulates the p53 pathway via targeting p21 in glioblastoma, however, their results also showed an induction of p53 following knockdown of miR-10b, implying that miR-10b may target p53 to modulate the functions of p53 pathway.

Several miRNAs have been shown to exert oncogenic functions via targeting p53 expression. For example, miR-504 modulate cisplatin resistance by inhibiting p53 expression in osteosarcoma cells (68), while miR-675 induces invasion and metastasis by inhibiting p53 expression in colorectal cancer cells (69). Furthermore, miR-122 promotes lung cancer cell development via inhibiting p53 expression (70). In addition, miR-125b induces cisplatin resistance via the blockade of p53/Bax/apoptosis pathways in nasopharyngeal cancer cells (71). The present study demonstrated that ectopic expression of miR-10b-agomir decreased cisplatin sensitivity of lung cancer cells by directly inhibiting p53 levels. Since p53

is the primary mediator of cellular transformation to tumor cells (72,73), overexpression of miRNAs in different types of tumor may confer malignancy by inhibiting p53 expression levels.

Despite the inverse association between miR-10b and p53 in a cellular model in previous study (67), these two genes did not demonstrate a negative correlation in a clinical investigation of patients with LUAD from the TCGA database. By contrast, miR-10b and p53 showed two distinct associations, termed cocurrent (positive correlation) and countercurrent (negative correlation), which, to the best of our knowledge, have not previously been observed between miR-10b and its target genes or upstream regulators. On the other hand, increasing evidence suggest an association between expression level of miR-10b or p53 with the prognosis of various types of cancer; for example, mir-10b is positively associated with the malignancies of melanoma, glioblastoma and clear cell carcinoma, as well as gastric, colorectal and lung cancer (35,54,67,74-76), while p53 is positively associated with the malignancies of colorectal (positive correlation), breast (negative correlation), gastric (positive correlation) and lung cancer (negative association) (49,77-79). However, the present results showed that neither miR-10b nor p53-alone exhibited prognostic value in patients with LUAD. Zhang *et al* reported that levels of miR-10b are correlated with lower survival rate in 73 patients with NSCLC (35). The present study analyzed 488 patients; larger sample sizes may reveal distinct patterns of miR-10b expression levels and prognosis in patients with NSCLC. The present results showed that patients with LUAD with cocurrence between miR-10b and p53 showed better prognosis than those with countercurrence. These results suggested that miR-10b and p53 may be mutually exclusive regulators or that other regulators may participate in modulating expression of both miR-10b and p53. p53 may serve as an upstream inhibitor of miR-10b following treatment with Withaferin A (46) which supports the hypothesis that p53 may downregulate miR-10b in the presence of cisplatin. However, Bisio *et al* demonstrated that p53 serves as an inducer of miR-10b expression in HCT cells (80). This difference may be due to the use of different cell lines and indicates the complexity of functional correlations between miR-10b and p53. Moreover, controversial studies also provide a potential explanation for the distinct expression trends between miR-10b and p53 and the subsequent survival ratio in patients with lung cancer. Further investigation of the role of miR10b and p53 in regulating tumorigenesis, malignancy and prognosis is required. Despite the fact that miR-10b exhibited an inverse correlation with p53 between non-tumor and tumorous cells, the highest level of miR-10b was observed in CL cells, which harbor an oncogenic-mutant form of p53 that promotes cell invasion and chemoresistance by induing Slug and Nrf2, respectively (21,81); whether miRNAs are involved in oncogenic mutant p53-primed malignancy remains unclear. Changing levels of miR-10b only marginally affected levels of p53 in CL cells (data not shown), which suggested different regulatory mechanisms between miR-10b and oncogenic mutant p53 may exist. In addition, correlation analysis between p53 mutations and miR-10b expression levels suggested no significant difference in miR-10b expression levels between WT and mutated p53 (data not shown). These results suggest that alternatively regulated mechanisms between p53 and miR-10b exist. Moreover, the expression of miR-10b was independent of the mutated status of p53 in patients with lung cancer, which implies that miR-10b may serve as an upstream regulator of p53 in patients with LUAD. Our previous report indicated that Aurora-A and p53 exhibit an inverse association in patients with NSCLC and that combining expression levels of p53 and Aurora-A provides better prognostic potential than individual consideration in patients with lung cancer (49). Similarly, the present study demonstrated that combining miR-10b and p53 may provide a more precise predictive value of patients with NSCLC with poor prognosis who may benefit from specific targeted therapy. One limitation of the present study is the lack of direct in vivo evidence to confirm the association between miR-10b and p53 in drug-tolerance. The role of miR-10b-p53 in mice bearing cisplatin-sensitive or resistant lung tumors and the effects of miR-10b-antagomir in treating mice with cisplatin-resistant lung tumor should be investigated in the future.

