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Circ_0110498 facilitates the cisplatin resistance of non-small cell lung cancer by mediating the miR-1287-5p/RBBP4 axis

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Abstract

Background: Circular RNAs (circRNAs) play vital roles in non-small cell lung cancer (NSCLC) progression. Our research analyzed the role of circ_0110498 on the cisplatin (DDP) resistance of NSCLC.

Methods: Cell glycolysis was analyzed by measuring glucose consumption and lactate production. Protein expression was determined by western blot analysis. The expression of circ_0110498, microRNA (miR)-1287-5p and RBBP4 was detected by RT-qPCR assay. Cell counting kit-8, colony formation and transwell assays, together with flow cytometry were conducted to analyze cell DDP resistance, proliferation, metastasis and apoptosis.

Results: Circ_0110498 expression was elevated in DDP-resistant NSCLC tissues and cells. Circ_0110498 silencing not only suppressed the DDP resistance of NSCLC cells by inhibiting cell growth, metastasis and glycolysis, but also enhanced the DDP sensitivity of NSCLC tumors. MiR-1287-5p was sponged by circ_0110498, and its inhibitor also reversed the effect of circ_0110498 silencing on the DDP resistance of NSCLC cells. MiR-1287-5p interacted with RBBP4, and RBBP4 overexpression partly reversed the inhibitory effect of miR-1287-5p on the DDP resistance of NSCLC cells.

Conclusion: Circ_0110498 facilitated DDP resistance partly through mediating the miR-1287-5p/RBBP4 signaling in NSCLC.

KEYWORDS
circ_0110498, cisplatin, miR-1287-5p, non-small cell lung cancer, RBBP4

INTRODUCTION

Lung cancer is a common fatal cancer, accounting for 18.4% of cancer-associated deaths worldwide,[1] among which non-small cell lung cancer (NSCLC) is the main pathological subtype.[2] Cisplatin (DDP) is a platinum-based antitumor drug, which is widely used in NSCLC treatment as the major adjuvant method following surgery.[3] However, the development of DDP resistance limits its therapeutic efficacy.[4] Hence, illustrating the mechanism behind DDP resistance is essential to provide new effective therapeutic strategies for NSCLC.

Accumulating evidence demonstrates that circular RNAs (circRNAs) are vital regulators in carcinogenesis, including NSCLC. For instance, circ_0001869 had been found to promote NSCLC malignant properties by the miR-638/FOSL2 axis.[5] Circ_0001946 has been reported to suppress NSCLC cell malignant behaviors and chemoresistance by targeting NER signaling.[6] A previous study found that circ_0110498 facilitated NSCLC cell glycolysis and proliferation abilities by targeting miR-515-5p/HDGF signaling.[7] However, the role of circ_0110498 in NSCLC chemoresistance is still largely unknown.

CircRNAs can act as microRNAs (miRNAs) molecular sponges.[8] MiRNAs are implicated in regulating cancer progression and chemoresistance.[9,10] By bioinformatic prediction, miR-1287-5p has been found to be a possible target of circ_0110498. MiR-1287 plays an antitumor role in NSCLC. For example, circ_0016760 aggravated NSCLC development through mediating the miR-1287/GAGE1 axis,[11] and circ_0026134 facilitated NSCLC development by sponging miR-1256.[12] In this study, we tested the target relationship between circ_0110498 and miR-1287-5p. MiRNAs can

regulate cell biological behaviors by binding to messenger RNAs (mRNAs).[13] RBBP4 was predicted as a potential downstream gene of miR-1287-5p by the TargetScan database. A previous study showed that RBBP4 facilitated NSCLC cell growth and metastasis.[14] In this study, we tested the binding relationship between RBBP4 and miR-1287-5p.

Here, we first analyzed the role of circ_0110498 in regulating the DDP resistance of NSCLC. Subsequently, the interaction and potential mechanism among miR-1287-5p, circ_0110498, and RBBP4 were explored.

METHODS

Clinical specimens

NSCLC specimens (DDP-resistant: $n = 30$; DDP-sensitive: $n = 24$) and adjacent healthy specimens ($n = 54$) were harvested at The First Affiliated Hospital of Zhengzhou University. This study was approved by the Ethics Committee of The First Affiliated Hospital of Zhengzhou University. All the participants signed their written informed consent.

Cell cultivation

NSCLC cell lines (A549 and H1299) and human bronchial epithelioid cell line 16HBE were purchased from BNCC (Beijing). A549/DDP and H1299/DDP cells were constructed as described in a previous study.[15] Cells were cultivated in RPMI-1640 (Hyclone) with 10% FBS (Hyclone) and 1% penicillin/streptomycin (Invitrogen).

Analysis of cell glycolysis

Cell glycolytic rate was assessed through analyzing glucose consumption and lactate production using a commercial glucose/lactate assay kit (BioVision).

Western blot assay

Protein samples were obtained with RIPA buffer (Beyotime). Samples were loaded onto 10% separating gel and blotted to PVDF membrane. The membrane was labeled with the specific primary antibodies (Abcam), including anti-HK2 (ab209847), anti-LDHA (ab101562), anti-RBBP4 (ab92344), and anti-β-actin (ab8226). After labeling with the respective secondary antibody, protein signal was detected via ECL reagent (Beyotime).

RT-qPCR

RNA extracted by TRIzol reagent (Invitrogen) was reverse transcribed using a Prime-Script RT master mix kit (Takara). Thermal cycling reaction was implemented using SYBR Green (Takara) and specific primers (Table 1). The fold change was analyzed by the $2^{-\Delta\Delta Ct}$ method. For RNase R treatment, 2 μg RNAs were mixed with 6 U RNase R and a quantitative reverse transcription PCR (RT-qPCR) assay was performed. RNAs in nucleus and cytoplasm were extracted using PARIS kit (Invitrogen) according to the protocol of a previous study.[16]

Oligonucleotides, plasmids, and transfection

Small interference (si)RNA of circ_0110498 (si-circ_0110498), short hairpin (sh)RNA of circ_0110498 (sh-circ_0110498), miR-1287-5p mimics, miR-1287-5p inhibitor (anti-miR-1287-5p), RBBP4 ectopic expression plasmid (RBBP4), and controls were synthesized by GenePharma.

Cell counting kit-8 (CCK8) assay

A cell counting kit-8 (CCK8) assay was adopted to assess cell chemoresistance and proliferation capacity. NSCLC

TABLE 1 Specific primers in the RT-qPCR assay

Gene	Direction	Sequence
circ_0110498	forward	5′-TCAGCAAGATGGAAAGAATGG-3′
	reverse	5′-CACTTGCTGCAGTTTGTGGT-3′
MAGI3	forward	5′-CCCCCAGGGATGGAGAAGTA-3′
	reverse	5′-AGAAGTTTCCATCATATGTCCCAC-3′
miR-1287-5p	forward	5′-GCGGTGCTGGATCAGTGG-3′
	reverse	5′-CAGTGCAGGGTCCGAGGTAT-3′
RBBP4	forward	5′-CCGACAAGGAAGCCTTCGAC-3′
	reverse	5′-TCACTGTCGTAGTGTGACGC-3′
GAPDH	forward	5′-TATGATGACATCAAGAAGGTGGT-3′
	reverse	5′-TGTAGCCAAATTCGTTGTCATAC-3′
U6	forward	5′-GCTTCGGCAGCACATATACTAAAAT-3′
	reverse	5′-CGCTTCACGAATTTGCGTGTCAT-3′

