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MiR-133a-3p overexpression-induced elevation of cisplatin-mediated chemosensitivity to non-small cell lung cancer by targeting replication factor C3

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ABSTRACT

Investigated the role of miR-133a-3p expression in NSCLC resistance to cisplatin (DDP) treatment and elucidate the possible mechanisms. MiR-133a-3p expression levels in DDP-resistant cells (SPC-1/DPP and A549/DDP) were measured using quantitative reverse-transcription polymerase chain reaction. Cell proliferation, cell apoptosis, cell cycle distribution, and DDP sensitivity were detected through flow cytometry, Cell Counting Kit-8 (CCK-8) and western blot analysis. In addition, databases were used to predict the miR-133a-3p targets, confirmed by dual-luciferase reporter assay. The miR-133a-3p expression levels obviously decreased in the A549/DDP and SPC-1/DPP cells [$^{**}P < 0.01$; ($n = 3$)]. Upregulation of the miR-133a-3p expression notably suppressed cell growth, enhanced cell apoptosis, and resulted in cell cycle arrest in the SPC-1/DPP and A549/DDP cells. CCK-8 assay for the detection of proliferation of the miR-133a-3p mimic-transfected A549/DDP and SPC-A1/DDP cells treated with different DDP doses revealed IC_{50} values of NC mimic group: 6.85 μg/mL; miR-133a-3p mimic: 3.9 μg/mL and NC mimic group: 6.9 μg/mL; miR-133a-3p mimic: 3.99 μg/mL, respectively]. By contrast, it elevated DDP sensitivity in the A549/DDP cells and DDP resistance in the SPC-1/DPP cells. Mechanically, miR-133a-3p negatively regulated replication factor C3 and promoted DDP sensitivity in the SPC-1/DPP and A549/DDP cells, 94 potential targets that might bind to miR-133a-3p were identified.

1. Introduction

Lung cancer (LC) represents a malignancy originating in the bronchial gland or mucosa or gland that has the highest mortality and incidence rates worldwide [1]. LC is estimated to cause 1.5 million deaths in the global population every year, which accounts for one-fifth of all tumor-related deaths [2]. In China, approximately 400,000 LC cases are diagnosed annually, and the incidence rate is 61.4 per 10 million population [3]. By 2025, the mortality rate from LC in China is estimated to reach millions [4]. Non-small cell LC (NSCLC) accounts for approximately 85 % of all LCs, and the 5-year survival of patients is approximately 15 % [5]. Currently, cisplatin (DDP)-based chemotherapy is the most commonly used treatment for advanced cases, but only 10–35 % of patients respond to DDP, with a median survival time of 6–12 months [6]. However, with the development of chemotherapy, DDP resistance has become increasingly prevalent, causing great difficulties in the treatment of NSCLC [7]. According to a survey of the American Cancer Association, >90 % of deaths among patients with NSCLC are highly related to drug resistance of tumor cells [8]. At present, simple and noninvasive biological indicators for the evaluation and prediction of the therapeutic effects of NSCLC chemotherapy are lacking. Therefore, this warrants the identification of effective predictors and therapeutic targets to improve the prognosis of patients with NSCLC.

MicroRNAs (miRNAs) are small RNA molecules with lengths ranging

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from 18 to 25 bp that do not encode protein and are closely involved in the post-transcriptional gene expression regulation [9]. miRNAs have been suggested to exert an important role in gene silencing through complete or incomplete complementary pairing with the 3′-untranslated region (UTR) of targets, further regulating cell growth, proliferation, differentiation, and apoptosis [10]. In addition, an increasing number of articles have revealed the role of miRNAs as tumor suppressors or oncogenes, exhibiting essential effects on tumorigenesis [11,12]. Moreover, in recent years, miRNAs have been reported to be closely related to the drug resistance (including DDP) of NSCLC [13]. For example, Blower et al. found that cell activity and drug sensitivity to DDP were changed after the overexpression or inhibition of miR-21, miR-16, and miR-7i in the NSCLC cell line A549 [14]. Moreover, Ceppi et al. confirmed that miR-200 expression is related to the resistance of NCI-H1299 to DDP [15]. In addition, Zhang et al. showed that miR-513a-3p mimics significantly enhanced DDP sensitivity in A549/CDDP cells in human lung adenocarcinoma [16]. The above-mentioned findings suggest the involvement of miRNAs in the chemotherapy resistance mechanism of tumors and can be used as biological indicators to predict the efficacy of and prognosis after tumor chemotherapy. miR-133a-3p, a common tumor-related miRNA, is weakly expressed in many malignant tumors and plays the role of tumor suppressor in the development of many malignancies such as pancreatic cancer, breast cancer, and gastric cancer [17]. Moreover, miR-133a mimics remarkably enhanced DDP sensitivity in A549/DDP cells [18]. Furthermore, the precise effect of miR-133a-3p on the regulation of NSCLC resistance to DDP and the related mechanisms remain largely unknown.