In summary, the present study identified p53 as a target gene of miR-10b and revealed a pathway to increase p53 following treatment of cisplatin via downregulating levels of miR-10b (Fig. 12). In addition, the present study also revealed a novel function of miR-10b in modulating drug tolerance in addition to promoting motility. Furthermore, clinical investigation revealed that the combination of miR-10b and p53 expression provided more precise prognosis of patients with lung cancer. These findings improve understanding of the role of miR-10b in regulating multiple malignancy in lung cancer cells and offer more valuable prognostic prediction for patients with lung cancer.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

CCL performed qPCR, western blotting and data analysis. TY, HJ and WT analyzed data. TY, SL and CCW participated in experimental design and manuscript drafting. CCW, CCL and TYY confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Bach PB, Mirkin JN, Oliver TK, Azzoli CG, Berry DA, Brawley OW, Byers T, Colditz GA, Gould MK, Jett JR, *et al*: Benefits and harms of CT screening for lung cancer: A systematic review. JAMA 307: 2418-2429, 2012.
2. Brenner DR, McLaughlin JR and Hung RJ: Previous lung diseases and lung cancer risk: A systematic review and meta-analysis. PLoS One 6: e17479, 2011.
3. Hutchinson L: Practical assay to predict survival. Nat Rev Clin Oncol 9: 127, 2012.
4. Ranpura V, Hapani S and Wu S: Treatment-related mortality with bevacizumab in cancer patients: A meta-analysis. JAMA 305: 487-494, 2011.
5. Seton-Rogers S: Metastasis: Opposing forces in invasion. Nat Rev Cancer 11: 624-625, 2011.
6. Bray F, Ferlay J, Soerjomataram I, Siegel R, Torre L and Jemal A: Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 68: 394-424, 2020.
7. Holohan C, Van Schaeybroeck S, Longley DB and Johnston PG: Cancer drug resistance: An evolving paradigm. Nat Rev Cancer 13: 714-726, 2013.
8. Camidge DR, Pao W and Sequist LV: Acquired resistance to TKIs in solid tumours: Learning from lung cancer. Nat Rev Clin Oncol 11: 473-481, 2014.
9. Wang H, Zhang G, Zhang H, Zhang F, Zhou B, Ning F, Wang HS, Cai SH and Du J: Acquisition of epithelial-mesenchymal transition phenotype and cancer stem cell-like properties in cisplatin-resistant lung cancer cells through AKT/β-catenin/Snail signaling pathway. Eur J Pharmacol 723: 156-166, 2014.
10. Arumugam T, Ramachandran V, Fournier KF, Wang H, Marquis L, Abbruzzese JL, Gallick GE, Logsdon CD, McConkey DJ and Choi W: Epithelial to mesenchymal transition contributes to drug resistance in pancreatic cancer. Cancer Res 69: 5820-5828, 2009.
11. Toledo F and Wahl GM: Regulating the p53 pathway: In vitro hypotheses, in vivo veritas. Nat Rev Cancer 6: 909-913, 2006.
