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Circ_0076305 regulates cisplatin resistance of non-small cell lung cancer via positively modulating STAT3 by sponging miR-296–5p

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ABSTRACT

Aims: Circular RNAs (circRNAs) acted as key regulators in the development of various human tumors. Our present study aimed to investigate the role and molecular mechanism of circ_0076305 in regulating cisplatin (DDP) resistance of non-small cell lung cancer (NSCLC).

Main methods: Using RT-qPCR, the expressions of circ_0076305 in NSCLC tissues and cells (A549, H1650, A549/DDP, H1650/DDP) were measured. Through loss-of-function and overexpression experiments, the role of circ_0076305 in DDP resistance of NSCLC was verified. Inhibitory rate and IC50 for DDP were detected using MTT method after DDP treatment. Western blotting was performed to evaluate protein levels of P-gp and MRP1. The bindings between circ_0076305 and miR-296–5p, as well as miR-296–5p and STAT3 were validated by bioinformatics, CircRIP, Pearson's correlation analysis and luciferase report vector assays.

Key findings: Circ_0076305 was upregulated in NSCLC, and more significantly elevated in DDP-resistant NSCLC tissues and cells. Further experiments discovered that circ_0076305 could regulate DDP resistance of NSCLC cells via binding to miR-296–5p. Directly targeted by miR-296–5p, STAT3 hindered the miR-296-5p-induced suppression on DDP resistance. Finally, the expression of circ_0076305 was found to have positive correlation with STAT3, and circ_0076305 was validated to regulate STAT3 via targeting miR-296–5p.

Significance: Our present study illustrated that circ_0076305 regulated STAT3 expression and DDP resistance of NSCLC cells via sponging miR-296–5p. These results suggested knockdown of circ_0076305 might provide an effective approach for NSCLC treatment strategy.

1. Introduction

Globally, lung cancer is a member of the most common malignant tumors, and is threatening human life. About 18 million patients were diagnosed with lung cancer every year, along with a worldwide death at 1.8 million [1]. In terms of the histological classifications, non-small cell lung cancer (NSCLC) is the most crucial one in lung cancer due to its 80% ~ 85% occupancy case rate [2]. In recent years, although medical researchers have implemented multiple modalities of methods in NSCLC diagnosis and treatment, NSCLC was still a terrible disease with a poor five-year survival rate.

Nowadays, more than 90% patients suffered lung cancer tend to receive chemotherapy [3]. Cisplatin (DDP) is the most commonly used chemotherapeutic drug for cancer therapy [4]. However, drug resistance is a tough problem during cancer treatment. P-gp and MRP1, ATP-binding cassette transporters, are implicated in multidrug resistance due to their function of pumping out cytotoxic drugs [5]. Elevating expressions of P-gp and MRP1 decreased DDP sensitivity in lung cancer [6]. Moreover, DDP resistance gradually develops during the course of chemotherapy, ultimately leads to the therapeutic failure [7]. Evidence showed that the different DDP sensitivities of patients varies the efficacy of DDP [8]. That is to say, overcoming the resistance to DDP of NSCLC patients is a crucial challenge for clinical therapy.

With the development of high-throughput sequencing technology, scientists discovered that circular RNA (circRNA) was a novel class of non-coding RNAs. Derived from introns, exons or intergenic regions, circRNA has a closed loop structure which is formed by back splicing its 3′ tails and 5′ caps [9–11]. Accumulating studies proposed that circRNA was a pivotal regulator in cell functions and the development of various tumors, NSCLC included. For instance, circ_HIPK3 served as a

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prognostic biomarker and exerted depressed effects on the tumor growth and metastasis of osteosarcoma [12]. Evidence showed that circRNA_100876 was an upregulated RNA molecule related to carcinogenesis of NSCLC [13]. Zhang et al. demonstrated that circRNA FOXO3 relieved its suppression on proliferation and invasion of NSCLC cells via regulating miR-155 [14]. The downregulation of hsa_circ_0001946 in NSCLC was validated by Huang and colleagues, and its participation in regulating DDP sensitivity of NSCLC cells [8]. Nevertheless, further explorations are still needed to be performed for the illustration of the biological function and potential regulation mechanism of circRNA in DPP resistance of NSCLC.

Our previous studies had found that the upregulation of circ_0076305 was relevant to the growth and metastasis of NSCLC tumor. In the present study, we validated the significant differential expressions of circ_0076305 in NSCLC tissues and cell lines. And also, circ_0076305 were found to be closely related to the DDP resistance of NSCLC cells. Besides, based on *in vitro* models of DDP-resistant A549 and H1650 cells, we verified the underlying molecular mechanism of circ_0076305, acting as a sponge of miR-296–5p to modulate STAT3 expression and DDP resistance in NSCLC cells.

2. Materials and methods

2.1. Patients and samples

To meet the ethical requirements, our research applied and obtained the approval of the Ethics Committee of the Second Affiliated Hospital of Xi'an Jiaotong University, and the experiments performed during the entire study was under the Declaration of Helsinki. Besides, the tumor and normal specimens were respectively collected from 56 cases of NSCLC patients and 32 healthy donators. The clinical characteristics of NSCLC were analyzed. Epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK) were considered as sensitive genes for NSCLC, and their mutations could be used in targeted drug therapy for tumors [15]. ADx-ARMS® kits (AmoyDx Biology, Xiamen, China) based on amplification refractory mutation system (ARMS) technology were used for mutation detection of EGFR and ALK genes. In addition, the patients were all received DDP chemotherapy at the Second Affiliated Hospital of Xi'an Jiaotong University from 2009 to 2016. The specific regimen of chemotherapy was as follows: the patients were treated with 75 mg/m^2 DDP by intravenous drip, and this treatment was repeated every 21 days. Six-cycles was maximum of treatment unless there significant progression or unacceptable toxicity appeared. Next, the classification of DDP sensitivity or DDP resistance in patients was based on their outcomes according to previous study [16]. We obtained NSCLC tissues after patients received resection surgery. However, because DDP-resistant patients did not undergo surgery, we used biopsy specimens retained from re-examination after chemotherapy. The tissues after resection and biopsy were stored at −80 °C by liquid nitrogen, immediately. More importantly, we got written informed consent for involvement from all patients before conducting research.

