

Original Research Article

Circ_0011385 increases cisplatin resistance in breast cancer via miR-615-5p suppression

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Abstract

Purpose: Circular RNAs (circRNAs) participate in chemo-resistance among different cancers, containing breast cancer (BC). Herein, this project aimed to investigate the role of circ_0011385 on cisplatin (DDP) resistance in BC cells.

Methods: circ_0011385 and microRNA (miR)-615-5p content in DDP-resistant BC cells and their parent cells (MCF-7) was calculated by RT-qPCR. CCK-8 assessed inhibition rate, and further to calculate half inhibitory concentration (IC₅₀). Clone formation and flow cytometry experiments detected clone numbers and apoptosis rate. Dual-luciferase reporter system validated targeting between circ_0011385 and miR-615-5p.

Results: circ_0011385 was upregulated, whereas miR-615-5p was decreased in MCF-7/DDP cells. Silencing circ_0011385 or miR-615-5p overexpression enhanced BC cell sensitivity to DDP, repress proliferation, and expedite apoptosis rate. Circ_0011385 directly targeted miR-615-5p.

CONCLUSION: Silencing circ_0011385 increases DDP sensitivity by targeting and up-regulating miR-615-5p expression in MCF-7/DDP.

Keywords: Breast cancer; circ_0011385; cisplatin resistance; miR-615-5p

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INTRODUCTION

As a prevalent occurring malignancy in females worldwide, breast cancer (BC) has gradually become a growing public health concern during recent decades [1]. Given the fact that BC is lacking specific symptoms and diagnostic markers in the early stages, most sufferers are always diagnosed in advanced stages with a poor prognosis [2, 3]. Currently, in spite of the

emergence of treatments like molecular targeted therapy, surgical resection, and adjuvant chemotherapy remain the principal approaches for BC [4]. Yet, managing patients continues to be challenging due to the aggressive nature and often chemotherapy-resistant nature of these tumors [5]. Of interest, resistance to cisplatin (DDP), an anti-cancer drug commonly in BC, is one of the primary reasons for treatment failure [6]. Hence, further investigation of the

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mechanism of chemo-resistance is imperative in BC treatment. Recently, featured with loop structure, circular RNA (circRNA) is generated from the back-spliced mechanism of pre-mRNA during the process of transcriptions [7]. Differentially expressed in diverse cancers, circRNAs engage in biological processes, thereby modulating tumorigenesis and chemo-resistance [8, 9]. Numerous evidence has suggested that circ_0011385 is often freakishly expressed and participates in controlling the malignant progression of many cancers [10, 11], but its role in BC has not been reported. In terms of molecular mechanisms, with miRNA binding sites, the majority of circRNAs can be utilized as miRNA sponges to dampen miRNA activity, which modulates downstream target gene expression and biological functions in human tumors [12]. Herein, target gene prediction to miR-615-5p as a potential target of circ_0011385. Beyond that, some literature has reported that miR-615-5p is a well-known tumor suppressor and represses the growth and metastasis of prostate cancer and colorectal cancer [13, 14]. Moreover, miR-615-5p is closely associated with DDP-insensitive gastric cancer cell apoptosis [15]. Here, we analyzed the influences of circ_0011385 lack on proliferation, apoptosis, and DDP resistance, aiming to provide available targets for reversing DDP resistance in BC.

EXPERIMENTAL

Reagents

MCF-7, MCF-7/DDP, and HEK-293T cells (ATCC, Manassas, VA, USA) were cultivated in DMEM (Gibco, Carlsbad, CA, USA). miRNA cDNA Synthesis Kit was provided by SHZY SW (Shanghai, China). TaKaRa (Dalian, China) supplied PrimeScript Reverse Transcription Kit and TB Green Premix Ex Taq II. CCK-8 reagent was purchased from Abace Biology (Beijing, China). Absin Bioscience (Shanghai, China) supplied Annexin V-FITC kit and TRIZOL Regent Kit. Sangon Biotech (Shanghai, China) provided luciferase reporter vector, si-circ_0011385, si-NC, miR-615-5p mimic/inhibitor, miR-NC/anti-miR-NC. Opti-MEM and Lipofectamine 2000 were respectively provided by Gibco and Invitrogen (Carlsbad, CA, USA).