12. Koumenis C, Alarcon R, Hammond E, Sutphin P, Hoffman W, Murphy M, Derr J, Taya Y, Lowe SW, Kastan M and Giaccia A: Regulation of p53 by hypoxia: Dissociation of transcriptional repression and apoptosis from p53-dependent transactivation. Mol Cell Biol 21: 1297-1310, 2001.
13. Lakin N and Jackson S: Regulation of p53 in response to DNA damage. Oncogene 18: 7644-7655, 1999.
14. Ravi R, Mookerjee B, Bhujwalla ZM, Sutter CH, Artemov D, Zeng Q, Dillehay LE, Madan A, Semenza GL and Bedi A: Regulation of tumor angiogenesis by p53-induced degradation of hypoxia-inducible factor 1alpha. Genes Dev 14: 34-44, 2000.
15. Wang X, Huang G, Mei S, Qian J, Ji J and Zhang J: Over-expression of C/EBP-alpha induces apoptosis in cultured rat hepatic stellate cells depending on p53 and peroxisome proliferator-activated receptor-gamma. Biochem Biophys Res Commun 380: 286-291, 2009.
16. Vilborg A, Glahder JA, Wilhelm MT, Bersani C, Corcoran M, Mahmoudi S, Rosenstierne M, Grandér D, Farnebo M, Norrild B and Wiman KG: The p53 target Wig-1 regulates p53 mRNA stability through an AU-rich element. Proc Natl Acad Sci USA 106: 15756-15761, 2009.
17. Vousden KH and Prives C: Blinded by the light: The growing complexity of p53. Cell 137: 413-431, 2009.
18. Soussi T and Beroud C: Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer 1: 233-240, 2001.
19. Brown JM and Attardi LD: The role of apoptosis in cancer development and treatment response. Nat Rev Cancer 5: 231-237, 2005.
20. Hwang CI, Matoso A, Corney DC, Flesken-Nikitin A, Körner S, Wang W, Boccaccio C, Thorgeirsson SS, Comoglio PM, Hermeking H and Nikitin AY: Wild-type p53 controls cell motility and invasion by dual regulation of MET expression. Proc Natl Acad Sci USA 108: 14240-14245, 2011.
21. Wang SP, Wang WL, Chang YL, Wu CT, Chao YC, Kao SH, Yuan A, Lin CW, Yang SC, Chan WK, *et al*: p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. Nat Cell Biol 11: 694-704, 2009.
22. Gibbons DL, Byers LA and Kurie JM: Smoking, p53 mutation, and lung cancer. Mol Cancer Res 12: 3-13, 2014.
23. Cuperus JT, Fahlgren N and Carrington JC: Evolution and functional diversification of MIRNA genes. The Plant Cell 23: 431-442, 2011.
24. Bartel DP: MicroRNAs: Target recognition and regulatory functions. Cell 136: 215-233, 2009.
25. Li Y and Kowdley KV: MicroRNAs in common human diseases. Genomics Proteomics Bioinformatics 10: 246-253, 2012.
26. Lange A, Prenzler A, Frank M, Golpon H, Welte T and von der Schulenburg JM: A systematic review of the cost-effectiveness of targeted therapies for metastatic non-small cell lung cancer (NSCLC). BMC Pulm Med 14: 192, 2014.
27. Durrant DE and Morrison DK: Targeting the Raf kinases in human cancer: The Raf dimer dilemma. Br J Cancer 118: 3-8, 2018.
28. Bouchie A: First microRNA mimic enters clinic. Nat Biotechnol 31: 577, 2013.
29. Iorio MV and Croce CM: MicroRNA dysregulation in cancer: Diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med 4: 143-159, 2012.
30. Raza U, Zhang JD and Şahin Ö: MicroRNAs: Master regulators of drug resistance, stemness, and metastasis. J Mol Med 92: 321-336, 2014.
31. Lu Y, Yao J, Yu J, Wei Q and Cao X: The association between abnormal microRNA-10b expression and cancer risk: A meta-analysis. Sci Rep 4: 7498, 2014.