2.2. Cell culture and treatment

In this study, human normal pulmonary epithelial cells (BEAS-2B) and two NSCLC cell lines (A549 and H1650) were selected for *in vitro* experiments. These cells were supplied by the Cell Resource Center of Chinese Academy of Sciences (Shanghai, China). To obtain DDP-resistant cell lines of A549 and H1650, the cells were continuously treated with DDP and selected as previously described [17]. Dulbecco's modified Eagle's medium (DMEM) contained 10% fetal bovine serum, 100 μg/mL streptomycin and 100 U/mL penicillin was where the cells were cultured. And the humidified atmosphere for culture contained 5% CO_2 with the temperature of 37 °C. DDP was prepared into solutions of different concentrations and added to the medium after all transfection assays were completed. All these reagents were purchased from Sigma-Aldrich (St. Louis, USA).

Table 1
Relevance between clinicopathological characteristics of NSCLC patients and circ_0076305 expressions.

Clinical and pathological characteristics	Cases	Circ_0076305 expression		P-value
		high	low	
Age				0.731
< 60	25	16	9	
≥60	31	20	11	
Sex				0.632
Female	16	10	6	
Male	39	26	13	
TNM stage				0.573
I-II	22	13	9	
III-IV	34	23	11	
DDP				0.027
Sensitive	32	14	18	
Resistant	24	22	2	
Smoking status				0.613
Smoker	41	27	14	
Non-smoker	15	9	6	
Lymph node metastasis				0.041
Yes	30	24	6	
No	26	12	14	
EGFR mutation				0.382
Wild type	48	31	17	
Mutant type	8	5	3	
ALK alteration				0.597
Yes	3	3	0	
No	53	33	20	

2.3. Cell transfection

The siRNAs for circ_0076305, miR-296–5p mimic and inhibitor were designed and synthesized by RiboBio (Guangzhou, China). To construct the overexpression plasmids of circ_0076305 and STAT3, we cloned the sequences of them onto pCDS-At cloning vector (BioVector, Beijing, China). Lipofectamine 3000 (Invitrogen, Carlsbad, USA) was used to implement transfections of siRNAs, (50 nM), miRNA mimic and inhibitor (50 nM), and pCDS plasmid (50 μg), in line with its manufacturer's protocol.

2.4. RNA extraction and RNase R treatment

Using TRIzol reagent (Invitrogen, Carlsbad, USA), the total RNA of specimens was isolated, and the reverse-transcription was conducted in line with a Reverse Transcription Kit (Takara, Dalian, China). RNase R digestion was carried out at 37 °C for 20 min by using 1 μL RNase R which was provided by Epicenter (Madison, USA). The digestion was based on 1 μg total RNA.

2.5. RT-qPCR

For the measurement of cDNA, we were guided by the instruction of a SYBR green kit whose producer was Takara (Dalian, China) to perform RT-qPCR on a platform purchased from Bio-Rad Laboratories (Berkeley, USA). The calculation of the relative level was followed the $2^{-\Delta\Delta Ct}$ method. And the normalization protein for circ_0076305 and STAT3 mRNA was GAPDH, while U6 was used for miR-296–5p.

2.6. DDP sensitivity assay

First, the different concentrations (0 μg/mL, 1 μg/mL, 2 μg/mL, 4 μg/mL, 8 μg/mL, 16 μg/mL, 32 μg/mL) of DDP were added to the freshly prepared medium. A 96-well plate was the place where to seed