Methods

RT-qPCR

Total RNA was extracted by TRIzol kit, and total RNA was reverse transcribed to cDNA using

miRNA cDNA synthesis kit and PrimeScript reverse transcription kit. After being mixed with TB Green Premix Ex Taq II, cDNA samples were used for RT-qPCR on an ABI 7500 fluorescent quantitative PCR RT-qPCR was performed. GAPDH or U6 was implemented as an internal reference for circ_0011385 or miR-615-5p assay, and data were calculated via the $2^{-\Delta\Delta Ct}$ method. U6: 5'-TGC GGG TGC TCG CTT CGG CA-3'(sense), 5'-CCA GTG CAG GGT CCG AGG T-3'(antisense). circ_0011385: 5'-TGA CAA CAA TGA GCC CTA CA-3'(sense), 5'-TTT CCT TGG CAC TAT ACT GG-3'(antisense). GAPDH: 5'-TGT TCG TCA TGG GTG TGA AC-3'(sense), 5'-ATG GCA TGG ACT GTG GTC AT-3'(antisense). miR-615-5p: 5'-TCC GAT TCT CCC TCT GGG TC-3'(sense), 5'-GTG CAG GGT CCG AGG T-3'(antisense).

Cell treatments

When cells grew to 80% confluence in a temperature chamber with 5% CO₂ at 37 °C, they were digested with trypsin for 5 min and passage according to 1:3. 2×10^4 MCF-7/DDP cells at the logarithmic phase and inoculated in 24-well plates. Transient transfection was carried out when the cells reached 50% confluence. Subsequently, Lipofectamine 2000 and oligonucleotides separately were diluted with serum-free Opti-MEM. After incubation for another 20 min, the transfection reagent mixture was added to cells in each well, which were divided into si-NC, si-circ_0011385, miR-615-5p, si-circ_0011385+anti-miR-NC, and si-circ_0011385+miR-615-5p inhibitor group. After being cultured for 48 h, RT-qPCR was performed to detect circ_0011385 or miR-615-5p expression.

CCK-8

After replacing using culture medium with 0, 10, 20, 40, 80, and 100 $\mu\text{mol/L}$ DDP for 24 h, 10 μL CCK-8 was mixed for another 2 h. Finally, the 96-well plate was removed and results were measured via an enzyme meter to calculate the cell inhibition rate. The half inhibitory concentration (IC₅₀) of DDP was calculated based on the cell inhibition rate. Cell inhibition rate (%) = $(1 - \text{Experiment A}/\text{Control A}) \times 100\%$.

Clone formation assay

After being digested with trypsin, 200 cells were inoculated in 6 cm dishes, followed by culture with complete medium containing 20 $\mu\text{mol/L}$ DDP for 2-3 weeks. After discarding, cells were subjected to PBS washing, 4% paraformal-

dehydrate fixture, and staining. At last, clones were analyzed under an inverted microscope.

Flow cytometry

Transfected MCF-7/DDP cells were incubated with complete medium containing 20 μmol/L DDP. After digestion and resuspending in 1x binding buffer, 500 μL cell suspensions were stained with Annexin V-FITC and PI and protected from light for 20 min. Apoptosis was assessed by flow cytometry within 1 h.

Dual-luciferase reporter assay

Firstly, the wild-type (WT) circ_0011385 sequence containing the miR-615-5p binding site and its corresponding mutant (MUT) were synthesized, followed by inserting into pmirGLO vectors. 2 × 10⁴ HEK-293T cells at 50% confluent were co-transfected with wt/mut-circ_0011385, and miR-NC or miR-149-5p in 24-well plates, and then analyzed using a dual luciferase reporter gene assay kit.

Statistical analysis

Three replicate wells were set up for each test and the experiment was repeated independently three times. *P*<0.05 was deemed statistically significant. Data were expressed as $\bar{x}\pm s$. SPSS

22.0 was applied for statistical analysis. Comparison of means between the two groups was performed by independent samples *t*-test.

RESULTS

Circ_0011385 was improved in MCF-7/DDP cells

Based on data exhibited in Table 1, circ_0011385 expression and IC₅₀ value of cisplatin were significantly higher in MCF-7/DDP cells, but miR-615-5p was decreased (*P*<0.05).