Thus, the present work aimed to elucidate the biological functions of miR-133a-3p in NSCLC resistance to DDP and the associated mechanisms. We identified that the miR-133a-3p expression level decreased in the DDP-resistant cells. MiR-133a-3p upregulation remarkably inhibited the proliferation of DDP-resistant NSCLC cells, promoted their apoptosis, induced cycle arrest, and enhanced their sensitivity to DDP. These might have been achieved by targeting replication factor C3 (RFC3), which implies the potential usefulness of miR-133a-3p as a novel target in the treatment of DDP-resistant NSCLC.

2. Materials and methods

2.1. Cell culture and transfection

Parental NSCLC cells (SPC-A1, A549) and those resistant to DDP (A549/DDP, SPC-A1/DDP) were provided by the American Type Culture Collection (USA) and cultivated in Dulbecco's modified Eagle's medium (Gibco, USA) containing 10 % fetal bovine serum (Invitrogen, USA) and 1% streptomycin-penicillin (Sigma-Aldrich, USA) under 37℃ and 5% CO_2 conditions.

pc-RFC3/pc-NC and NC/miR-133a-3p mimics were obtained from Ambion (Austin, USA) and transfected into DDP-resistant cells (5×10^5) maintained in six-well plates using Lipofectamine 3000 (Invitrogen). The miR-133a-3p mimic sequence was 5′-UUUGGUCCCCUUCAACCAGCUG-3′, and the mimic control sequence was 5′-UUUUCCGAACGUUCACGUTT-3′. The cells were transfected with 50 nM mimic or 2-μL plasmid per well in the six-well plate. The transfection efficiency was examined using quantitative reverse-transcription polymerase chain reaction (qRT-PCR) after 48 h.

2.2. Cell counting Kit-8 assay

A DDP sensitivity analysis was performed as previously described [19]. DDP-resistant NSCLC cells (1×10^4 cells per well) were maintained in 96-well plates and then incubated with DDP (0/2/4/6/8 μg/mL) for 48 h. For cell viability assessment, after 48 h of transfection, Cell Counting Kit-8 assay (CCK-8; Beyotime Biotechnology, China) was performed to detect cell viability. In this study, we measured the absorbance (optical density) value at 490 nm. The half minimal inhibitory concentration (IC_{50}) was calculated.

2.3. Flow cytometry

To analyze cell apoptosis, DDP-resistant NSCLC cells from different groups and culture media were collected. Then, annexin V conjugated with fluorescein isothiocyanate and propidium iodide (PI) were added and allowed to rest for 10 min. The apoptosis rate was determined using flow cytometry for cell cycle analysis. DDP-resistant NSCLC cells were harvested from the different groups, followed by overnight fixation with 70 % EtOH and 30 min of PI staining. Finally, flow cytometry was performed to identify the cell cycle distribution, in which a minimum of 5000 events were recorded.

2.4. RNA isolation and qRT-PCR

RNA isolation was performed using TRIzol and chloroform as previously described. In brief, cells were washed with phosphate-buffered saline and subsequently lysed in TRIzol. RNA was isolated with chloroform, precipitated using isopropanol, washed twice with ethanol, dried, and resuspended in water. After the extraction of total RNA, cDNA, as the starting material for qRT-PCR using the Step One System (Life Technologies Corp., California, USA), was prepared with the extracted total RNA by using the RNeasy plus micro kit through reverse transcription in accordance with specific instructions. The sequences of all the primers were designed using the Primer Premier 4.0 software (Premier, Canada) as follows: miR-133a-3p forward, 5′-UUUGGUCCCCUUCAACCAGCUG-3′ and reverse, 5′-UAAACCAAGGUAAAAUGGUCGA-3′; U6 forward, 5′-CGCTTCGGCAGCACATATAC-3′ and reverse, 5′-TTCACGAATTTGCGTGTCAT-3′; RFC3 forward, 5′-TCCCTGCTTCTGATTTCCTTTACC-3′ and reverse, 5′-GGCTTCCCTGACCACCCTATTTA-3′; and β-actin forward, 5′-CTGGAACGGTGAAGGTGACA-3′ and reverse, 5′-AAGGGACTTCCTGTAACAATGCA-3′. U6 or β-actin were normalized using the $2^{-\Delta\Delta CT}$ approach.