32. Ma Z, Chen Y, Min L, Li L, Huang H, Li J, Yan Q, Song P, Dai L and Yao X: Augmented miR-10b expression associated with depressed expression of its target gene KLF4 involved in gastric carcinoma. Int J Clin Exp Pathol 8: 5071-5079, 2015.
33. Ma L, Teruya-Feldstein J and Weinberg RA: Tumour invasion and metastasis initiated by microRNA-10b in breast cancer. Nature 449: 682-688, 2007.
34. Guo Y, Lang X, Lu Z, Wang J, Li T, Liao Y, Jia C, Zhao W and Fang H: MiR-10b directly targets ZEB1 and PIK3CA to curb adenomyotic epithelial cell invasiveness via upregulation of E-cadherin and inhibition of Akt phosphorylation. Cell Physiol Biochem 35: 2169-2180, 2015.
35. Zhang J, Xu L, Yang Z, Lu H, Hu D, Li W, Zhang Z, Liu B and Ma S: MicroRNA-10b indicates a poor prognosis of non-small cell lung cancer and targets E-cadherin. Clin Transl Oncol 17: 209-214, 2015.
36. Moriarty CH, Pursell B and Mercurio AM: miR-10b targets Tiam1 implications for Rac activation and carcinoma migration. J Biol Chemistry 285: 20541-20546, 2010.
37. Ahmad A, Ginnebaugh KR, Yin S, Bollig-Fischer A, Reddy KB and Sarkar FH: Functional role of miR-10b in tamoxifen resistance of ER-positive breast cancer cells through down-regulation of HDAC4. BMC Cancer 15: 540, 2015.
38. Nishida N, Yamashita S, Mimori K, Sudo T, Tanaka F, Shibata K, Yamamoto H, Ishii H, Doki Y and Mori M: MicroRNA-10b is a prognostic indicator in colorectal cancer and confers resistance to the chemotherapeutic agent 5-fluorouracil in colorectal cancer cells. Ann Surg Oncol 19: 3065-3071, 2012.
39. Wu K, Hu Y, Yan K, Qi Y, Zhang C, Zhu D, Liu D and Zhao S: microRNA-10b confers cisplatin resistance by activating AKT/mTOR/P70S6K signaling via targeting PPARγ in esophageal cancer. J Cell Physiol 235: 1247-1258, 2020.
40. Setoyama T, Zhang X, Natsugoe S and Calin GA: microRNA-10b: A new marker or the marker of pancreatic ductal adenocarcinoma? Clin Cancer Res 17: 5527-5529, 2011.
41. Huang J, Sun C, Wang S, He Q and Li D: MicroRNA miR-10b inhibition reduces cell proliferation and promotes apoptosis in non-small cell lung cancer (NSCLC) cells. Mol Biosyst 11: 2051-2059, 2015.
42. Fang CY, Yu CC, Liao YW, Hsieh PL, Ohiro Y, Chu PM, Huang YC, Yu CH and Tsai LL: miR-10b regulated by Twist maintains myofibroblasts activities in oral submucous fibrosis. J Formos Med Assoc 119: 1167-1173, 2020.
43. Tsukerman P, Stern-Ginossar N, Gur C, Glasner A, Nachmani D, Bauman Y, Yamin R, Vitenshtein A, Stanietsky N, Bar-Mag T, *et al*: MiR-10b downregulates the stress-induced cell surface molecule MICB, a critical ligand for cancer cell recognition by natural killer cells. Cancer Res 72: 5463-5472, 2012.

44. Wang L, Liu W, Zhang Y, Hu Z, Guo H, Lv J and Du H: Dexmedetomidine had neuroprotective effects on hippocampal neuronal cells via targeting lncRNA SHNG16 mediated microRNA-10b-5p/BDNF axis. Mol Cell Biochem 469: 41-51, 2020.
45. Yu X, Li Z, Chen G and Wu WK: MicroRNA-10b induces vascular muscle cell proliferation through Akt pathway by targeting TIP30. Curr Vasc Pharmacol 13: 679-686, 2015.