Abbreviation: RT-qPCR, quantitative reverse transcription PCR

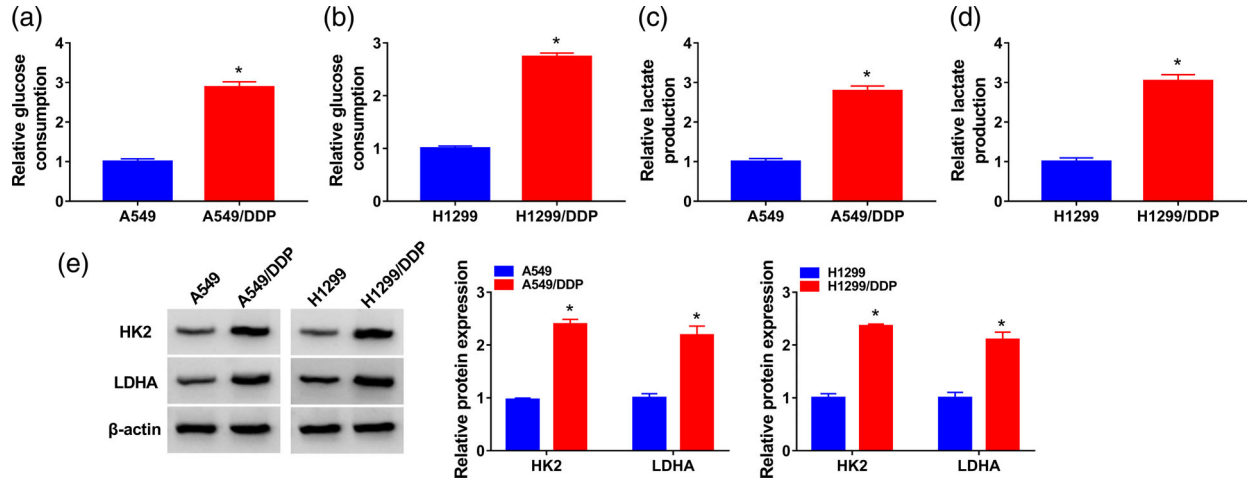

FIGURE 1 Glycolytic metabolism is enhanced in DDP-resistant non-small cell lung cancer (NSCLC) cells. (a–d) The glucose consumption and lactate production were determined using a commercial glucose/lactate assay kit. (e) Western blot assay was conducted to examine the expression of HK2 and LDHA. *$p < 0.05$

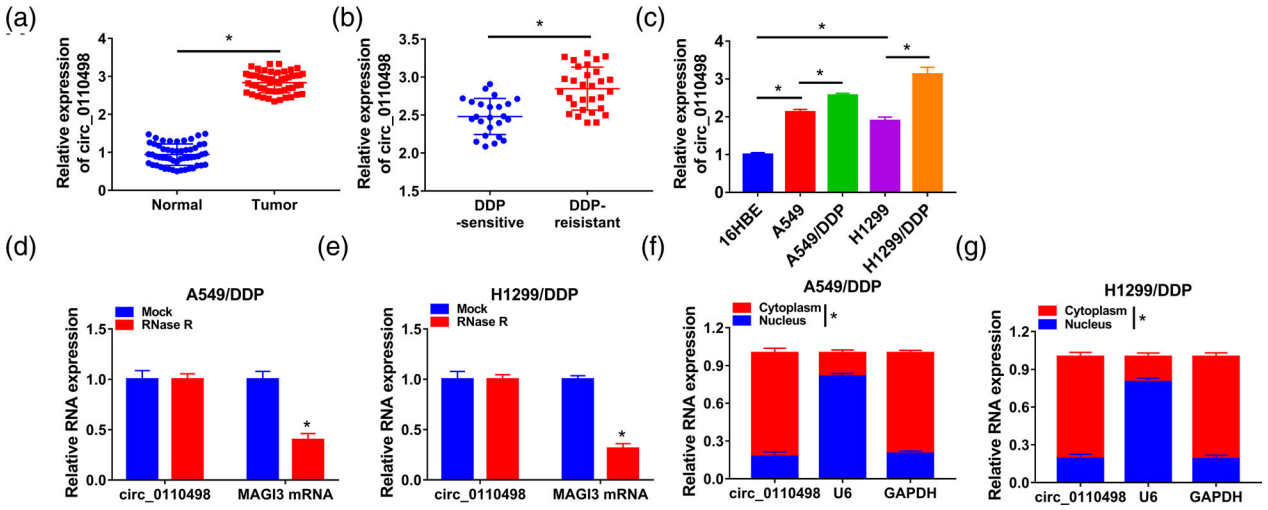

FIGURE 2 Circ_0110498 expression is elevated in DDP-resistant non-small cell lung cancer (NSCLC) tissues and cell lines. (a) The expression of circ_0110498 was determined in 54 pairs of NSCLC tissues and adjacent normal tissues by quantitative reverse transcription PCR (RT-qPCR) assay. (b) The expression of circ_0110498 in DDP-sensitive NSCLC tissues ($n = 24$) and DDP-resistant NSCLC tissues ($n = 30$) was analyzed by RT-qPCR. (c) The level of circ_0110498 was examined in parental NSCLC cell lines, DDP-resistant NSCLC cell lines, and 16HBE cell line by RT-qPCR. (d and e) RNase R treatment. (f and g) Subcellular localization assay. *$p < 0.05$

cells were seeded onto 96-well plates. After the indicated treatment, 20 μl CCK8 solution (Beyotime) was pipetted to the wells and the cells were incubated for 4 h. The optical density at 450 nm was detected.

Colony formation assay

Cells were dispersed in the medium at low density. Cell suspension was added to a 12-well plate. NSCLC cells were cultivated for 2 weeks, and the colonies were immobilized, stained and counted.

Transwell assays

Uncoated transwell chambers (Corning) were adopted to analyze cell migration, and chambers coated with Matrigel (Corning) were utilized to assess cell invasion. Transfected cells (transwell migration assay: 2×10^4 cells/well; transwell invasion

assay: 8×10^4 cells/well) with serum-free medium were added to the upper chambers. After 24 h, migrated or invaded cells were dyed using crystal violet, and cells were counted at 100×.

Flow cytometry

NSCLC cells were seeded onto six-well plates. After the indicated transfection, NSCLC cells were dyed with Annexin V-FITC and PI (BestBio). The apoptotic cells were captured via the flow cytometer.

Xenograft tumor model

A cell inoculation method was used for constructing the animal model. A total of 24 BALB/c nude mice were arbitrarily divided into four groups ($n = 6$ in each group): sh-NC + PBS group (mice were inoculated with A549/DDP cells stably expressing sh-NC followed by the administration of PBS), sh-NC + DDP group (mice were inoculated with A549/DDP cells stably expressing sh-NC followed by the administration of DDP), sh-circ_0110498 + PBS group (mice were inoculated with A549/DDP cells stably expressing sh-circ_0110498 followed by the administration of PBS), and sh-circ_0110498 + DDP group (mice were inoculated with A549/DDP cells stably expressing sh-circ_0110498 followed by the administration of DDP). Tumor volume was analyzed every 7 days. After 28 days, the mice were sacrificed and tumor weight was recorded. The procedures were endorsed by the Animal Ethics Committee of The First Affiliated Hospital of Zhengzhou University.