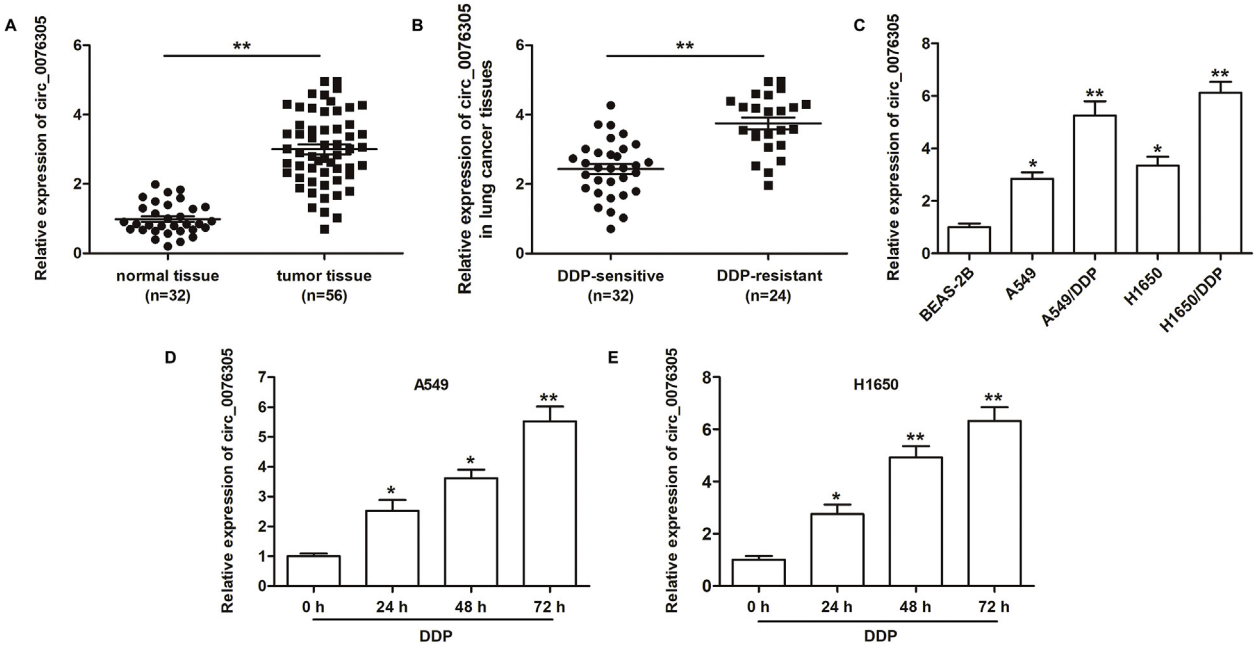

Fig. 1. Circ_0076305 was upregulated in NSCLC and was related to DDP resistance. The expression of circ_0076305 were detected using RT-qPCR. (A and B) The differential expressions of circ_0076305 between normal and tumor tissues, also DDP-sensitive and DDP-resistance tissues. (C) The expressions of circ_0076305 in various NSCLC cell lines; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus BEAS-2B. (D and E) A549 and H1650 cells were treated with 5 μg/mL DDP for different times, and RT-qPCR determined the expression of circ_0076305; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus 0 h. The statistical results were obtained from at least three experimental repeats. Data were exhibited as mean ± SEM.

the cells after transfection assays. And the cells were pre-incubated overnight at 37 °C and 5% CO_2. The treatment of DDP lasted for 48 h at the atmosphere of 37 °C and was followed by the incubation of 20 μL MTT reagent (5 mg/mL) according to its guidelines. Finally, the absorbance at 570 nm was measured and data was presented as inhibitory rate. The IC50 for DDP was calculated on GraphPad Prism 5 (GraphPad Software, San Diego, USA).

2.7. Western blotting

To detect the protein expression of target genes, western blotting assay was conducted under the guidance of manufacturer's protocol and a previous study [18]. The primary antibodies (anti-P-gp, anti-MRP1, anti-STAT3) and the second antibody (HRP-conjugated anti-mouse secondary antibody) were purchased from Abcam (Cambridge, USA). Besides, the GAPDH protein was used as negative control.

2.8. CircRIP

GenePharma (Shanghai, China) provided the design and synthesis of circ_0076305 probe which was labelled by biotin. Cells were inoculated in a 10-cm dish for 48 h, and the logarithmic growth period was the best time for growth termination. Next, we transfected the cells with specific biotin-tagged probe or control probe (200 nM) for 24 h at 37 °C and 5% CO_2. The cross-linking reaction was completed by the incubation of formaldehyde for 10 min and terminated by glycine solution. After that, the cells were rinsed with PBS and lysed by sonication. The input sample was retained after centrifugation, while the remaining supernatant was mixed with the streptavidin labelled magnetic beads (M − 280, Invitrogen, CA, USA) and incubated overnight at room temperature. Then, the mixture was washed and reversed crosslinking by lysis buffer which contained proteinase K. Eventually, miRNeasy Mini Kit (Qiagen, Düsseldorf, Germany) was utilized to acquire total RNA for the follow-up quantitative detection.

2.9. Bioinformatics prediction

There were three bioinformatics prediction platforms we utilized for the potential binding miRNAs of circ_0076305, TargetScanHuman Release 7.2 (http://www.targetscan.org/vert_72/), RNAhybird (https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid/) and miRanda (http://www.microrna.org/microrna/getDownloads.do). And the target protein of miR-296–5p, STAT3, was predicted by TargetScanHuman Release 7.2 (http://www.targetscan.org/vert_72/).

2.10. Mutation and luciferase report vector assays

For the mutation of the binding sequences to miR-296–5p on STAT3 3′UTR, we designed specific primers (Invitrogen, Carlsbad, USA) which contained the mutant sites and cloned the mutant STAT3 3′UTR by fusion PCR. Then, the 3′UTR mutant type (MUT) and wild type (WT) of STAT3 were both introduced onto the sequence of pMIR-Report Luciferase vector (Ambion, Foster City, USA). Cells were co-transfected with negative control (NC) mimic or miR-296–5p mimic along with pMIR-LUC STAT3 3′UTR MUT/WT vector. Forty-eight hours after transfection, the luciferase intensity was measured according to the protocol of dual-luciferase assay kit (Promega, Madison, USA).

2.11. Statistical analysis

All the experiments in this study were conducted independently at least three times. The means ± SEM was the final form to present all data. For the difference comparison of the results, the two-tailed Student's t-test analysis of variance was conducted. And the P value which was less than 0.05 presented that there was statistical difference. SPSS 25.0 software package was utilized for data analysis during the whole experiment.