2.5. Western blot analysis

Total cell protein was extracted using a radioimmunoprecipitation assay lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA). Protein was isolated from DDP-resistant NSCLC cells and measured with the BCA kit (Beyotime Biotechnology). Protein was extracted using 12 % sodium dodecyl sulfate polyacrylamide gel electrophoresis and then shifted into poly (vinylidene fluoride) membranes (Millipore, Bedford, USA). Next, the membranes were incubated with 5% skimmed milk and then with primary antibodies under 4℃ (overnight). After the membranes were rinsed, they were further maintained for 1 h using horseradish peroxidase-labeled secondary antibody (1:4,000; SA00004−10, Proteintech, China) under ambient temperature. Finally, an enhanced chemiluminescence kit (Millipore, Bedford, USA) was used to observe protein blots, and the ImageJ version 4.3 software (NIH) was used for quantification. The primary antibodies used in the study included anti-PCNA (1:2,000; 10205−2-AP, Proteintech), anti-Ki-67 (1:2,000; 27309−1-AP, Proteintech), anti-cyclin A1 (1:1,000; ab53699, Abcam, USA), anti-cyclin B1 (1:2,000; 55004−1-AP, Proteintech), anti-cyclin D2 (1:2,000; 10934−1-AP, Proteintech), anti-p27 (1:2,000; 25614−1-AP, Proteintech), anti-Bax (1:2,000; 50599−2-Ig, Proteintech), anti-Bcl-2 (1:2,000; 12789−1-AP, Proteintech), anti-cleaved-caspase-3 (1:1,000; ab32042, Abcam), anti-cleaved-caspase-9 (1:1,000; ab2324, Abcam), anti-RFC3 (1:2,000; 11814−1-AP, Proteintech), and anti-β-actin (1:5,000; 66009−1-Ig, Proteintech), with β-actin as the endogenous control. Thorough washings were carried out at each treatment to the membrane.

2.6. Target gene prediction and luciferase reporter assay

As a result of the analysis using the ENCORI, miRBD, and miRWalk

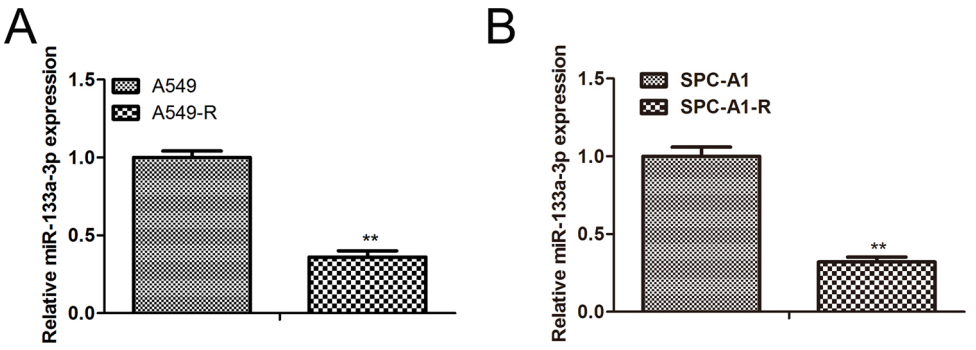

Fig. 1. MiR-133a-3p expression is downregulated in DDP-resistant NSCLC cells. The miR-133a-3p expression in the (A) A549/DDP and (B) SPC-A1/DDP cells was determined using a quantitative reverse-transcription polymerase chain reaction assay. $^{**}P < 0.01$, compared with A549 or SPC-A1 cells. All data are presented as mean ± SD ($n = 3$). NSCLC: non-small cell lung cancer; DDP: cisplatin.