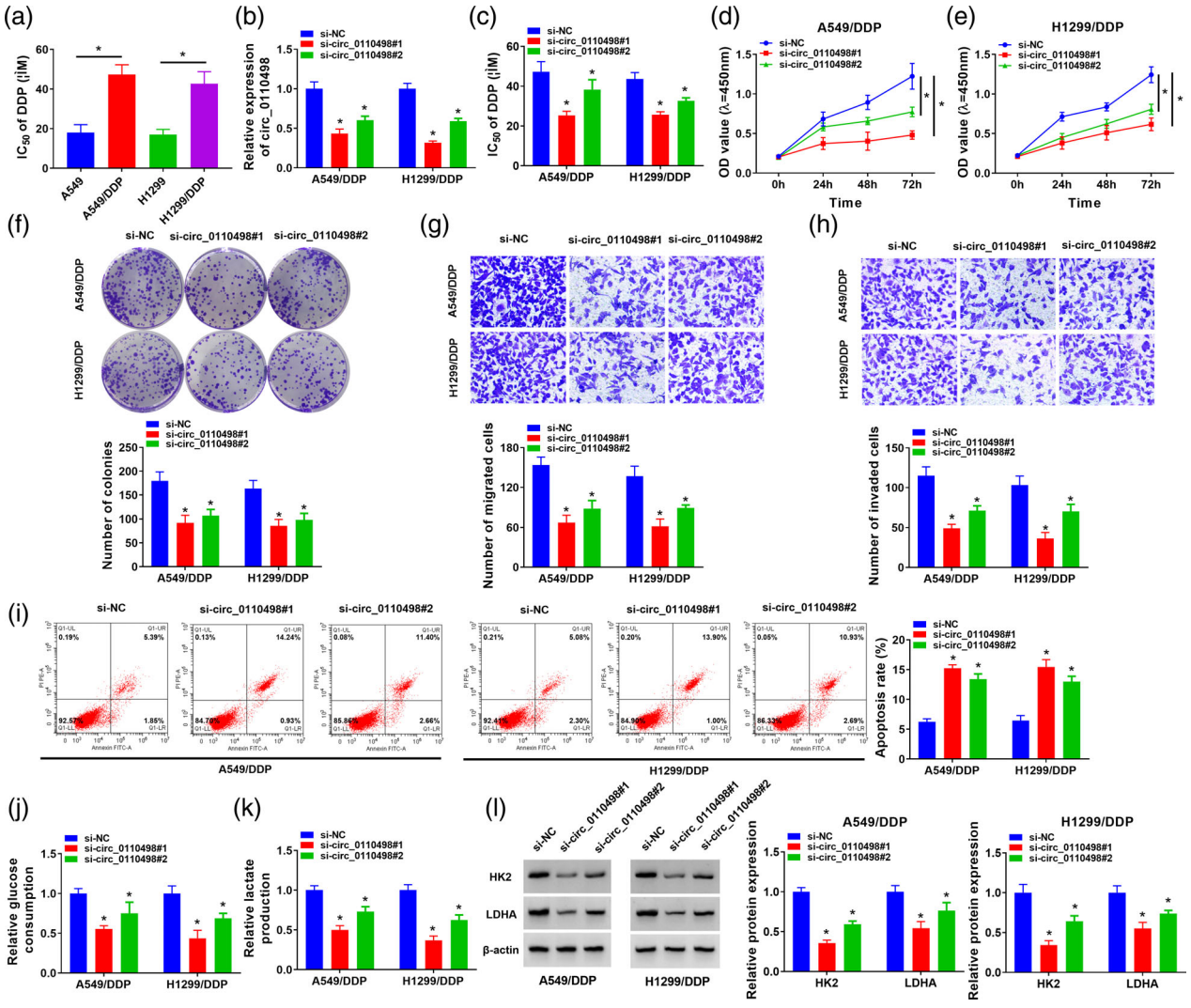

FIGURE 3 Circ_0110498 knockdown elevates the DDP sensitivity and suppresses the malignant behaviors of non-small cell lung cancer (NSCLC) cells. (a) Cell counting kit-8 (CCK8) assay was performed to analyze the IC_{50} value of DDP. (b–l) A549/DDP and H1299/DDP cells were transfected with si-NC, si-circ_0110498#1, or si-circ_0110498#2. (b) Quantitative reverse transcription PCR (RT-qPCR) assay was performed to measure the expression of circ_0110498 in transfected NSCLC cells. (c) CCK8 assay. (d and e) CCK8 assay. (f) Colony formation assay. (g and h) Transwell assay. (i) Flow cytometry. (j and k) The glucose consumption or lactate production were analyzed using commercial glucose/lactate assay kit. (l) Western blot assay. $^*p < 0.05$

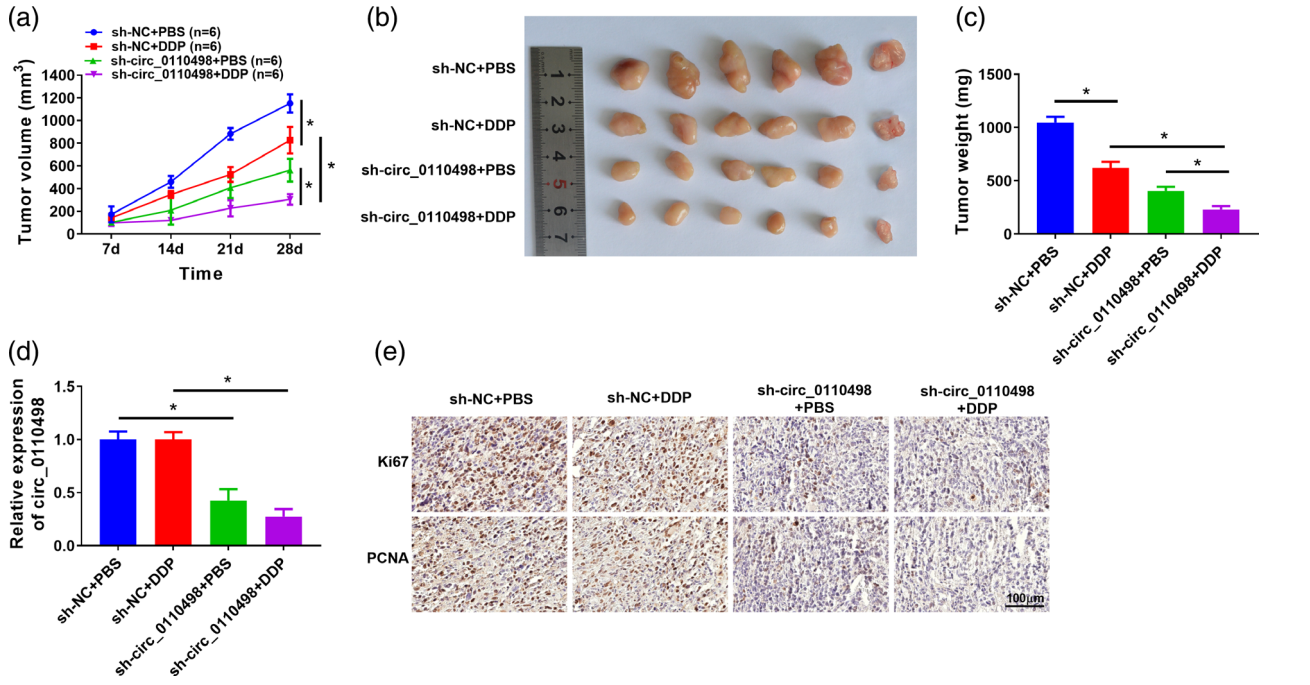

FIGURE 4 Circ_0110498 silencing elevates the sensitivity of DDP-resistant non-small cell lung cancer (NSCLC) tumors to DDP in vivo. A549/DDP cells stably expressing sh-NC or sh-circ_0110498 were inoculated into the BALB/c nude mice ($n = 6$/group). DDP (5 mg/kg) or PBS was administered every 3 days at 7 days post-inoculation. (a) Tumor volume was measured every 7 days as length × width^2 × 0.5. (b and c) Mice were sacrificed at 28 days post-inoculation, and resected tumors were imaged and weighed. (d) The expression of circ_0110498 was determined in tumors by quantitative reverse transcription PCR (RT-qPCR). (e) An immunohistochemical (IHC) assay was performed to analyze the protein levels of Ki67 and PCNA in tumor tissues. *$p < 0.05$

Dual-luciferase reporter assay

Circinteractome software was utilized to establish the association between circ_0110498 and miRNAs, and TargetScan database was adopted to establish miR-1287-5p-mRNAs associations. The sequence of circ_0110498 or RBBP4 (wild-type or mutant) was inserted into pmirGLO vector. WT-circ_0110498, MUT-circ_0110498, WT-RBBP4 3' UTR, and MUT-RBBP4 3' UTR were introduced into NSCLC cells with miR-1287-5p or miR-NC. The luciferase activities were examined.

RIP assay

Magna RNA immunoprecipitation (RIP) kit was utilized in this assay. Cell extracts were incubated with magnetic beads labeled with AGO2 antibody or IgG antibody. The enriched RNAs were analyzed by RT-qPCR.