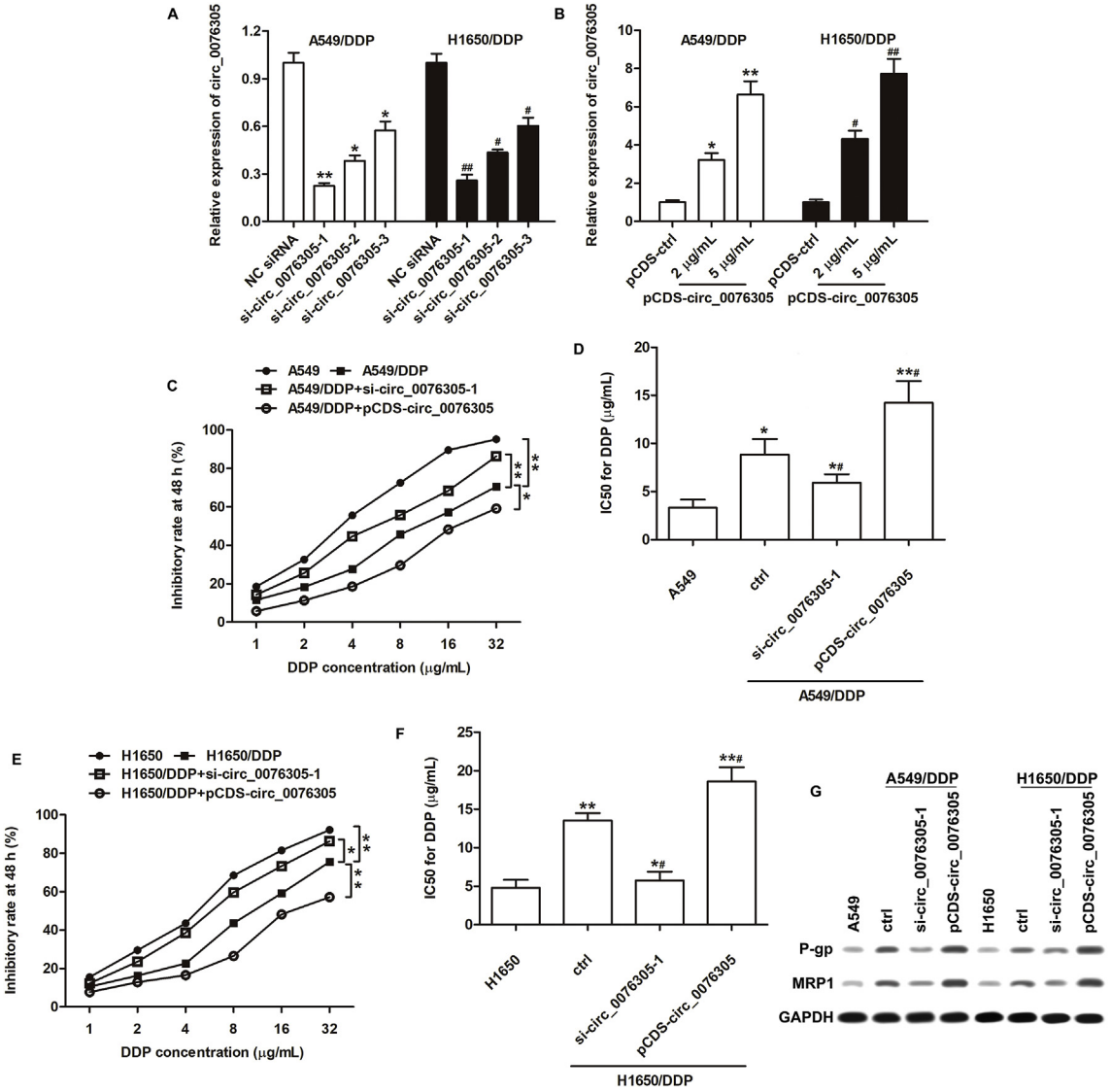

Fig. 2. Circ_0076305 increased DDP resistance in NSCLC cells. A549/DDP and H1650/DDP cells were transfected with 50 nM siRNAs or pCDS-circ_0076305 vector at different concentrations for 24 h before detections. (A and B) The expression levels of circ_0076305 in A549/DDP and H1650/DDP cells after transfections were determined by RT-qPCR; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus ctrl or NC group of A549/DDP; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ versus ctrl or NC group of H1650/DDP. (C and E) The inhibitory rate of cells treated with different concentrations of DDP at 48 h was detected by MTT assay; $^{*}P < 0.05$; $^{**}P < 0.01$. (D and F) Using GraphPad Prism, the IC50 for DDP in cells of every group was calculated; $^{*}P < 0.05$ and $^{**}P < 0.01$ versus A549 or H1650; $^{\#}P < 0.05$ versus ctrl group of A549/DDP or H1650/DDP. (G) The protein expressions of P-gp and MRP1 were determined using western blotting. All the detections were conducted at least triple repeats. The exhibition form of data was mean ± SEM.

3. Results

3.1. Circ_0076305 was upregulated in NSCLC and related to DDP resistance

To illustrate the role which circ_0076305 played in the DDP resistance of NSCLC, we typed NSCLC patients according to DDP sensitivity and detected the expression of circ_0076305 in the tissues of patients with different NSCLC typing and normal tissues. The clinicpathological characteristics and EGFR and ALK gene alterations of patients were presented in Table 1. As shown in Fig. 1A and B, the expression of circ_0076305 was significantly upregulated in NSCLC tissues, and the relative level was nearly one-fold higher in DDP-resistant tissues than DDP-sensitive tissues. Besides, both A549/DDP and H1650/DDP showed significant upregulations of circ_0076305 compared with normal cell lines (Fig. 1C). In A549 and H1650 cells, DDP treatment effectively elevated the expression of circ_0076305 in a time-dependent manner (Fig. 1D and E).

3.2. Circ_0076305 increased the DDP resistance of NSCLC cells

Based on the two DDP-resistant cell lines constructed in this study, we investigated the effect of circ_0076305 exerted on DDP resistance of NSCLC. The transfection of si-circ_0076305-1 had the most effective inhibition on the expression of circ_0076305 in DDP-resistant NSCLC cells among these three siRNAs, as shown in Fig. 2A. Meanwhile, pCDS-circ_0076305 stimulated the upregulation of circ_0076305 in A549/DDP and H1650/DDP cells in dose-dependent manner (Fig. 2B). The interference of circ_0076305 significantly enhanced the inhibitory effect of DDP on DDP-resistant A549 and H1650 cells, while circ_0076305 overexpression presented the opposite effect (Fig. 2C and E). Also, our results presented that the transfection of pCDS-circ_0076305 effectively blocked the elevations of IC50 and the protein expressions of P-gp and MRP1 in DDP-resistant cells compared with normal cells, while the