46. Lin CC, Yang TY, Lu HJ, Wan CK, Hsu SL and Wu CC: Attenuating role of withaferin A in the proliferation and migration of lung cancer cells via a p53-miR-27a/miR-10b pathway. Oncol Lett 21: 232, 2021.
47. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
48. Falasca M, Raimondi C and Maffucci T: Boyden chamber. In: Cell Migration Springer, pp87-95, 2011.
49. Yang TY, Teng CLJ, Lin TCC, Chen KC, Hsu SL and Wu CC: Transcriptional repression of Aurora-A gene by wild-type p53 through directly binding to its promoter with histone deacetylase 1 and mSin3a. Int J Cancer 142: 92-108, 2018.
50. Anaya J: OncoLnc: Linking TCGA survival data to mRNAs, miRNAs, and lncRNAs. PeerJ Computer Sci 2: e67, 2016.
51. Mavrevski R, Traykov M, Trenchev I and Trencheva M: Approaches to modeling of biological experimental data with GraphPad Prism software. WSEAS Transactions Systems Control 13: 242-247, 2018.
52. Lund AH: miR-10 in development and cancer. Cell Death Differ 17: 209-214, 2010.
53. Liao CG, Kong LM, Zhou P, Yang XL, Huang JG, Zhang HL and Lu N: miR-10b is overexpressed in hepatocellular carcinoma and promotes cell proliferation, migration and invasion through RhoC, uPAR and MMPs. J Transl Med 12: 234, 2014.
54. Wang Y, Li Z, Zhao X, Zuo X and Peng Z: miR-10b promotes invasion by targeting HOXD10 in colorectal cancer. Oncol Lett 12: 488-494, 2016.
55. Liu Y, Zhao J, Zhang PY, Zhang Y, Sun SY, Yu SY and Xi QS: MicroRNA-10b targets E-cadherin and modulates breast cancer metastasis. Med Sci Monit 18: BR299-BR308, 2012.
56. Lee DH, Kim C, Zhang L and Lee YJ: Role of p53, PUMA, and Bax in wogonin-induced apoptosis in human cancer cells. Biochem Pharmacol 75: 2020-2033, 2008.
57. Fan S, Smith ML, Rivet DJ II, Duba D, Zhan Q, Kohn KW, Fornace AJ Jr and O'Connor PM: Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. Cancer Res 55: 1649-1654, 1995.
58. Vazquez A, Bond EE, Levine AJ and Bond GL: The genetics of the p53 pathway, apoptosis and cancer therapy. Nat Rev Drug Discov 7: 979-987, 2008.
59. Huang Q, Song Q, Zhong W, Chen Y and Liang L: MicroRNA-10b and the clinical outcomes of various cancers: A systematic review and meta-analysis. Clin Chim Acta 474: 14-22, 2017.
60. Chen H, Fan Y, Xu W, Chen J, Xu C, Wei X, Fang D and Feng Y: miR-10b inhibits apoptosis and promotes proliferation and invasion of endometrial cancer cells via targeting HOXB3. Cancer Biother Radiopharm 31: 225-231, 2016.
61. Wang S, Wu Y, Xu Y and Tang X: miR-10b promoted melanoma progression through Wnt/β-catenin pathway by repressing ITCH expression. Gene 710: 39-47, 2019.
62. Han X, Yan S, Weijie Z, Feng W, Liuxing W, Mengquan L and Qingxia F: Critical role of miR-10b in transforming growth factor-β1-induced epithelial-mesenchymal transition in breast cancer. Cancer Gene Ther 21: 60-67, 2014.
63. Bourguignon LY: Matrix hyaluronan-CD44 interaction activates microRNA and lncRNA signaling associated with chemoresistance, invasion, and tumor progression. Front Oncol 9: 492, 2019.
64. El-Deiry WS: The role of p53 in chemosensitivity and radiosensitivity. Oncogene 22: 7486-7495, 2003.
65. Ho J, Ma W, Mao D and Benchimol S: p53-Dependent transcriptional repression of c-myc is required for G1 cell cycle arrest. Mol Cell Biol 25: 7423-7431, 2005.