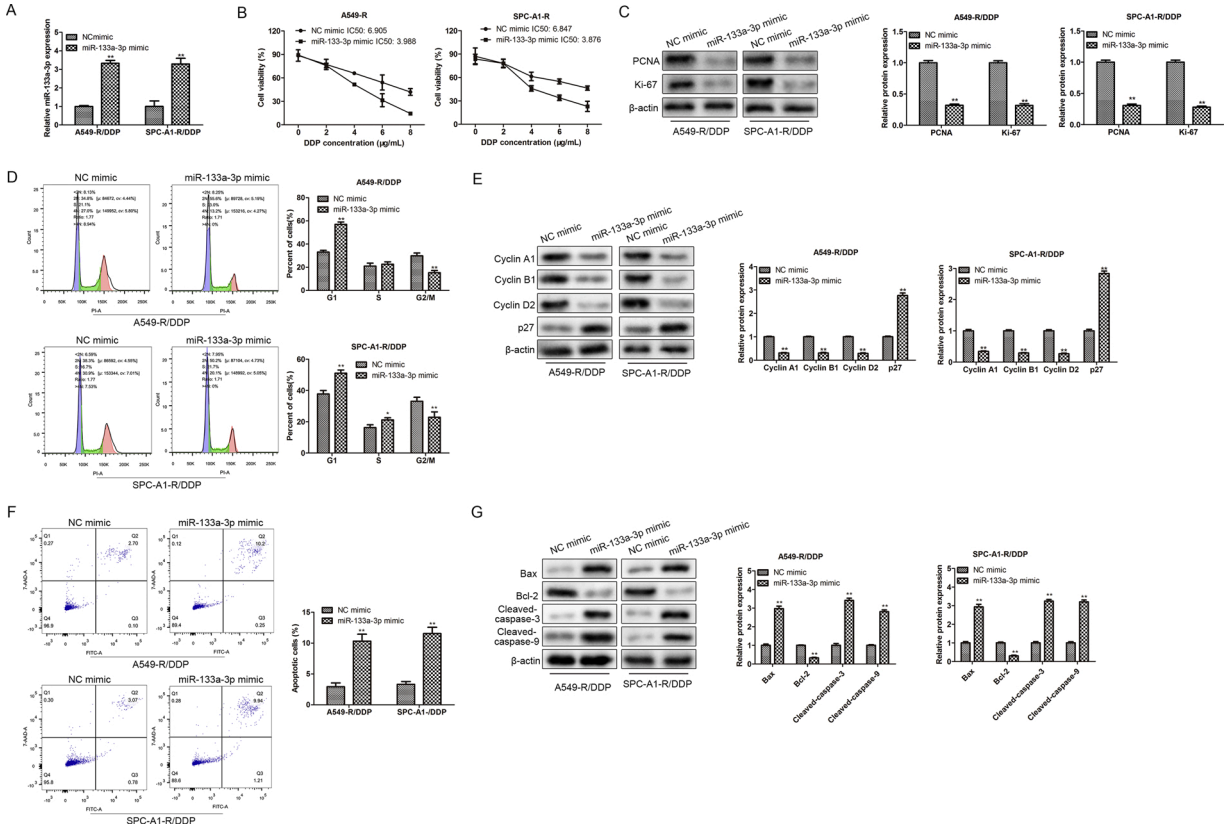

Fig. 2. Upregulation of miR-133a-3p expression enhances the DDP sensitivity, inhibits the proliferation, promotes the apoptosis, and triggers the cell cycle arrest of DDP-resistant NSCLC cells. (A) Quantitative reverse-transcription polymerase chain reaction assay for evaluating the miR-133a-3p expression in SPC-A1/DDP and A549/DDP cells transfected with the miR-133a-3p mimic. (B) CCK-8 assay for the detection of the proliferation of the miR-133a-3p mimic-transfected A549/DDP and SPC-A1/DDP cells treated with different DDP doses. The (C) cell proliferation, (E) cell cycle distribution, and (G) cell apoptosis-related protein expressions in the SPC-A1/DDP and A549/DDP cells transfected with the miR-133a-3p mimic were analyzed using a western blot assay. (D) Cell cycle distribution and (F) flow cytometry assay were used to analyze the cell apoptosis of the SPC-A1/DDP and A549/DDP cells transfected with the miR-133a-3p mimic. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the NC mimic group. All data are presented as mean ± SD ($n = 3$). NSCLC: non-small cell lung cancer; DDP: cisplatin; CKK: Cell Counting Kit.

databases, 94 potential targets that might bind to miR-133a-3p were identified. RFC3 was chosen as the target. The pmirGLO dual-luciferase vector (150 ng; Promega, USA) was subcloned with RFC3 WT/MUT to generate pmirGLO-RFC3 WT/MUT. Then, the cells were co-transfected with the miR-133a-3p mimic and pmirGLO-RFC3 WT/MUT or NC mimic for 48 h. The dual-luciferase reporter system (Promega) was used to detect relative luciferase activity.

2.7. Statistical analysis

Data were analyzed using GraphPad Prism 5.0 and presented as mean ± SD. Differences between the groups were compared using analysis of variance and Tukey post hoc analysis. P values < 0.05 indicated statistical significance. Each experiment was repeated three times, including three biological replicates and three technical replicates.