Statistical analysis

Data were analyzed by GraphPad Prism software and expressed as mean ± SD. Student's *t*-test and ANOVA were utilized to analyze the differences. $p < 0.05$ was considered statistically significant.

RESULTS

Glycolytic metabolism is enhanced in DDP-resistant NSCLC cells

Accumulating evidence has revealed an association between glycolytic metabolism and chemoresistance.[17,18] The glycolytic metabolism of DDP-resistant NSCLC cells was significantly enhanced, as evidenced by the increased glucose consumption and lactate production (Figure 1a–d). Moreover, the expression of HK2 and LDHA was markedly elevated in DDP-resistant NSCLC cells (Figure 1e).

Circ_0110498 expression is elevated in DDP-resistant NSCLC tissues and cells

Circ_0110498 expression was significantly upregulated in NSCLC tissues ($n = 54$) (Figure 2a), and was elevated in DDP-resistant NSCLC tissues ($n = 30$) (Figure 2b). Circ_0110498 level was increased in parental NSCLC cells relative to 16HBE cells, and its expression was elevated in DDP-resistant NSCLC cells (Figure 2c). Circ_0110498 expression was unaffected upon RNase R digestion (Figure 2d, e). Similar to GAPDH, circ_0110498 was majorly localized in the cytoplasm (Figure 2f, g).

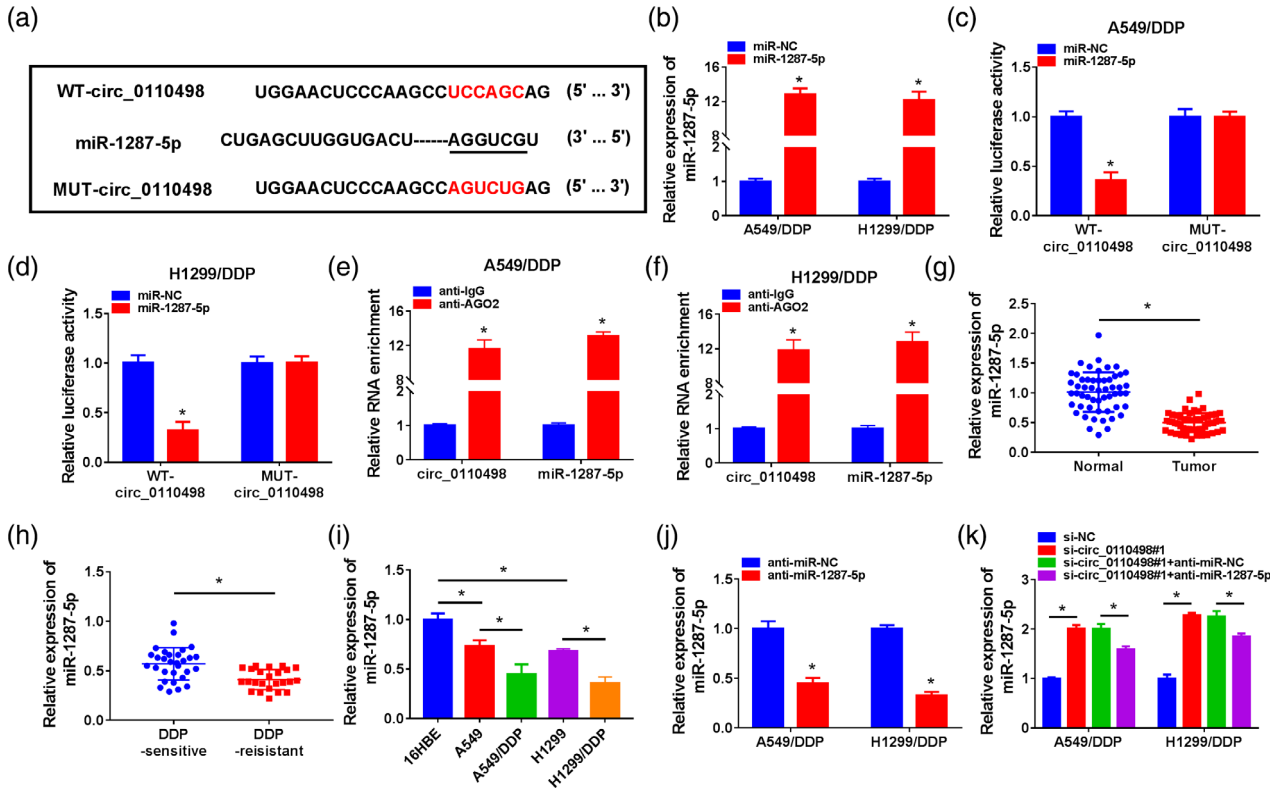

FIGURE 5 miR-1287-5p is a target of circ_0110498. (a) Circ_0110498-miRNA interactions were predicted, and the putative binding sites between circ_0110498 and miR-1287-5p are shown. (b) The transfection efficiency of miR-1287-5p mimics in A549/DDP and H1299/DDP was analyzed by RT-qPCR. (c and d) Dual-luciferase reporter assay. (e and f) RNA immunoprecipitation (RIP) assay. (g) Quantitative reverse transcription PCR (RT-qPCR) was performed to measure the expression of miR-1287-5p in non-small cell lung cancer (NSCLC) tissues ($n = 54$) and adjacent normal tissues ($n = 54$). (h) The level of miR-1287-5p was examined in DDP-sensitive NSCLC tissues ($n = 24$) and DDP-resistant NSCLC tissues ($n = 30$) by RT-qPCR. (i) The level of miR-1287-5p was determined by RT-qPCR. (j) The transfection efficiency of anti-miR-1287-5p was assessed by RT-qPCR. (k) RT-qPCR assay was conducted to measure the expression of miR-1287-5p in transfected NSCLC cells. $^*p < 0.05$

Circ_0110498 knockdown elevates the DDP sensitivity of NSCLC cells

The IC_{50} value of DDP was notably elevated in A549/DDP and H1299/DDP cells (Figure 3a). We silenced circ_0110498 using si-circ_0110498#1 or si-circ_0110498#2 to analyze the role of circ_0110498 in the DDP sensitivity of NSCLC. RT-qPCR assay showed that the transfection efficiencies of si-circ_0110498#1 and si-circ_0110498#2 were both high in NSCLC cells (Figure 3b). Circ_0110498 knockdown reduced the IC_{50} value of DDP (Figure 3c). Circ_0110498 silencing significantly repressed the OD value after 72-h transfection (Figure 3d, e), demonstrating that circ_0110498 silencing suppressed NSCLC cell proliferation. Circ_0110498 silencing decreased the number of colonies, migrated cells and invaded cells (Figure 3g, h). Cell apoptosis was notably triggered in si-circ_0110498-transfected group (Figure 3i). Circ_0110498 knockdown markedly restrained glucose uptake, lactate production, HK2 and LDHA expression in NSCLC cells (Figure 3j–l).

Circ_0110498 silencing elevates the DDP sensitivity of NSCLC tumor

DDP treatment suppressed tumor volume, size and weight, and these effects were further restrained in sh-circ_0110498 + DDP group (Figure 4a–c). Circ_0110498 expression was notably decreased in the tumor tissues of sh-circ_0110498 + PBS group and sh-circ_0110498 + DDP group compared with their corresponding control groups (Figure 4d). In addition, immunohistochemical (IHC) staining results showed that the positive cells of proliferation markers Ki67 and PCNA were reduced in the tumor tissues of sh-circ_0110498 + DDP group (Figure 4 e).