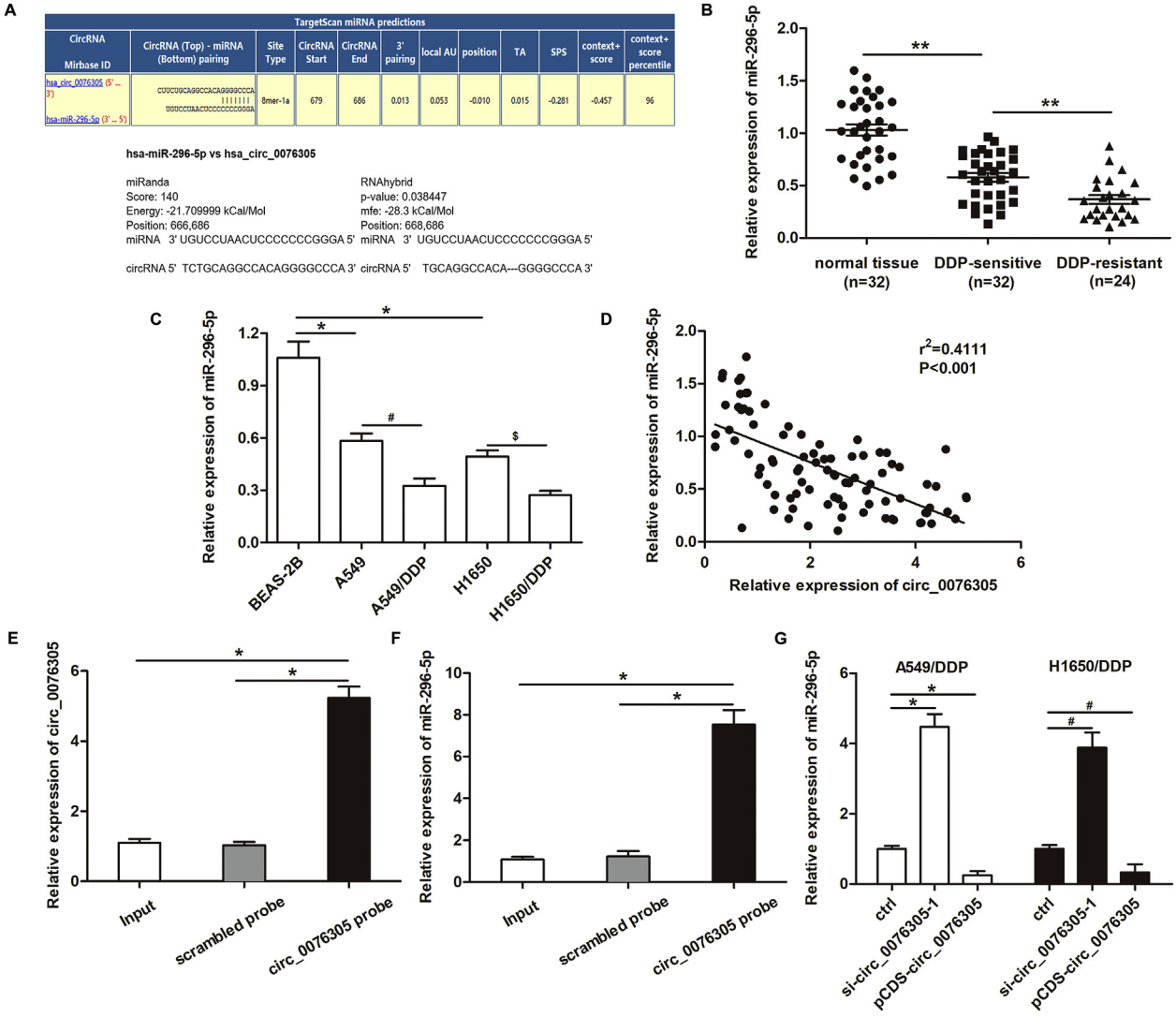

Fig. 3. MiR-296–5p was targeted by circ_0076305 and was the downstream effector of circ_0076305. (A) The results of bioinformatics analysis (TargetScan, miRanda and RNAhybird) showed the combination between circ_0076305 and miR-296–5p. (B and C) The expressions of miR-296–5p in normal, chemo-resistance and chemo-sensitive tissues, also different cell lines were determined by RT-qPCR. (D) Pearson's correlation analysis exhibited the correlation between the expressions of circ_0076305 and miR-296–5p. (E) Circ_0076305 in cell lysates was pulled down and enriched with a circ_0076305-specific probe and then detected by qPCR. (F) RT-qPCR showed the relative expression of miR-296–5p in cells transfected by si-circ_0076305–1 or pCDS-circ_0076305 with or without miR-296–5p mimic. Cells were transfected by pCDS-circ_0076305 with or without miR-296–5p mimic in G ~ J: (G and H) The result of MTT assay exhibited the inhibitory rate of cells in different groups. (I) The IC50 was obtained using GraphPad Prism. (J) Western blotting showed the expressions of P-gp and MRP1. Triple repeats were conducted. Data were expressed as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{\#}P < 0.05$; $^{\$}P < 0.05$.

siRNA for circ_0076305 significantly worsened the adverse effects of DDP resistance (Fig. 2D, F and 2G). Taken together, these consequences indicated that circ_0076305 played a crucial part in the regulation of the sensitivity to DDP in NSCLC cells.

3.3. MiR-296–5p was targeted by circ_0076305

To further explore the mechanism of circ_0076305 modulations, we predicted the possible targeted miRNA using various bioinformatics prediction software. The results presented that the binding sequence of circ_0076305 to miR-296–5p was GGGGCCC (Fig. 3A). Besides, we found that the relative level of miR-296–5p expression was down-regulated and the downregulation was aggravated as the enhancement of DDP resistance in both NSCLC tissues and cells (Fig. 3B and C). Pearson's correlation analysis showed that the expression levels of circ_0076305 were negatively correlated with miR-296–5p in NSCLC tissues (Fig. 3D). Furthermore, we enriched the expressions of circ_0076305 and miR-296–5p in circ_0076305-tagged RNA which was purified by specific circ_0076305 probe compared to controls (Fig. 3E and F). To confirm the combination between circ_0076305 and miR-296–5p, we synthesized miR-296–5p mimic and performed transfection assays. The results showed us that the knockdown of circ_0076305 obviously raised the expression of miR-296–5p in A549/DDP and H1650/DDP cells, while pCDS-circ_0076305 reduced miR-296–5p expression (Fig. 3G).