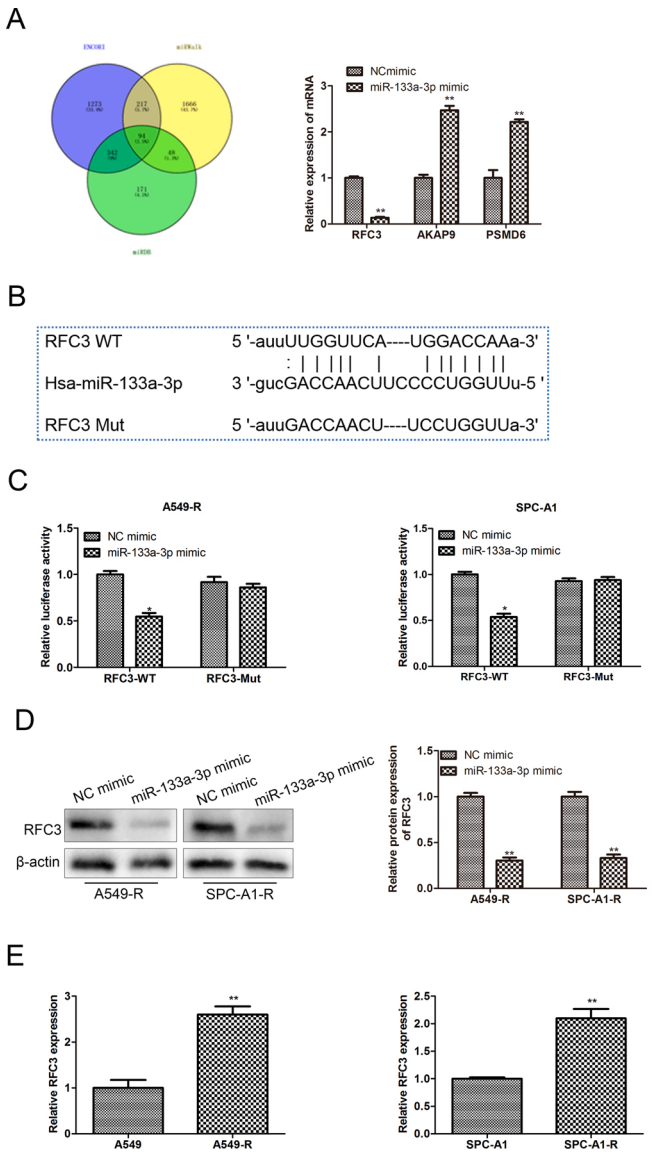

Fig. 3. RFC3 is a miR-133a-3p target and negatively associated with miR-133a-3p expression. (A) The candidate targets regulated by miR-133a-3p that were predicted using the ENCORI, miRWalk, and miRDB databases. (B) The binding sites between miR-133a-3p and RFC3. (C) Dual-luciferase reporter analysis to validate the coactions of miR-133a-3p and RFC3. (D) Western blot assay for the analysis of the protein expressions of RFC3 in A549/DDP and SPC-A1/DDP cells transfected with the miR-133a-3p mimic. $^{*}P < 0.05$, $^{**}P < 0.01$, compared with the NC mimic group. (E) Quantitative reverse-transcription polymerase chain reaction assay for the quantification of the mRNA expression levels of RFC3 in the A549/DDP and SPC-A1/DDP cells. $^{**}P < 0.01$, compared with A549 or SPC-A1 cells. All data are presented as mean ± SD ($n = 3$). NSCLC: non-small cell lung cancer; DDP: cisplatin; CKK: Cell Counting Kit; RFC: replication factor C.

3. Results

3.1. MiR-133a-3p expression level decreased in the NSCLC cells with DDP resistance

First, in this study, we performed qRT-PCR to detect miR-133a-3p expressions in parental NSCLC cells (SPC-A1, A549) and NSCLC cells with DDP resistance (A549/DDP, SPC-A1/DDP). As presented in Figs. 1A and 1B, miR-133a-3p was weakly expressed in the SPC-A1/DDP and A549/DDP cells relative to its expression levels in the SPC-A1 and A549 cells. These data suggest the potential role of miR-133a-3p in the chemoresistance of NSCLC.

3.2. MiR-133a-3p upregulation enhanced the DDP sensitivity, suppressed the growth, enhanced the apoptosis, and induced the cell cycle arrest of NSCLC cells with DDP resistance