MiR-1287-5p is sponged by circ_0110498

The possible miRNA targets of circ_0110498 was predicted using Circinteractome software. Among all the predicted targets, we selected miR-142-3p,[19] miR-145-5p,[20]

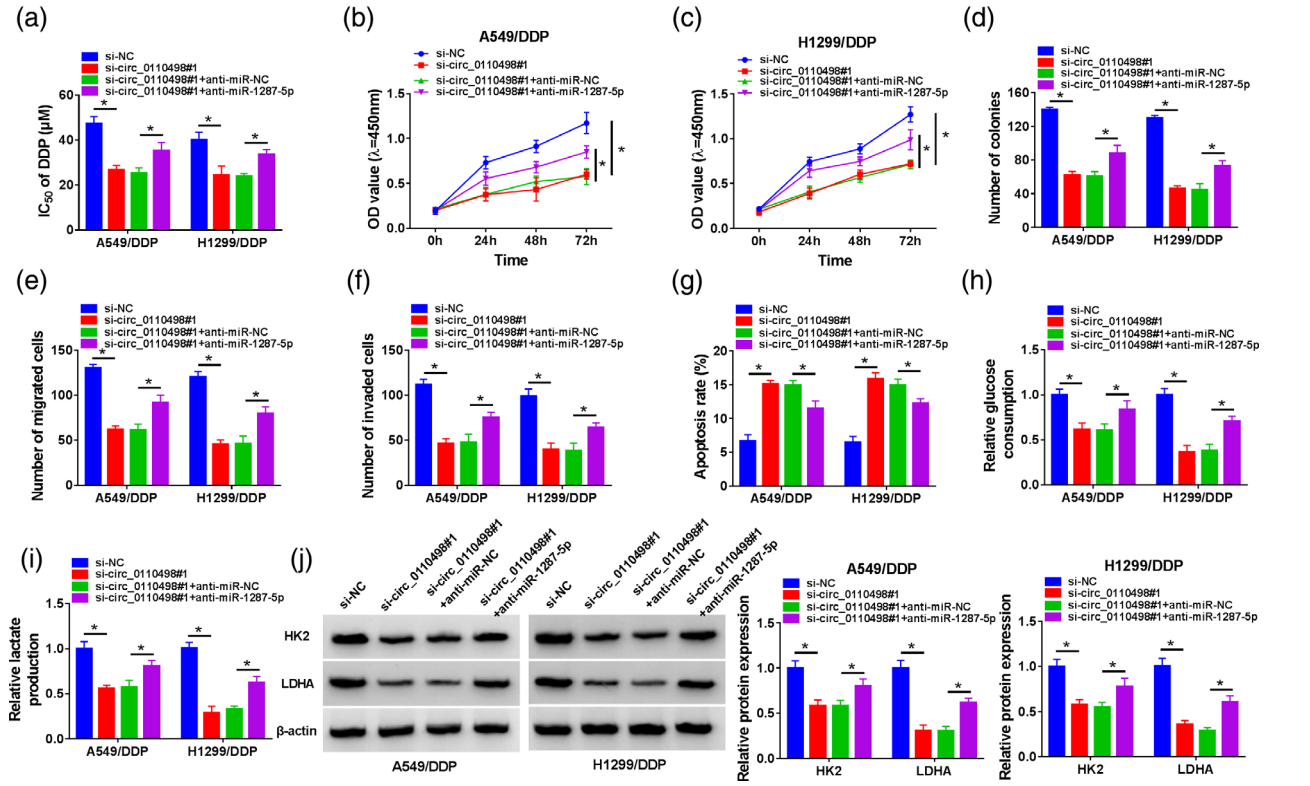

FIGURE 6 Circ_0110498 knockdown suppresses the DDP resistance of non-small cell lung cancer (NSCLC) cells by upregulating miR-1287-5p. (a–j) A549/DDP and H1299/DDP cells were transfected with si-circ_0110498 alone or together with anti-miR-1287-5p. (a) Cell counting kit-8 (CCK8) assay. (b–d) CCK8 assay and colony formation assay. (e and f) Transwell assay. (g) Flow cytometry. (h and i) The glucose consumption or lactate production were analyzed using a commercial glucose/lactate assay kit. (j) Western blot assay. $^*p < 0.05$

miR-149,[21] miR-331-3p,[22] miR-1287-5p,[11,12] miR-515-5p,[23] and miR-873-3p[24] for further experiments. The expression of miR-149, miR-1287-5p, miR-515-5p, and miR-873-3p was upregulated in circ_0110498-silenced NSCLC cells, especially miR-1287-5p (Figure S1A, B). Therefore, we further assessed the target relationship between circ_0110498 and miR-1287-5p. The putative binding sites between circ_0110498 and miR-1287-5p are shown in Figure 5a. High transfection efficiency of miR-1287-5p mimics was verified by RT-qPCR (Figure 5b). MiR-1287-5p overexpression reduced the luciferase activity of WT-circ_0110498 vector (Figure 5c, d). Both circ_0110498 and miR-1287-5p were pulled down when using AGO2 antibody (Figure 5e, f). MiR-1287-5p expression was reduced in NSCLC tissues (Figure 5g) and was negatively correlated with circ_0110498 expression (Figure S2A). Furthermore, miR-1287-5p level was decreased in DDP-resistant NSCLC tissues (Figure 5h). MiR-1287-5p expression was downregulated in DDP-resistant NSCLC cells (Figure 5i). In addition, we confirmed that the knockdown efficiency of anti-miR-1287-5p was high (Figure 5j). In H1299/DDP and A549/DDP cells transfected with si-circ_0110498#1 and anti-miR-1287-5p, we found that circ_0110498 silencing upregulated miR-1287-5p expression, and anti-miR-1287-5p reduced this effect (Figure 5k).

Circ_0110498 knockdown suppresses the DDP resistance of NSCLC cells by miR-1287-5p

We then analyzed whether circ_0110498 sponged miR-1287-5p to regulate NSCLC cell DDP resistance via rescue experiments. With the transfection of anti-miR-1287-5p, the IC_{50} value of DDP was partly rescued (Figure 6a). Cell proliferation was partly recovered in si-circ_0110498 and anti-miR-1287-5p cotransfected group (Figure 6b–d). Also, circ_0110498 knockdown induced suppressive effects on cell migration and invasion were partly diminished by silencing miR-1287-5p (Figure 6e, f). Circ_0110498 silencing-induced cell apoptosis was partly attenuated by miR-1287-5p inhibitor (Figure 6g). Circ_0110498 silencing inhibited the glucose consumption and lactate production, which were alleviated by anti-miR-1287-5p (Figure 6h, i). The introduction of anti-miR-1287-5p also partly rescued HK2 and LDHA expression in circ_0110498-silenced cells (Figure 6J).

RBBP4 is targeted by miR-1287-5p

The bioinformatic software TargetScan was utilized to predict the possible mRNA targets of miR-1287-5p. Among all the predicted targets, we selected six mRNAs due to their