66. Lu C, Jiang W, Hui B, Rong D, Fu K, Dong C, Tang W and Cao H: The circ_0021977/miR-10b-5p/P21 and P53 regulatory axis suppresses proliferation, migration, and invasion in colorectal cancer. J Cell Physiol 235: 2273-2285, 2020.
67. Sun B, Zhao X, Ming J, Liu X, Liu D and Jiang C: Stepwise detection and evaluation reveal miR-10b and miR-222 as a remarkable prognostic pair for glioblastoma. Oncogene 38: 6142-6157, 2019.
68. Hu W, Chan CS, Wu R, Zhang C, Sun Y, Song JS, Tang LH, Levine AJ and Feng Z: Negative regulation of tumor suppressor p53 by microRNA miR-504. Mol Cell 38: 689-699, 2010.
69. Liu C, Chen Z, Fang J, Xu A, Zhang W and Wang Z: H19-derived miR-675 contributes to bladder cancer cell proliferation by regulating p53 activation. Tumor Biol 37: 263-270, 2016.
70. Manfè V, Biskup E, Rosbjerg A, Kamstrup M, Skov AG, Lerche CM, Lauenborg BT, Odum N and Gniadecki R: miR-122 regulates p53/Akt signalling and the chemotherapy-induced apoptosis in cutaneous T-cell lymphoma. PLoS One 7: e29541, 2012.
71. Le MT, Teh C, Shyh-Chang N, Xie H, Zhou B, Korzh V, Lodish HF and Lim B: MicroRNA-125b is a novel negative regulator of p53. Genes Dev 23: 862-876, 2009.
72. Muller PA and Vousden KH: p53 mutations in cancer. Nat Cell Biol 15: 2-8, 2013.
73. Stiewe T: The p53 family in differentiation and tumorigenesis. Nat Rev Cancer 7: 165-167, 2007.
74. Bai M, Zhang H, Si L, Yu N, Zeng A and Zhao R: Upregulation of serum miR-10b is associated with poor prognosis in patients with melanoma. J Cancer 8: 2487-2491, 2017.
75. Khella HW, Daniel N, Youssef L, Scorilas A, Nofech-Mozes R, Mirham L, Krylov SN, Liandeau E, Krizova A, Finelli A, *et al*: miR-10b is a prognostic marker in clear cell renal cell carcinoma. J Clin Pathol 70: 854-859, 2017.
76. Wang YY, Ye ZY, Zhao ZS, Li L, Wang YX, Tao HQ, Wang HJ and He XJ: Clinicopathologic significance of miR-10b expression in gastric carcinoma. Hum Pathol 44: 1278-1285, 2013.
77. Wang P, Liang J, Wang Z, Hou H, Shi L and Zhou Z: The prognostic value of p53 positive in colorectal cancer: A retrospective cohort study. Tumor Biol 39: 1010428317703651, 2017.
78. Santoro A, Vlachou T, Luzi L, Melloni G, Mazzarella L, D'Elia E, Aobuli X, Pasi CE, Reavie L, Bonetti P, *et al*: p53 loss in breast cancer leads to Myc activation, increased cell plasticity, and expression of a mitotic signature with prognostic value. Cell Rep 26: 624-638.e8, 2019.
79. Yildirim M, Kaya V, Demirpence O, Gunduz S and Bozcuk H: Prognostic significance of p53 in gastric cancer: A meta-analysis. Asian Pac J Cancer Prev 16: 327-332, 2015.
80. Bisio A, De Sanctis V, Del Vescovo V, Denti MA, Jegga AG, Inga A and Ciribilli Y: Identification of new p53 target microRNAs by bioinformatics and functional analysis. BMC Cancer 13: 552, 2013.
81. Tung MC, Lin PL, Wang YC, He TY, Lee MC, Yeh SD, Chen CY and Lee H: Mutant p53 confers chemoresistance in non-small cell lung cancer by upregulating Nrf2. Oncotarget 6: 41692-41705, 2015.