3.4. MiR-296–5p targeted to STAT3 and declined the DDP resistance of NSCLC cells

The subsequent prediction using TargetScan release 7.2 exhibited that miR-296–5p had the complementary sites (GGGGCCC) with 3′UTR of STAT3 (Fig. 4A), so that we suggested STAT3 as a putative target of miR-296–5p. RT-qPCR detected dramatically upregulated STAT3 expression in tumor tissues compared with normal group, and discovered much higher mRNA level of STAT3 in tissues of DDP-resistant NSCLC patients (Fig. 4B). Besides, the protein expression of STAT3 at cellular

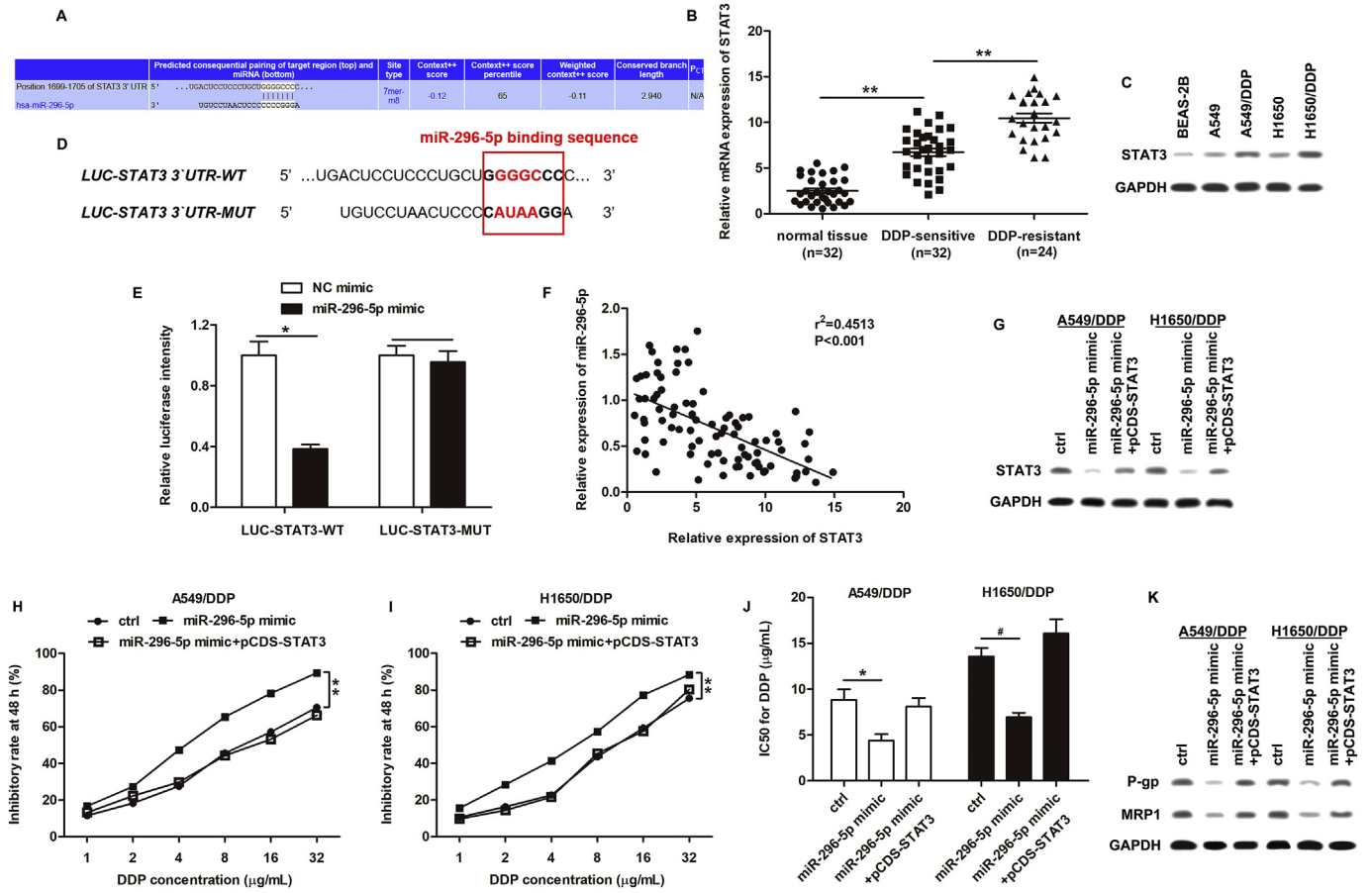

Fig. 4. MiR-296–5p targeted to STAT3 and declined the DDP resistance of NSCLC cells. (A) The diagram exhibited the predicted result of combination between miR-296–5p and STAT3 from TargetScan. (B) The expressions of STAT3 in normal and tumor tissues were determined by RT-qPCR. (C) Western blotting visualized the protein expression of STAT3 in normal and cancer cell lines. (D) The mutant nucleotides in the binding sites between STAT3 3′UTR and miR-296–5p. (E) The luciferase intensities of reporter vectors were measured after miR-296–5p mimic transfection. (F) The correlation between the expressions of STAT3 and miR-296–5p was analyzed by Pearson's analysis. Cells were transfected by miR-296–5p mimic with or without pCDS-STAT3 in G ~ K: (G and K) The expressions of target proteins were detected using western blotting. (H and I) The inhibitory rate of cells was detected by MTT method. (J) The IC50 for DDP of every group was calculated on GraphPad Prism. The experiments were conducted triple repeats. Data were showed as mean ± SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{\#}P < 0.05$.