Transfection efficiency of circ_0011385

As shown in Table 2, circ_0011385 content was reduced in si-circ_0011385-transfected MCF-7/DDP cells (*P*<0.05), suggesting knockdown efficiency is available.

Circ_0011385 absence enhanced DDP sensitivity

Data from Figure 1 and Figure 2, and Table 3 exhibited that MCF-7/DDP cell inhibition and apoptosis were obviously higher in si-circ_0011385 group (*P*<0.05), and clone formation number and IC₅₀ value to DDP were apparently lower (*P*<0.05).

Table 1 circ_0011385 and miR-615-5p content in MCF-7/DDP cells and MCF-7 cells

Groups	n	circ_0011385	miR-615-5p	IC ₅₀ (μmol/L)
MCF-7	9	1.00±0.00	1.00±0.00	20.18±1.24
MCF-7/DDP	9	2.42±0.10*	0.42±0.03*	88.47±4.66*
<i>t</i>		42.600	58.000	42.485
<i>P</i>		<0.001	<0.001	<0.001

**P*<0.05, MCF-7/DDP group versus MCF-7 group

Table 2 Detection of circ_0011385 expression

Groups	n	circ_0011385
si-NC	9	1.00±0.00
si-circ_0011385	9	0.26±0.04*
<i>t</i>		55.500
<i>P</i>		<0.001

**P*<0.05, si-circ_0011385 group compared with si-NC group

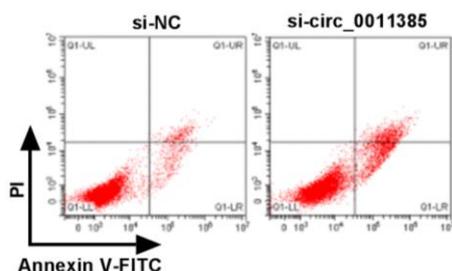


Figure 1: Effect of silencing circ_0011385 on MCF-7/DDP apoptosis

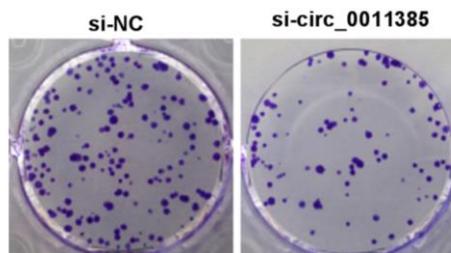
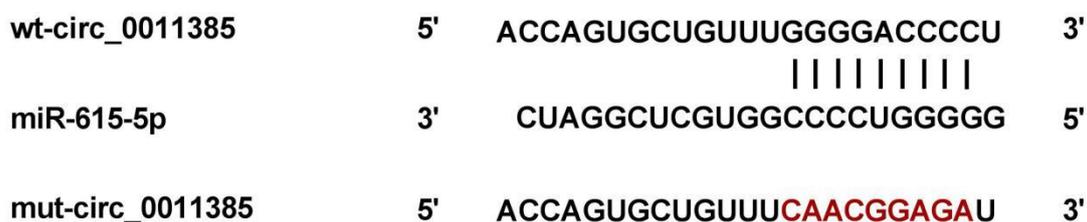


Figure 2: Effect of silencing circ_0011385 on MCF-7/DDP clone formation

Table 3: Detection of cisplatin resistance to MCF-7/DDP by silencing circ_0011385

Groups	n	Inhibition rate (%)	Apoptotic rates (%)	The number of cell colonies	IC ₅₀ (μmol/L)
si-NC	9	0.00±0.00	7.86±0.42	123.89±6.33	89.03±6.09
si-circ_0011385	9	56.54±2.41*	23.81±1.23*	53.89±1.73*	26.17±2.84*
<i>t</i>		70.382	36.815	32.002	28.064
<i>P</i>		<0.001	<0.001	<0.001	<0.001

P*<0.05, si-circ_0011385 group versus si-NC groupFigure 3:** Complementary sequences of circ_0011385 and miR-615-5p**Table 4:** Dual luciferase reporter assay

