SNHG15 Contributes To Cisplatin Resistance In Breast Cancer Through Sponging miR-381

Hailong Mi*
Xiaochun Wang*
Fang Wang
Lin Li
Mingzhi Zhu
Nan Wang
Youyi Xiong
Yuanting Gu

Department of Breast Surgery, The First Affiliated Hospital of Zhengzhou University, Zhengzhou 475000, People’s Republic of China

*These authors contributed equally to this work

Background: Increasing evidence implies the participation of long non-coding RNAs (lncRNAs) in chemoresistance to cancer treatment. Their role and molecular mechanisms in breast cancer chemoresistance, nevertheless, are yet not considerably elucidated. In this work, we research the function of small nucleolar RNA host gene 15 (SNHG15) in cisplatin (DDP) resistance of breast cancer and uncover the underlying molecular mechanism.

Methods: SNHG15 and miR-381 expression levels were detected using Quantitative real-time PCR (qRT-PCR) analysis. The functional roles of SNHG15 and miR-381 in breast cancer were determined using MTT assay and flow cytometry analysis. The effect of SNHG15 on miR-381 expression was determined using Luciferase reporter assay, RNA immunoprecipitation (RIP) assay and qRT-PCR analysis.

Results: SNHG15 was found to be up-regulated in cisplatin resistant breast cancer tissues and cell lines. Breast cancer patients with high SNHG15 expression had a poor prognosis. SNHG15 silencing enhanced cisplatin sensitivity of MCF-7/DDP and MDA-MB-231/DDP cells. Additionally, SNHG15 could function as a miR-381 sponge. miR-381 overexpression could overcome cisplatin resistance. miR-381 knockdown countered SNHG15 knockdown-mediated enhancement of cisplatin sensitivity in MCF-7/DDP and MDA-MB-231/DDP cells. Besides, SNHG15 knockdown facilitated cisplatin sensitivity of cisplatin resistant breast cancer cells in vivo.

Conclusion: In summary, SNHG15 knockdown overcame cisplatin resistance of breast cancer by sponging miR-381, providing a novel therapeutic target for breast cancer.

Keywords: breast cancer, cisplatin, small nucleolar RNA host gene 15, miR-381

Introduction

Breast cancer is a most common malignancy in women worldwide and a major serious health threat to women.1 Even though impressive improvements have been achieved in diagnosis and therapy of breast cancer in the past decade, prognosis for advanced breast cancer remains relatively poor.2 Platinum-based chemotherapy is a significant therapeutic strategy for breast cancer patients.3 Nevertheless, chemoresistance remains a key barrier to the efficacy of chemotherapy drugs for cancers, including breast cancer.4,5 Hence, to sequentially elucidate the underlying mechanism and discover new therapeutic targets are essential for developing effective therapies for breast cancer patients.

Long non-coding RNAs (lncRNAs) are a group of endogenous non-protein-coding RNAs with more than 200 nucleotides in length.6 Recently, lncRNAs were discovered to play crucial regulatory roles in various biological processes, including tumorigenesis process.7 Increasing evidence indicates that lncRNAs are usually deregulated in malignant tumors’ progression.8–10 Moreover, dysregulated lncRNAs have been reported to be implicated with cisplatin resistance in various cancers.11 LncRNA small nucleolar RNA...
host gene 15 (SNHG15), an intergenic lncRNA located on chromosome 7p13, belongs to a family of non-coding RNAs that hosting snoRNAs.\textsuperscript{12} Previous studies suggest that SNHG15 could promote cancer cell proliferation and invasiveness and is able to play as a crucial determinant role in the chemoresistance.\textsuperscript{13} However, the functional role of SNHG15 in cisplatin (DDP) resistance in breast cancer has not been evaluated.

miRNAs are a class of small, non-coding RNA with about 22 nucleotides in length, which contribute to their target genes’ inhibition, through mRNA degradation or translation inhibition.\textsuperscript{14,15} MiRNAs is recently implicated by more and more reports as critical determinants that are involved in various cellular processes, including chemoresistance and tumorigenesis.\textsuperscript{16} MiRNAs’ aberrant expression has been regarded as a powerful regulator of CDDP resistance. For instance, miR-34a has been discovered to be down-regulated and contributed to DDP resistance in DDP-resistant prostate cancer cells.\textsuperscript{17} miR-381 could overcome cisplatin resistance in non-small cell lung cancer (NSCLC) cells through inactivating nuclear factor-κB signaling.\textsuperscript{18} Moreover, miR-381 overexpression could improve DDP sensitivity of breast cancer cells through targeting MDR1.\textsuperscript{19} However, how miR-381 was regulated in breast cancer cisplatin resistance remains largely unknown.