level was also proved to be associated with DDP resistance in NSCLC cells (Fig. 4C). Fig. 4D presented the specific mutant nucleotides on the sequence of MUT-STAT3 3′UTR compared with the wild type. Luciferase report analysis revealed that the luciferase activity in the reporter group of miR-296–5p mimic and STAT3 3′UTR-WT was remarkably reduced compared with other groups (Fig. 4E). Next, besides the negative correlation between the expression of STAT3 and miR-296–5p (Fig. 4F), we also found that the protein levels of STAT3 in A549/DDP and H1650/DDP cells were significantly decreased by miR-296–5p mimic, and this inhibition was reversed by pCDS-STAT3 (Fig. 4G). The functional experiments similarly indicated that the overexpression of STAT3 reversed the suppression of DDP resistance in A549/DDP and H1650/DDP cells which was induced by miR-296–5p (Fig. 4H, I, 4J and 4K).

3.5. Cric_0076305 regulated STAT3 expression and DDP resistance in NSCLC via sponging to miR-296–5p

In line with the result of Pearson's correlation analysis on NSCLC tissues, the expression of circ_0076305 was proved to be positively correlated with STAT3 expression (Fig. 5A). The present study also documented that the expression of STAT3 was dramatically restrained in si-circ_0076305–1 transfected A549/DDP and H1650/DDP cells, and STAT3 expression in miR-296–5p inhibitor or pCDS-STAT3 co-transfected cells had no obvious difference from the control group (Fig. 5B). Moreover, the transfection of siRNA for circ_0076305 effectively depressed the enhanced inhibitory effect of DDP, the declined IC50 for DDP and the decreased protein expressions of P-gp and MRP1 in both A549/DDP and H1650/DDP cells; the suppressed effects of si-circ_0076305–1 on DDP resistance in NSCLC cells was dramatically abrogated by miR-296–5p inhibitor and pCDS-STAT3 (Fig. 5C, D, 5E, 5F and 5G). These results suggested that circ_0076305/miR-296–5p/STAT3 axis was pivotal for modulating DDP resistance of NSCLC cells.

4. Discussion

As one of the most prevalent malignant tumors, lung cancer is threatening the human life in the world. The clinical practice in recent years have proved that DDP was a frequently used chemotherapeutic drug for the treatment of lung cancer and has achieved certain curative effect [19]. But, the clinical efficacy was restricted by the resistance of patients to DDP, endogenously or exogenously [20].

In previous studies, circRNAs were reported to serve as novel biomarkers for the diagnosis and prognosis of multiple cancers due to the involvement of their abnormal expressions in modulating the sensitivity to chemotherapeutic drugs and pathogenesis [21–23]. Xue et al. demonstrated that the hyper-expression of hsa_circ_0081143 significantly reduced the sensitivity of gastric cancer cells to DDP by targeting miR-646/CDK6 pathway [24]. It was worth mentioning that Zhou and colleagues identified a novel circular RNA, circ_0004015, which was