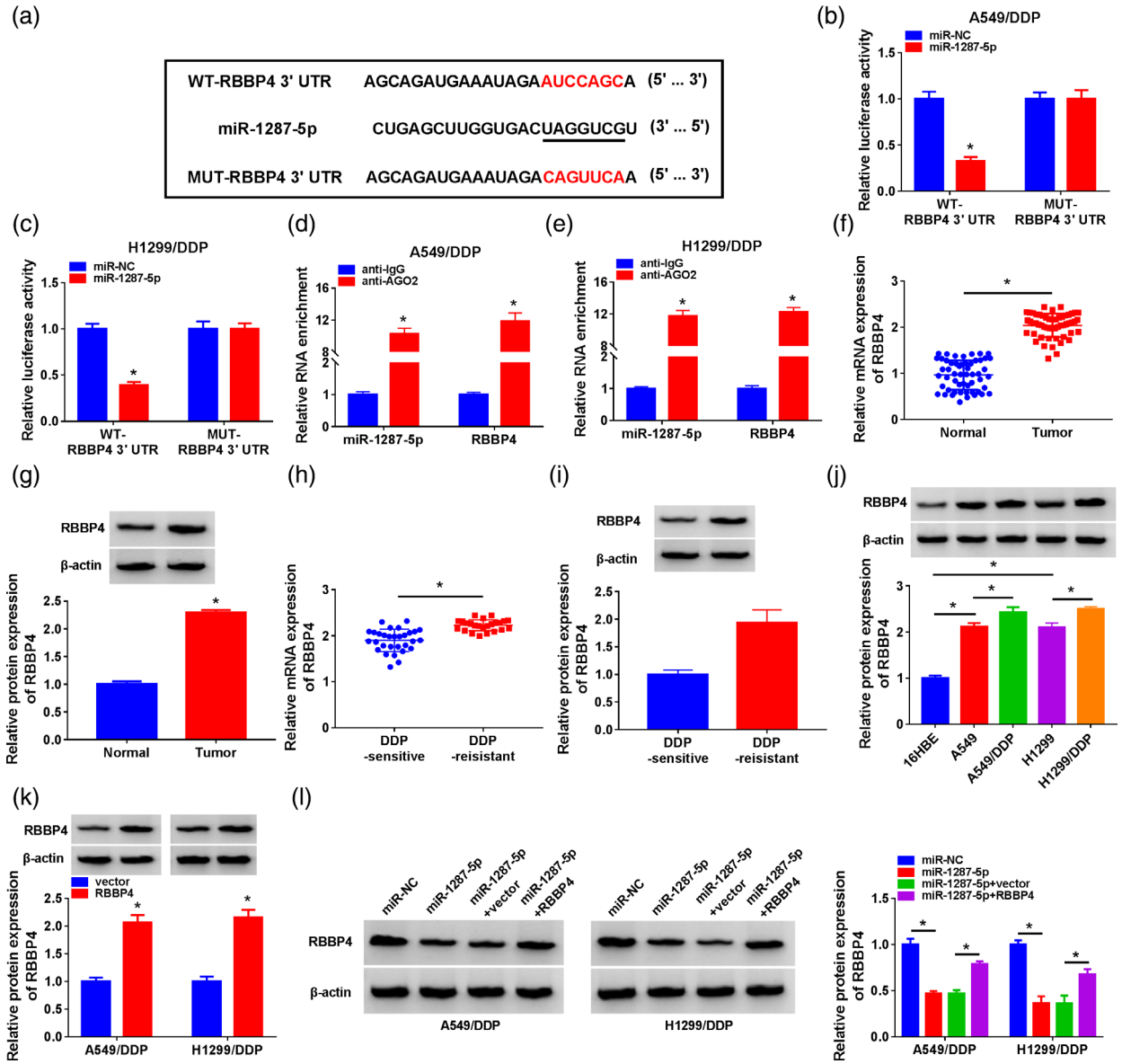

FIGURE 7 RBBP4 is a target of miR-1287-5p. (a) The potential target relation between miR-1287-5p and RBBP4 was predicted. (b and c) Dual-luciferase reporter assay. (d and e) RNA immunoprecipitation (RIP) assay. (f and g) Quantitative reverse transcription PCR (RT-qPCR) and Western blot assay were conducted to measure the mRNA and protein expression of RBBP4. (h and i) The mRNA and protein expression of RBBP4 in DDP-sensitive non-small cell lung cancer (NSCLC) tissues and DDP-resistant NSCLC tissues was examined by RT-qPCR and Western blot assay. (j) Western blot assay was performed to determine the protein level of RBBP4. (k) The transfection efficiency of RBBP4 plasmid was analyzed by Western blot assay. (l) The protein expression of RBBP4 was measured by Western blot assay. $^*p < 0.05$

high expression characteristic or oncogenic role in NSCLC, including FOSL2,[5] NOVA2,[25] RBBP4,[14] CORO1C,[26] CCND1,[27] and RASAL2.[28] After overexpressing miR-1287-5p, we found that the expression of RBBP4, CORO1C, and CCND1 was reduced, especially RBBP4 (Figure S1C, D). The potential binding sites between miR-1287-5p and RBBP4 are shown in Figure 7a. The luciferase activity was notably reduced in WT-RBBP4 3' UTR group following the overexpression of miR-1287-5p (Figure 7b, c). RIP assay further confirmed the interaction between miR-1287-5p and RBBP4 (Figure 7d, e). The mRNA and protein expression of RBBP4 was upregulated in NSCLC tumor tissues (Figure 7f, g). The level of RBBP4 mRNA was negatively correlated with miR-1287-5p level (Figure S2B), and positively correlated with circ_0110498 level (Figure S2C). The mRNA and protein expression of RBBP4 was elevated in DDP-resistant NSCLC tissues (Figure 7h, i). RBBP4 protein expression was increased in DDP-resistant NSCLC cell lines (Figure 7j). The transfection efficiency of RBBP4 plasmid was significant in NSCLC cells (Figure 7k). MiR-1287-5p mimic reduced RBBP4 protein expression, and its protein level was rescued by RBBP4 overexpression (Figure 7l).

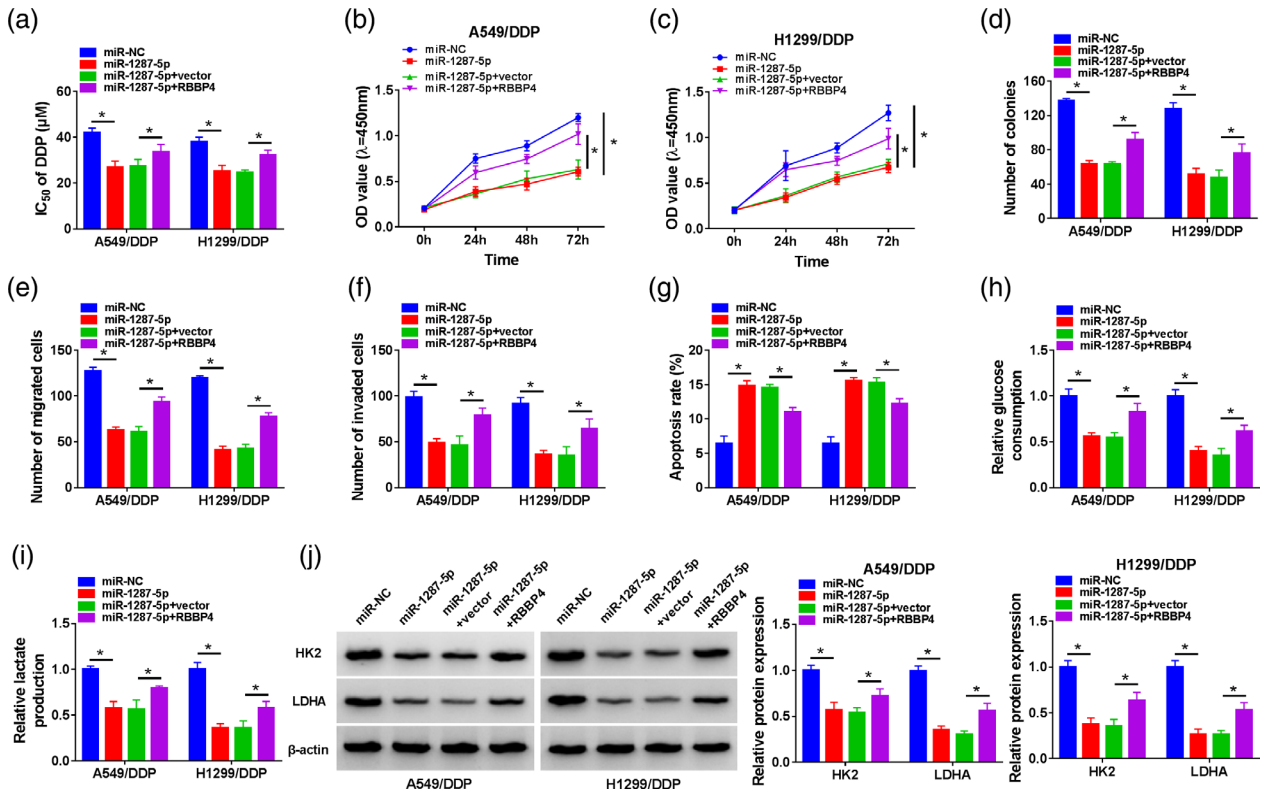

FIGURE 8 MiR-1287-5p plays a tumor suppressor role in non-small cell lung cancer (NSCLC) cells partly by downregulating RBBP4. (a–j) A549/DDP and H1299/DDP cells were transfected with miR-1287-5p alone or together with RBBP4 plasmid. (a) The resistance of NSCLC cells to DDP was assessed by cell counting kit-8 (CCK8) assay. (b–d) CCK8 assay and colony formation assay. (e and f) Transwell assay. (g) Flow cytometry. (h and i) Cell glycolytic metabolism was analyzed using a commercial glucose/lactate assay kit. (j) Western blot assay. $^*p < 0.05$