In this study, we aimed to investigate the expression pattern and functional role and underlying molecular mechanism of SNHG15 in breast cancer cisplatin resistance. Our study revealed that SNHG15 expression was up-regulated in breast cancer tissues and cell lines, especially in DDP-resistant breast cancer tissues and cells. Functionally, SNHG15 knockdown re-sensitized DDP-resistant breast cancer cells to DDP. Mechanically, knockdown of SNHG15 facilitated the sensitivity of breast cancer cells towards cisplatin through increasing miR-381 expression. Our work demonstrated a novel SNHG15/miR-381 regulatory axis, overcoming cisplatin resistance in breast cancer.

Materials And Methods
Tumor Tissue Samples And Cells
The paired tumor tissues (n=42) and adjacent normal tissues (n=42) were obtained from breast cancer patients who underwent surgery at the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all participants. This study had acquired the approval of the ethics committee of the First Affiliated Hospital of Zhengzhou University. Normalized RNA-seq data of Breast adenocarcinoma (BRCA) were downloaded from the TCGA data portal website (https://cancergenome.nih.gov/).

Human breast cancer cell lines (MCF-7 and MDA-MB-231) and human normal breast epithelial cell line MCF-10A were obtained from ATCC (Manassas, VA, USA). DDP-resistant cell lines (MCF-7/DDP and MDA-MB-231/DDP) were selected from MCF-7 and MDA-MB-231 cells after continuous exposure to stepwise increasing concentrations of DDP for 12 months. All cells were grown in RPMI-1640 medium (Gibco BRL, Grand Island, NY, USA) supplement with 10% FBS at 37°C with 5% CO₂.

Cell Transfection
Mimic control (miR-con), miR-381 mimic (miR-381) and miR-381 inhibitor (anti-miR-381) were purchased from GenePharma (Shanghai, China). SNHG15 overexpressing vector pcDNA3.1-SNHG15 (SNHG15) or the empty vector pcDNA3.1 (Vector) or and small interfering RNAs against SNHG15 (si-SNHG15 #1, si-SNHG15 #2, and si-SNHG15 #3) and their negative control (si-con) were designed and synthesized by GenePharma (China). Lipofectamine 2000 (Invitrogen) was used for cell transfections.

Quantitative Real-Time PCR (qRT-PCR)
Total RNA extraction was carried out through using Trizol reagent (TaKaRa, Tokyo, Japan). Then, the RNA was reverse transcribed to cDNA using PrimeScript RT Reagent Kit (TaKaRa). The qRT-PCR was carried out using SYBR green qRT-PCR assay. GAPDH and U6 were used for internal controls for SNHG15 and miR-381, respectively. The data was analyzed using the 2\textsuperscript{−ΔΔCt} method.

DDP Sensitivity Assay
The sensitivity of breast cancer cells was evaluated by MTT assay. DDP sensitivity was determined using the IC50 value (half maximal inhibitory concentration). GraphPad Prism 7.0 Software was used to calculate the IC50 value.

Flow Cytometric Analysis
Cell apoptosis was identified using Annexin V-FITC/PI apoptosis detection kit (MultiScience Biotech, Hangzhou, China) as described previously.\textsuperscript{20}

Subcellular Fraction Assays
Cytoplasmic & Nuclear RNA Purification Kit (Norgen, Belmont, CA, USA) was used to separate the nuclear
and cytoplasmic RNA of MCF-7/DDP and MDA-MB-
231/DDP cells.

**Luciferase Reporter Assay**

The wild or mutant SNHG15 sequence containing predicted
miR-381 binding site were subcloned respectively into the
luciferase reporter vectors to generate SNHG15-WT and
SNHG15-MUT vectors. Then, MCF-7/DDP and MDA-MB-
231/DDP cells were co-transfected with these constructs and
miR-381 or miR-con. Finally, luciferase activity in MCF-7/
DDP and MDA-MB-231/DDP cells was determined using
Luciferase Reporter assay system (Promega, Madison,
WI, USA).