To determine the impact of miR-133a-3p expression on chemoresistance, NC or miR-133a-3p mimic was transfected into SPC-A1/DDP and A549/DDP cells. The miR-133a-3p level sharply increased in the cells transfected with the miR-133a-3p mimic, whereas no remarkable changes were observed in those transfected with the NC mimic (Fig. 2A). Furthermore, we performed a CCK-8 assay to evaluate the role of miR-133a-3p in DDP sensitivity. On the basis of our results, DDP significantly decreased the IC_{50} value in the miR-133a-3p mimic-transfected cells compared with the NC mimic-transfected cells (Fig. 2B). Moreover, we performed a western blot assay to assess the role of miR-133a-3p in the expressions of proliferation-related proteins. As shown in Fig. 2C, compared with the NC mimic, the miR-133a-3p mimic inhibited the protein expressions of PCNA and Ki-67. In addition, we performed a flow cytometry analysis to determine the role of miR-133a-3p in the cell cycle distribution and apoptosis of SPC-A1/DDP and A549/DDP cells. The data shown in Figs. 2D and 2E indicate that compared with the NC mimic, the miR-133a-3p mimic caused G1 phase arrest in the cell cycle and enhanced the A549/DDP and SPC-A1/DDP cell apoptosis. Furthermore, western blot assay was used to determine the role of miR-133a-3p in cell cycle and cell apoptosis-related protein expression. As depicted in Fig. 2F and G, the miR-133a-3p mimic decreased the protein expressions of cyclin A1, cyclin B1, cyclin D2, and Bcl-2 but increased those of p27, cleaved-caspase-3/cleaved-caspase-9, and Bax. These results confirm that the upregulation of miR-133a-3p expression enhanced the DDP sensitivity, suppressed the growth, enhanced the apoptosis, and induced the cell cycle arrest of NSCLC cells with DDP resistance.

3.3. RFC3 acted as a miR-133a-3p target and showed a negative association with miR-133a-3p expression

To ascertain the possible miR-133a-3p targets, bioinformatic tools, including ENCORI, miRBD, and miRWalk were jointly utilized. As a result, 94 potential targets that might bind to miR-133a-3p were identified. By reviewing articles on PubMed, we found that RFC3, AKAP9, and PSMD6 may be related to tumor progression. Furthermore, through the qRT-PCR experiment, we found that RFC3 was negatively related to miR-133a-3p expression (Fig. 3A and B). Moreover, the result of the dual-luciferase reporter analysis further validated the association of miR-133a-3p with RFC3 (Fig. 3C). Furthermore, the RFC3 levels in the miR-133a-3p mimic-transfected cells were measured using western blot assay. The data presented in Fig. 3D show that the RFC3 protein expression level remarkably decreased in the miR-133a-3p mimic-transfected cells. In addition, the RFC3 levels in the parental and DDP-resistant NSCLC cells were measured using qRT-PCR. As shown in Fig. 3E, compared with the parental cells, the DDP-resistant cells showed high RFC3 expression. These data demonstrate RFC3 as a direct target of miR-133a-3p in DDP-resistant NSCLC cells.

3.4. RFC3 mediated miR-133a-3p functions in DDP sensitivity, proliferation, apoptosis, and cell cycle arrest of NSCLC cells with DDP resistance

To illustrate the role of miR-133a-3p expression in regulating chemoresistance by targeting RFC3, miR-133a-3p or/and pc-RFC3 mimics were transfected into SPC-A1/DDP and A549/DDP cells (Fig. 4A). According to the CCK-8 assay result, DDP had a much higher IC_{50} value in the SPC-A1/DDP and A549/DDP cells under co-transfection with miR-133a-3p and pc-RFC3 mimics than in the miR-133a-3p mimic-transfected cells (Fig. 4B). Moreover, according to our flow cytometry analysis, upregulation of RFC3 expression remarkably restored the function