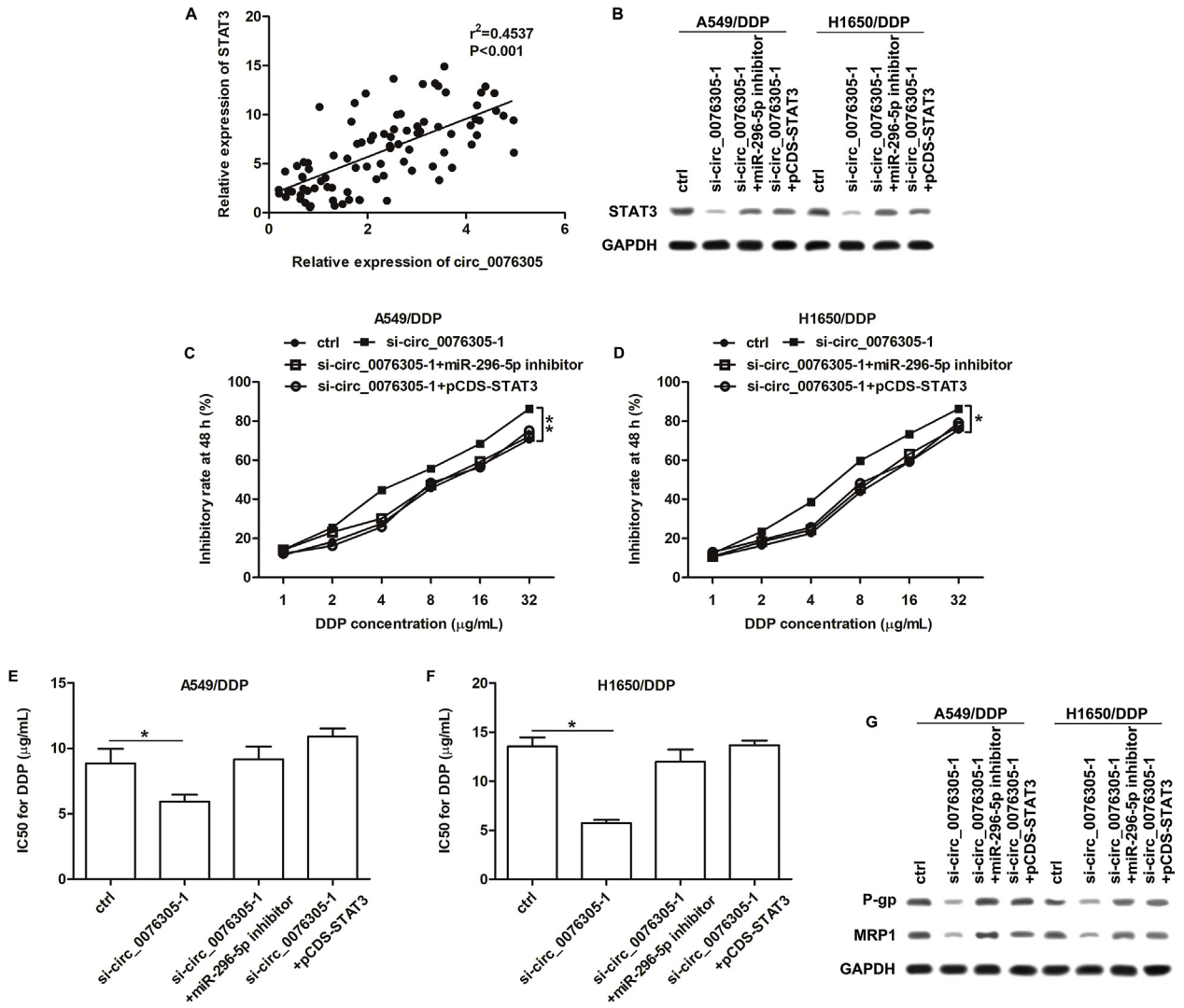

Fig. 5. Cric_0076305/miR-296–5p/STAT3 axis regulated DDP resistance in NSCLC. (A) The result of Pearson's analysis on the combination between expressions of circ_0076305 and STAT3. A549/DDP and H1650/DDP cells were transfected with si-circ_0076305–1 and miR-296–5p inhibitor or pCDS-STAT3: (B and F) Western blot assay provided the expressions of target proteins. (C, D and E) The inhibitory effect of DDP on cell survival and IC50 for DDP were measured using MTT method and GraphPad Prism. Triple repeats were needed. Mean ± SEM was the way to present data. $^{*}P < 0.05$ and $^{**}P < 0.01$ versus ctrl.

upregulated in NSCLC tissues, and circ_0004015 participated in the modulation of NSCLC progression, TKI drug resistance included, via miR-1183/PDPK1 signaling [25]. There was also evidence indicated that silencing circ_0001946 resulted in increased DDP resistance of A549 cells, while NER signaling pathway was activated [8]. Consistently, the present study exhibited that circ_0076305 was upregulated in NSCLC tissues and cell lines, and its expression had close correlation-ship with the regulation of DDP resistance in NSCLC.

Moreover, our study further explored that circ_0076305 regulated STAT3 expression by sponging miR-296–5p, thus to affected the DDP resistance of NSCLC cells. Our research documented a pattern through which circRNA could regulate biological functions, by acting as miRNA sponges. Consistent with our study, a study focused on circRNA and lung cancer proved that circFADS2 could sponge to miR-498 by a complementary binding region and exerted effective promotion on cancer progression [26]. Another research found that has_circ_103809 sequestered the expression of miR-4302, thus to elevate the relative level of ZNF121 and MYC, and finally resulted in enhanced proliferation and invasion of lung cancer cells [27]. Although the way through which circRNA participated in gene regulation was mainly acting as ceRNA of miRNAs, the regulation mechanism of circRNA were diverse, such as interacting with RNA-binding proteins, exerting as transcriptional regulators and being translated into proteins [28]. A recent study suggested that there existed IRES, ORF and m^6A modification in the structure of circPVRL3 which provided the potential ability for circPVRL3 to encode proteins [29]. However, these astonishing functions of circRNA were still unfamiliar and remained to be further explored.

Based on the previous research evidence, miR-296–5p was validated to have associations with abnormalities of various cellular processes during tumorigenesis. For example, Würdinger et al. found that the transfection of miR-296–5p inhibitor in human glioblastoma model significantly reduced the volume of tumors, and this inhibitory effect was achieved by increasing the expression of HGS and weakening the angiogenesis of tumors [30]. The downregulation of miR-296–5p was detected in NSCLC samples *in vivo* and *in vitro*, and overexpressing miR-296–5p inhibited cell growth of NSCLC via decreasing PLK1 [18]. Besides, in a study concentrated on colorectal cancer, miR-296–5p was shown to affect the prognosis of patients those received DDP-based chemotherapy regimen, and *in vitro* experiments validated that the overexpression of miR-296–5p significantly inhibited the viability of cancer cells [31]. Our work validated the improving role which miR-296–5p played in the regulation of DDP resistance in NSCLC cells. Except that, STAT3 was well accepted as a transcriptional activator of

oncogenes in a variety of cancers [32]. However, there was evidence demonstrated that STAT3 protein was highly expressed in head and neck squamous cell carcinoma, and had association with the DDP resistance of patients [33]. A recent study also detected high expression of STAT3 protein, and validated that STAT3 facilitated liver cancer metastasis via mediating the expression lncRNA HOXD-AS1 which was a ceRNA of SOX4 [34]. Moreover, the study of Zhao et al. identified STAT3 as a NSCLC promoter by aggravating tumor cell viability, migration and invasion which was suppressed by miR-202 [35]. According to the consequences of our research, we verified the downregulation of miR-296–5p and upregulation of STAT3 in NSCLC; also, we illustrated that miR-296–5p could target to and inhibited STAT3 expression, so as to improve the DDP sensitivity of NSCLC cells.

5. Conclusion

Our study focused on a novel circRNA, circ_0076305, and confirmed the upregulation of circ_0076305 in NSCLC. Moreover, the *in vitro* investigations based on DDP-resistant NSCLC cells, A549/DDP and H1650/DDP, suggested that circ_0076305 increased the DDP resistance of NSCLC cells via serving as miR-296–5p sponge to releasing STAT3 expression. This may provide a new sight for the diagnosis and therapy of NSCLC.

Declaration of competing InterestCOI

The authors declare that there are no conflicts of interest.

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