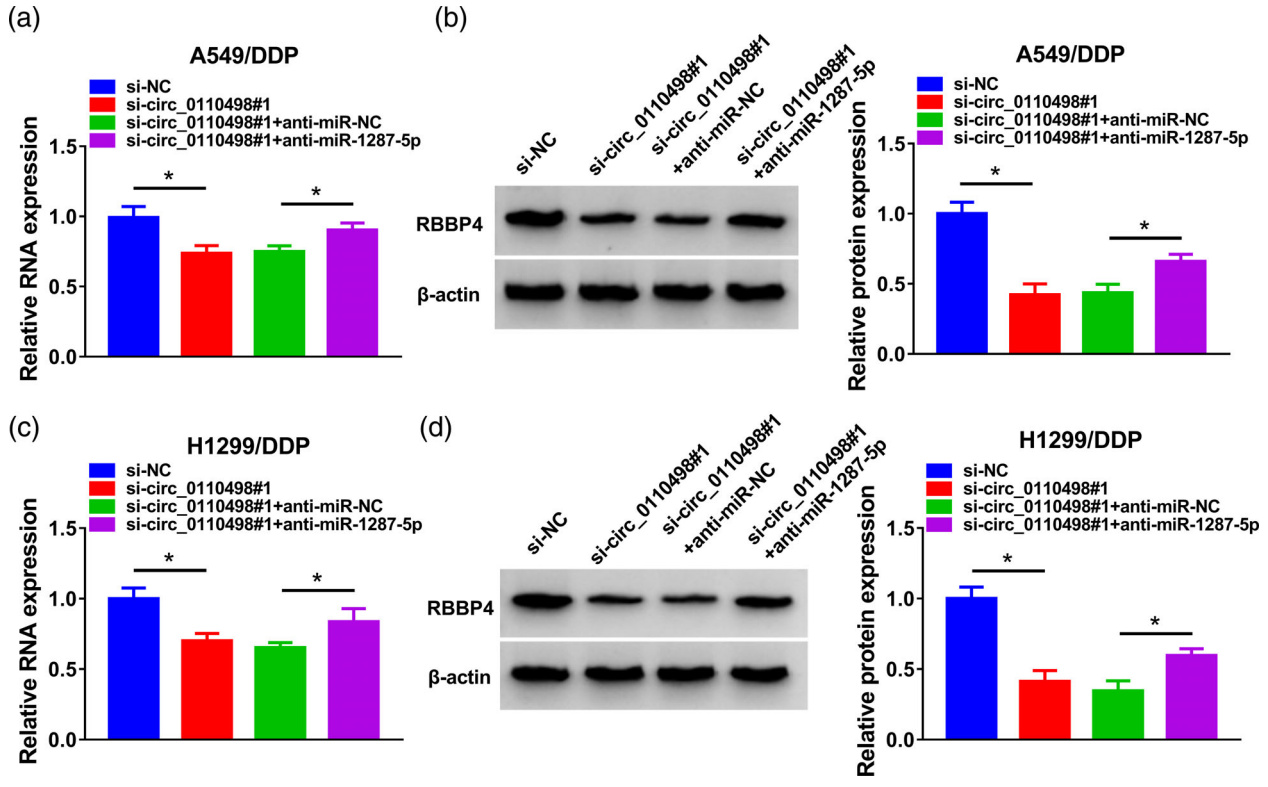

FIGURE 9 Circ_0110498 silencing reduces RBBP4 expression partly by upregulating miR-1287-5p in non-small cell lung cancer (NSCLC) cells. (a–d) The mRNA and protein expression of RBBP4 was examined in A549/DDP and H1299/DDP cells transfected with si-circ_0110498 alone or together with anti-miR-1287-5p by quantitative reverse transcription PCR (RT-qPCR) and Western blot assay. $^*p < 0.05$

MiR-1287-5p plays a tumor suppressor role in NSCLC cells partly by RBBP4

MiR-1287-5p overexpression reduced the DDP resistance of NSCLC cells, and RBBP4 plasmid recovered this effect (Figure 8a). MiR-1287-5p overexpression inhibited cell proliferation ability, which was overturned by the overexpression of RBBP4 (Figure 8b–d). MiR-1287-5p overexpression suppressed cell migration and invasion, and the addition of RBBP4 plasmid partly rescued cell metastasis (Figure 8e, f). MiR-1287-5p overexpression induced cell apoptosis, while this effect was overturned by RBBP4 overexpression (Figure 8g). MiR-1287-5p overexpression inhibited cell glycolysis, and the addition of RBBP4 plasmid partly recovered this effect (Figure 8h–j).

Circ_0110498 silencing reduces RBBP4 expression partly by miR-1287-5p

Circ_0110498 silencing reduced the mRNA and protein expression of RBBP4, and anti-miR-1287-5p partly rescued this effect (Figure 9a–d). These results suggested that circ_0110498 indirectly regulated RBBP4 expression by serving as miR-1287-5p sponge in NSCLC cells. To clarify the functional correlation between circ_0110498 and RBBP4 in NSCLC, we performed rescue experiments. Circ_0110498 silencing suppressed the DDP resistance of NSCLC cells, which were largely reversed by overexpressing RBBP4 (Figure S3A–J). In addition, we detected circ_0110498, miR-1287-5p and RBBP4 expression in A549 and H1299 cells treated with or without DDP, and confirmed that circ_0110498 and RBBP4 were significantly upregulated, while miR-1287-5p was markedly downregulated in A549 and H1299 cells treated with DDP (Figure S4A–C).

DISCUSSION

Accumulating evidence have previously reported the "miRNA sponge" role of circRNAs in regulating multiple pathological processes.[8,29] Here, we aimed to explore the role of circ_0110498 in the DDP resistance of NSCLC and its associated mechanism. A previous study demonstrated that circ_0110498 promoted NSCLC cell glycolysis and proliferation.[7] Consistent with a former study, circ_0110498 expression was elevated in NSCLC tissues. Moreover, circ_0110498 level was increased in DDP-resistant NSCLC tissues and cells. Circ_0110498 knockdown suppressed cell DDP resistance by inhibiting cell growth, metastasis, and glycolysis. In vivo experiments illuminated that circ_0110498 silencing markedly enhanced the DDP sensitivity of NSCLC tumors.

Most circRNAs are localized in the cytoplasmic fraction of eukaryotic cells in the form of exon cyclization.[30] Here, we confirmed that circ_0110498 was majorly localized in the cytoplasm of NSCLC cells. To investigate the mechanism by which circ_0110498 promoted the DDP resistance of NSCLC cells, we intended to seek the miRNA targets of circ_0110498. MiR-1287-5p was verified to be sponged by circ_0110498. As for NSCLC, circ_0016760 contributed to NSCLC progression via absorbing miR-1287.[11] In addition, circ_0026134 sponged miR-1287 to aggravate NSCLC progression.[12] In this, miR-1287-5p mimic restrained the DDP resistance of NSCLC cells. In addition, the regulation of circ_0110498 silencing on the DDP resistance of NSCLC cells were partly counteracted by miR-1287-5p inhibitor, revealing that circ_0110498 knockdown suppressed NSCLC progression partly by sponging miR-1287-5p.