Groups	n	wt-circ_0011385	mut-circ_0011385
miR-NC	9	1.03±0.13	1.00±0.12
miR-615-5p	9	0.44±0.05*	0.96±0.11
<i>t</i>		12.708	0.737
<i>P</i>		<0.001	0.472

P*<0.05, miR-615-5p group in comparison with miR-NC groupTable 5:** circ_0011385 modulates miR-615-5p

Groups	n	miR-615-5p
si-NC	9	1.00±0.00
si-circ_0011385	9	3.25±0.16 [#]
<i>t</i>		42.188
<i>P</i>		0.000

[#]*P*<0.05, si-circ_0011385 group relative to si-NC group

Circ_0011385 targets and regulates miR-615-5p

Existence of complementary sequences between circ_0011385 and miR-615-5p was discovered (Figure 3). Beyond that, miR-615-5p mimic greatly declined the luciferase activity of wt-circ_0011385 in MCF-7/DDP cells (*P*<0.05) (Table 4). MiR-615-5p expression level in si-circ_0011385 group was clearly enhanced, as presented in Table 5.

miR-615-5p regulated MCF-7/DDP proliferation, apoptosis, and DDP resistance

Based on the results displayed in Figure 4 and Table 6, miR-615-5p expression level and apoptosis rate of MCF-7/DDP cells in the miR-615-5p mimic group were strikingly increased (*P*<0.05), and cell proliferation, clone formation number, and the IC₅₀ value to DDP were remarkably decreased (*P*<0.05).

miR-615-5p knockdown might abolish the influences of circ_0011385 deficiency on the DDP resistance

As presented in Figure 5 and Table 7, miR-615-5p content and apoptosis in MCF-7/DDP cells in si-circ_0011385+miR-615-5p inhibitor group were lower (*P*<0.05), and clone formation number, and IC₅₀ value to cisplatin were elevated (*P*<0.05).

DISCUSSION

Herein, our findings presented that circ_0011385 expression was increased in MCF-7/DDP cells. Functional and mechanistic studies indicated that circ_0011385 contributed to DDP resistance of BC cells possibly through targeting miR-615-5p.

Convincing evidence has implied that numerous circRNAs perform essential roles in the progression and chemo-resistance in different human tumors, containing BC [16, 17]. Herein,

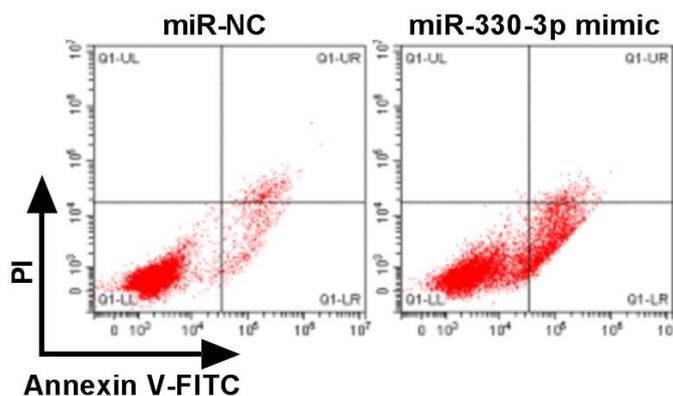


Figure 4: Effect of miR-615-5p on cisplatin resistance in MCF-7/DDP

Table 6: Detection of MCF-7/DDP cisplatin resistance by miR-615-5p

Groups	n	miR-615-5p	Inhibition rate (%)	Apoptotic rates (%)	The number of cell colonies	IC ₅₀ (μmol/L)
miR-NC	9	1.00±0.00	0.00±0.00	7.72±0.47	120.33±7.27	88.79±6.25
miR-615-5p mimic	9	2.64±0.09*	44.50±1.55*	20.23±0.99*	66.44±2.75*	36.41±2.75*
<i>t</i>		54.667	86.129	34.246	20.800	23.013
<i>P</i>		<0.001	<0.001	<0.001	<0.001	<0.001