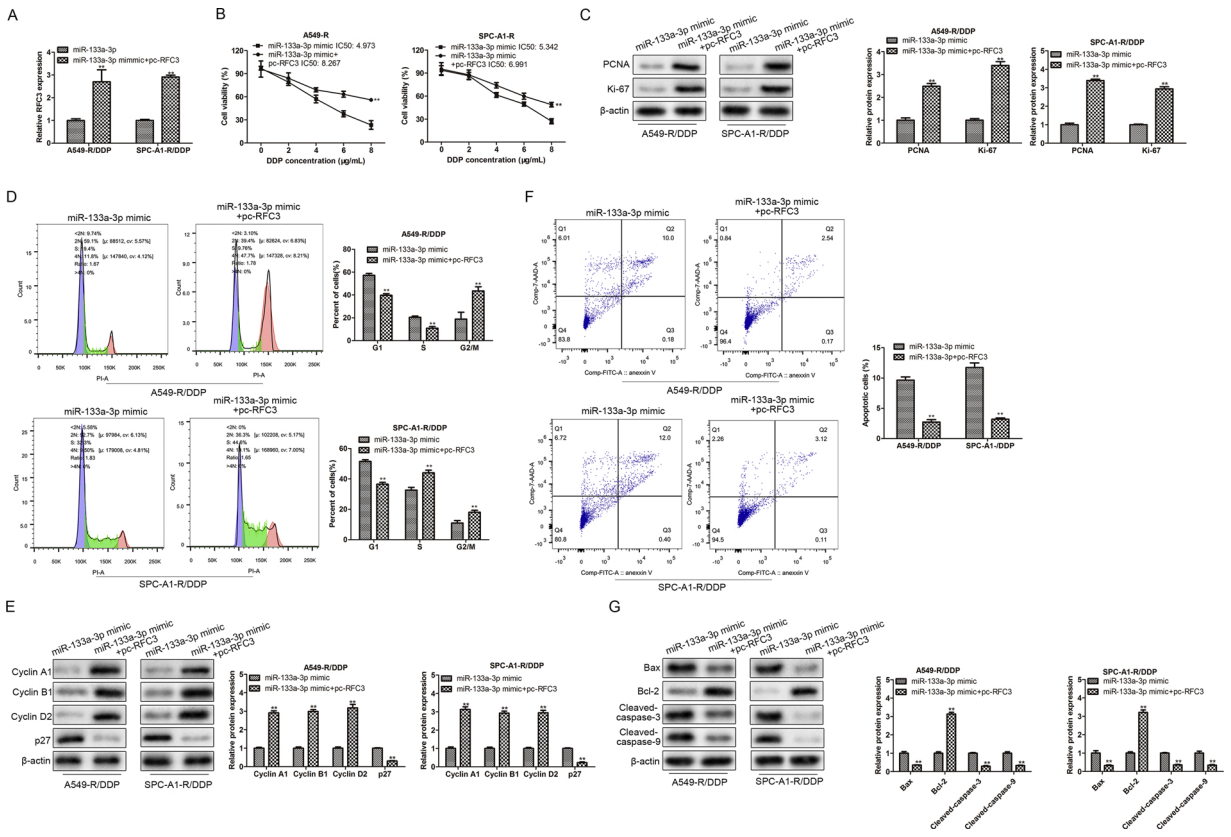

Fig. 4. RFC3 mediates the effects of miR-133a-3p expression on the DDP sensitivity, proliferation, apoptosis, and cell cycle arrest of DDP-resistant NSCLC cells. (A) The relative expression of RFC3 in A549/DDP and SPC-A1/DDP cells transfected with the miR-133a-3p and/or pc-RFC3 mimics. (B) CCK-8 assay for the detection of the proliferation of transfected A549/DDP and SPC-A1/DDP cells treated with different DDP doses. The (C) cell proliferation, (E) cell cycle distribution, and (G) cell apoptosis-related protein expressions of the A549/DDP and SPC-A1/DDP cells after transfection were analyzed using a western blot assay. The (D) cell cycle distribution and (F) cell apoptosis of the A549/DDP and SPC-A1/DDP cells after transfection was analyzed using flow cytometry. $^{**}P < 0.01$, compared with the miR-133a-3p mimic group. NSCLC: non-small cell lung cancer; DDP: cisplatin; CKK: Cell Counting Kit; RFC: replication factor C.

of the miR-133a-3p mimic in the cell cycle distribution and apoptosis of the SPC-A1/DDP and A549/DDP cells (Figs. 4D and 4 F). Moreover, the western blot assay data indicated that pc-RFC3 notably reversed the effect of the miR-133a-3p mimic on the protein expression related to the cell proliferation, apoptosis, and cycle distribution of the SPC-A1/DDP and A549/DDP cells (Fig. 4C, E, and G). These data elucidate that RFC3 overexpression partially mediated the functions of miR-133a-3p in DDP sensitivity, proliferation, cell cycle arrest, and apoptosis of DDP-resistant NSCLC cells.

4. Discussion

Globocan predicted that by 2008, the number of patients with cancer worldwide would reach 12.7 million, of whom 7.6 million would die of cancer. Up to now, cancer remains a threat to human beings [20]. LC is one of the leading cancers owing to its high incidence and mortality rates and early diagnosis. Among LCs, 85 % are NSCLC [21]. Surgical resection is still the preferred curative management for early NSCLC, whereas passive chemotherapy and radiotherapy are indicated for patients who lose the opportunity to undergo surgery. As a first-line chemotherapy drug commonly used for NSCLC, DDP inhibits DNA replication and triggers apoptosis of cancer cells. However, chemoresistance has become an obstacle in chemotherapy for NSCLC. Therefore, further studies are required to determine the mechanisms of chemoresistance of NSCLC to improve treatment options and identify the effective treatments for individual patients with NSCLC [22].