RBBP4 was validated as a downstream target of miR-1287-5p. A previous study demonstrated that RBBP4 overexpression promoted NSCLC progression, confirming the oncogenic role of RBBP4 in NSCLC.[14] The role of RBBP4 on the chemotherapy of cancer patients has also been reported. RBBP4 silencing elevated temozolomide sensitivity of glioblastoma cells by targeting DNA repair-related proteins.[31] Here, miR-1287-5p suppressed cell DDP resistance partly by downregulating RBBP4 expression, suggesting that RBBP4 might be a novel target to overcome the chemoresistance of NSCLC. Furthermore, we confirmed that circ_0110498 sponged miR-1287-5p to positively regulate RBBP4.

In summary, circ_0110498 facilitated the DDP resistance of NSCLC via promoting cell growth, metastasis and glycolysis by miR-1287-5p/RBBP4 pathway. Circ_0110498/miR-1287-5p/RBBP4 axis might be a novel therapeutic target for overcoming the DDP resistance of NSCLC.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

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REFERENCES

1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68:394–424.
2. Postmus PE, Kerr KM, Oudkerk M, Senan S, Waller DA, Vansteenkiste J, et al. Early and locally advanced non-small-cell lung cancer (NSCLC): ESMO clinical practice guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;28:iv1–iv21.
3. Scagliotti GV, Parikh P, von Pawel J, Biesma B, Vansteenkiste J, Manegold C, et al. Phase III study comparing cisplatin plus gemcitabine with cisplatin plus pemetrexed in chemotherapy-naive patients with advanced-stage non-small-cell lung cancer. J Clin Oncol. 2008; 26:3543–51.
4. Sarin N, Engel F, Kalayda GV, Mannewitz M, Cinatl J, Rothweiler F, et al. Cisplatin resistance in non-small cell lung cancer cells is associated with an abrogation of cisplatin-induced G2/M cell cycle arrest. PLoS One. 2017;12:e0181081.
5. Xu P, Wang L, Xie X, Hu F, Yang Q, Hu R, et al. Hsa_circ_0001869 promotes NSCLC progression via sponging miR-638 and enhancing FOSL2 expression. Aging. 2020;12:23836–48.

6. Huang MS, Liu JY, Xia XB, Liu YZ, Li X, Yin JY, et al. Hsa_circ_0001946 inhibits lung cancer progression and mediates cisplatin sensitivity in non-small cell lung cancer via the nucleotide excision repair signaling pathway. Front Oncol. 2019;9:508.
7. Guo F, Li S, Guo C, Xu X, Zhou X, Ma D, et al. Circular RNA circMAGI3 accelerates the glycolysis of non-small cell lung cancer through miR-515-5p/HDGF. Am J Transl Res. 2020;12:3953–63.
8. Hansen TB, Jensen TI, Clausen BH, Bramsen JB, Finsen B, Damgaard CK, et al. Natural RNA circles function as efficient microRNA sponges. Nature. 2013;495:384–8.
9. Sun H, Chen Y, Fang YY, Cui TY, Qiao X, Jiang CY, et al. Circ_0000376 enhances the proliferation, metastasis, and chemoresistance of NSCLC cells via repressing miR-384. Cancer Biomark. 2020;29:463–73.
10. Zhang J, Hua X, Qi N, Han G, Yu J, Yu Y, et al. MiR-27b suppresses epithelial-mesenchymal transition and chemoresistance in lung cancer by targeting Snail1. Life Sci. 2020;254:117238.
11. Li Y, Hu J, Li L, Cai S, Zhang H, Zhu X, et al. Upregulated circular RNA circ_0016760 indicates unfavorable prognosis in NSCLC and promotes cell progression through miR-1287/GAGE1 axis. Biochem Biophys Res Commun. 2018;503:2089–94.
12. Chang H, Qu J, Wang J, Liang X, Sun W. Circular RNA circ_0026134 regulates non-small cell lung cancer cell proliferation and invasion via sponging miR-1256 and miR-1287. Biomed Pharmacother. 2019;112:108743.
13. Fabian MR, Sonenberg N, Filipowicz W. Regulation of mRNA translation and stability by microRNAs. Annu Rev Biochem. 2010;79:351–79.
14. Cao X, Li F, Shao J, Lv J, Chang A, Dong W, et al. Circular RNA hsa_circ_0102231 sponges miR-145 to promote non-small cell lung cancer cell proliferation by up-regulating the expression of RBBP4. J Biochem. 2021;169:65–73.
15. Jiang Z, Yin J, Fu W, Mo Y, Pan Y, Dai L, et al. MiRNA 17 family regulates cisplatin-resistant and metastasis by targeting TGFbetaR2 in NSCLC. PLoS One. 2014;9:e94639.
16. Zhang S, Cheng J, Quan C, Wen H, Feng Z, Hu Q, et al. circCELSR1 (hsa_circ_0063809) contributes to paclitaxel resistance of ovarian cancer cells by regulating FOXR2 expression via miR-1252. Mol Ther Nucleic Acids. 2020;19:718–30.
17. Akram M. Mini-review on glycolysis and cancer. J Cancer Educ. 2013;28:454–7.
18. Bhattacharya B, Mohd Omar MF, Soong R. The Warburg effect and drug resistance. Br J Pharmacol. 2016;173:970–9.
19. Jin C, Xiao L, Zhou Z, et al. MiR-142-3p suppresses the proliferation, migration and invasion through inhibition of NR2F6 in lung adenocarcinoma. Hum Cell. 2019;32:437–46.
20. Pei X, Chen SW, Long X, Zhu SQ, Qiu BQ, Lin K, et al. circMET promotes NSCLC cell proliferation, metastasis, and immune evasion by regulating the miR-145-5p/CXCL3 axis. Aging. 2020;12:13038–58.
21. Zhao L, Liu L, Dong Z, Xiong J. miR-149 suppresses human non-small cell lung cancer growth and metastasis by inhibiting the FOXM1/cyclin D1/MMP2 axis. Oncol Rep. 2017;38:3522–30.
22. Tian QQ, Xia J, Zhang X, Gao BQ, Wang W. miR-331-3p inhibits tumor cell proliferation, metastasis, invasion by targeting MLLT10 in non-small cell lung cancer. Cancer Manag Res. 2020;12:5749–58.
23. Rong F, Liu L, Zou C, Zeng J, Xu Y. MALAT1 promotes cell tumorigenicity through regulating miR-515-5p/EEF2 Axis in non-small cell lung cancer. Cancer Manag Res. 2020;12:7691–701.
24. Cheng Z, Yu C, Cui S, Wang H, Jin H, Wang C, et al. circTP63 functions as a ceRNA to promote lung squamous cell carcinoma progression by upregulating FOXM1. Nat Commun. 2019;10:3200.
25. Xiao H. MiR-7-5p suppresses tumor metastasis of non-small cell lung cancer by targeting NOVA2. Cell Mol Biol Lett. 2019;24:60.
26. Liao M, Peng L. MiR-206 may suppress non-small lung cancer metastasis by targeting CORO1C. Cell Mol Biol Lett. 2020;25:22.
27. Huang SR, Jin SS, Xu B, Wang RP. Puerarin alleviates the progression of non-small cell lung cancer by regulating the miR-342/CCND1 axis. Neoplasma. 2020;67:1244–55.
28. Xiong J, Xing S, Dong Z, et al. miR-654-3p suppresses cell viability and promotes apoptosis by targeting RASAL2 in non-small-cell lung cancer. Mol Med Rep. 2021;23:124.
29. Panda AC. Circular RNAs act as miRNA sponges. Adv Exp Med Biol. 2018;1087:67–79.
30. Chen LL, Yang L. Regulation of circRNA biogenesis. RNA Biol. 2015;12:381–8.
31. Kitange GJ, Mladek AC, Schroeder MA, Pokorny JC, Carlson BL, Zhang Y, et al. Retinoblastoma binding protein 4 modulates temozolomide sensitivity in glioblastoma by regulating DNA repair proteins. Cell Rep. 2016;14:2587–98.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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