**P*<0.05, miR-615-5p mimic group relative to miR-NC group

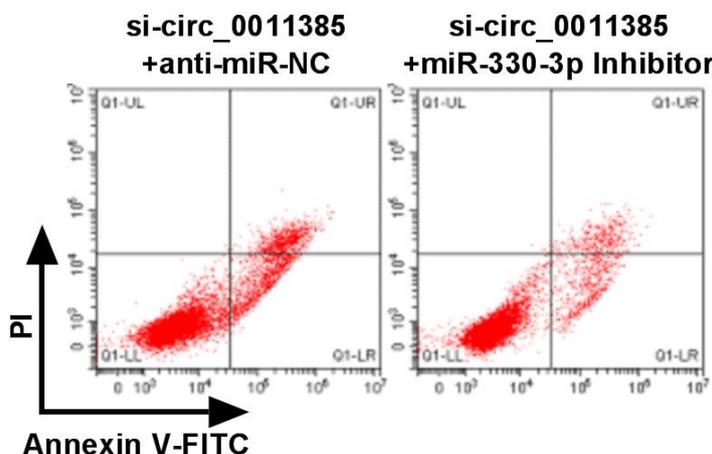


Figure 5: miR-615-5p inhibition and silencing circ_0011385 regulated MCF-7/DDP apoptosis

Table 7: Inhibiting miR-615-5p reverse silencing circ_0011385 on MCF-7/DDP cisplatin resistance

Groups	n	miR-615-5p	Inhibition rate (%)	Apoptotic rates (%)	The number of cell colonies	IC ₅₀ (μmol/L)
si-circ_0011385+anti-miR-NC	9	1.00±0.00	56.56±1.9	23.77±1.5	53.56±2.50	26.27±1.19
si-circ_0011385+miR-615-5p Inhibitor	9	0.37±0.04*	24.47±1.7	12.33±0.7	110.11±5.2	61.03±3.67*
<i>t</i>		47.250	36.706	20.244	29.130	27.029
<i>P</i>		<0.001	<0.001	<0.001	<0.001	<0.001

**P*<0.05, si-circ_0011385+miR-615-5p inhibitor group compared with si-circ_0011385+anti-miR-NC group

the significantly higher circ_0011385 expression level was found in MCF-7/DDP cells, which showed apparently higher IC₅₀ values for DDP relative to MCF-7 cells. These findings

suggested that aberrant expression of circ_0011385 may mediate DDP resistance in BC cells. Loss-of-function experiments displayed that silencing circ_0011385 might effectively

elevate the inhibitory role of DDP on MCF-7/DDP cell proliferation, induced apoptosis, and decreased DDP resistance. Besides, previous research presented that circ_0011385 expression was increased in cervical cancer and partake in malignant behaviors via targeting miR-149-5p [18], which was consistent with the anticancer effect of silencing circ_0011385 in this study. The above results verified that silencing circ_0011385 was able to increase DDP sensitivity by inhibiting proliferation and inducing apoptosis.

Up to now, considerable laboratory work has revealed that circRNAs have biological functions, such as miRNA sponges [19]. Herein, circ_0011385 targeted miR-615-5p. Some works of literatures have indicated that miR-615-5p overexpression might constrain colorectal cancer and nasopharyngeal carcinoma cell growth and metastasis [14, 20]. Enhanced miR-615-5p might repress DDP-insensitive gastric cancer cell growth and facilitate apoptosis [15]. Consistent with these studies, elevated miR-615-5p prominently hindered BC cell proliferation, promote apoptosis, and decline DDP resistance. Lack of circ_0011385 might reinforce miR-615-5p content, and the oncogenic effects of overexpressing miR-615-5p and silencing circ_0011385 were similar, suggesting that the proliferation inhibition, apoptosis promotion, and DDP sensitization effects of silencing circ_0011385 were achieved by up-regulating miR-615-5p expression. In-depth analysis revealed that inhibition of miR-615-5p expression significantly attenuated circ_0011385 absence-mediated proliferation, apoptosis, and IC50 values of DDP-activated MCF-7/DDP cells, which further confirms that circ_0011385 mediates DDP resistance of BC cells by targeting miR-615-5p.

CONCLUSION

Silencing circ_0011385 increased DDP sensitivity of BC cells by upregulating miR-615-5p expression, providing an important target for reversing DDP resistance in BC.

DECLARATIONS

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Ethical approval

None provided.

Availability of data and materials

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

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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