The DDP resistance of NSCLC is a complex process related to many factors, including miRNAs [23–25]. The expression of the tumor suppressor miR-133a-3p is closely related to the chemoresistance of tumors, including hepatocellular carcinoma, glioblastoma, and breast cancer [26–28]. Similarly, our data show that the miR-133a-3p expression level decreased in the A549/DDP and SPC-A1/DDP cells, confirming the results of previous studies. This suggests the possible role of miR-133a-3p expression in NSCLC chemoresistance. To better understand the biological functions of miR-133a-3p in DDP-resistant NSCLC cells, a series of assays were performed. As expected, miR-133a-3p upregulation increased the DDP sensitivity, suppressed the growth, caused the cell cycle arrest, and enhanced the apoptosis of DDP-resistant NSCLC cells. This moderately suggests that miR-133a-3p expression positively affected tumor chemoresistance. For example, miR-133a-3p showed a low expression level in the doxorubicin-resistant breast cancer cell cline MCF-7/Dox. MiR-133a-3p upregulation sensitized the reaction to treatment with doxorubicin. In addition, the miR-133a-3p expression level decreased in the hepatocellular carcinoma cell line HepG2, and miR-133a-3p mimic obviously sensitized HepG2 cells to adriamycin. These findings are basically consistent with our findings and suggest that miR-133a-3p upregulation possibly exerts a vital role in NSCLC chemoresistance.

MiRNAs exhibit their functional roles usually by regulating downstream targets [29]. This regulation is observed during the development of diseases. Chemosensitivity to DDP has been reported to be enhanced in NSCLC by targeting DCLK1 [30]. The miRNA 17 family regulates DDP resistance and metastasis by targeting TGFbetaR2 in NSCLC [31]. Li et al. found that high LncrnaSNHG14 expression, low miR-133a expression in DDP-resistant NSCLC, and miR-133a overexpression could increase the chemical sensitivity of A549/DDP cells, consistent with our conclusion [21]. In this study, we found that the miR-133a-3p expression was downregulated in the DDP-resistant NSCLC cells.

MiR-133a-3p negatively regulated RFC3 and promoted DDP sensitivity in the SPC-1/DPP and A549/DDP cells.

Abnormal miRNA expression occurs during tumorigenesis and cancer development. Hypermethylation of the miR-192 promoter occurs in pancreatic ductal adenocarcinoma and chronic pancreatitis, which is the main reason for miR-192 expression downregulation. The change in the miR-133a-3p expression level in chemoresistant cancer cells may also be related to the change of the expression level of its promoter, but this requires further study.

In the present study, we predicted that the 3′-UTR region of RFC3 contained complementary sites of miR-133a-3p, which suggests the potential of RFC3 as a miR-133a-3p target, as validated through the dual-luciferase reporter analysis. As a member of the RFC family and as a component of the eukaryotic DNA polymerase, RFC3 exerts a vital role in DNA duplication, injury repair, and checkpoint control [32–34]. RFC3 downregulation may suppress cancer cell multiplication [35]. Moreover, RFC3 has been suggested to show a close association with many malignant tumors such as ovarian and liver cancers and to play a vital role in tumor cell proliferation, metastasis, and invasion [36,37]. For instance, decreased RFC3 expression level significantly attenuates triple-negative breast cancer progression and metastasis by regulating the epithelial-to-mesenchymal transition signal pathway [38]. In addition, the RFC3 expression is upregulated in OVCAR-3 cells. RFC3 silencing restrains the viability and proliferation, induces the apoptosis, and causes the cell cycle arrest of OVCAR-3 cells [36]. Moreover, RFC3 overexpression shortens the survival time of patients with lung adenocarcinoma and increases the metastasis of A549 cells by regulating epithelial-to-mesenchymal transition progression through the wnt/β-catenin signaling pathway [39]. However, whether miR-133a-3p regulated the chemoresistance of NSCLC cells with DDP resistance by targeting RFC3 remains unclear. In this study, we performed diverse rescue assays. As expected, RFC3 overexpression partially restored the functions of the miR-133a-3p mimic in the DDP sensitivity, cell proliferation, apoptosis, and cell cycle arrest of the NSCLC cells with DDP resistance. However, the specific mechanism thereby RFC regulates chemotherapy resistance is still unknown. The Wnt/β-catenin signaling pathway plays an important role in the mechanism of DDP resistance. We speculate that RFC3 may play a role in the mechanism thereby miR-133a-3p enhances DDP sensitivity through the wnt/β-catenin signaling pathway. Further, earlier studies on these and current aspects will provide ways to proceed for finding new avenues [40–42].

In conclusion, the miR-133a-3p expression level decreased, whereas the RFC3 expression level was increased in the DDP-resistant NSCLC cells. Moreover, miR-133a-3p expression showed a close correlation with the DDP sensitivity, cell proliferation, apoptosis, and cycle distribution of the DDP-resistant NSCLC cells. Thus, this study provides an experimental basis of the potential usefulness of miR-133a-3p as a novel marker of NSCLC resistance to DDP.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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