**RNA Immunoprecipitation (RIP) Assays**

RIP experiments were performed using the Magna RIP RNA-
bounding protein immunoprecipitation kit (Millipore, Billerica,
MA, USA) and antibodies against Ago2 and IgG (Cell
Signaling Technology, Danvers, MA, USA). The co-precipi-
tated RNAs were purified and subjected to qRT-PCR analysis.

**Animal Experiments**

The animal experiments were conducted in strict accordance
with the guiding principles of the institutional animal ethics
committee and approved by the institutional research committee
of the First Affiliated Hospital of Zhengzhou University. MCF-
7/DDP cells stably expressing sh-SNHG15 were transplanted
into BALB/c nude mice (six-week-old) from Slac Laboratory
(Shanghai, China), and then mice were injected intraperitone-
ally with 5 mg/kg cisplatin or same volume of PBS every seven
days. Every seven days, tumor sizes were monitored, and tumor
volumes were calculated based on the formula: volume = 0.5 ×
length × width². Mice were euthanized at 35th day post injection
and tumors were excised and weighed.

**Statistical Analysis**

All data were evaluated as means±standard deviation
(SD). The statistic difference was calculated using
Student’s t-test and one-way ANOVA. P value <0.05 was
considered statistically significant.

**Results**

**SNHG15 Was Up-Regulated In DDP-
Resistant Breast Cancer Tissues And Cell
Lines**

To investigate the function of SNHG15 in breast cancer,
we firstly examined the expression of SNHG15 in breast
cancer tissues from TCGA databases. Compared with nor-
mal tissues, SNHG15 expression was dramatically
increased in breast cancer tumor tissues (Figure 1A). To
further prove the result from TCGA databases, SNHG15
expression in breast cancer tumor tissues (n=42) and adja-
cent normal tissues (n=42) was further determined by qRT-
PCR analysis. Consistently, SNHG15 was higher in breast
cancer tissues than that in adjacent normal tissues
(Figure 1B). Additionally, SNHG15 expression was extre-
remely increased in DDP-resistant breast cancer tissues
when compared with DDP-sensitive breast cancer tissues
(Figure 1C). Furthermore, the expression of SNHG15 was
significantly improved in MCF-7 and MDA-MB-231 cells
compared with normal MCF-10A cells (Figure 1D and E).
Notably, compared with their parental cells, MCF-7/DDP
and MDA-MB-231/DDP cells displayed high SNHG15
expression level (Figure 1D and E). Moreover, the breast
cancer patients with high SNHG15 level had a poor prog-
nosis (P = 0.0162) (Figure 1F). Collectively, these data
suggested that up-regulated SNHG15 may be implicated
with cisplatin resistance in breast cancer.

**SNHG15 Knockdown Overcame
Cisplatin Resistance Of Breast Cancer
Cells**

To evaluate the resistance of MCF-7/DDP and MDA-MB-231/
DDP cells to DDP, IC50 of DDP was measured by MTT assay
in DDP-resistant MCF-7/DDP and MDA-MB-231/DDP cells
and parental MCF-7 and MDA-MB-231 cells. Compared with
the parental cells, MCF-7/DDP and MDA-MB-231/DDP cells
displayed poor response to DDP (Figure 2A). To further con-
firm the role of SNHG15 in DDP-resistant breast cancer cells,
MCF-7/DDP and MDA-MB-231/DDP cells were transfected
with SNHG15 siRNAs (si-SNHG15 #1, si-SNHG15 #2 or si-
SNHG15 #3) or si-con. qRT-PCR analysis indicated that intro-
duction of SNHG15 siRNAs evidently declined SNHG15
expression in MCF-7/DDP and MDA-MB-231/DDP cells
(Figure 2B), especially in si-SNHG15 #2 treated group.
Therefore, si-SNHG15 #2 (si-SNHG15) was used for further
experiments. Remarkably, SNHG15 silencing suppressed the
cell viability and enhanced cisplatin sensitivity in MCF-7/DDP
and MDA-MB-231/DDP cells (Figure 2C and D). To further
determine the role of SNHG15 in DDP-induced apoptosis, flow
cytometry analysis was conducted in MCF-7/DDP and MDA-
MB-231/DDP cells with or without 10 μM DDP treatment.
SNHG15 knockdown could increase cell apoptosis in MCF-7/
DDP and MDA-MB-231/DDP cells (Figure 2E and F).
Prominently, inhibition of SNHG15 in combination with DDP exposure could exert their synergistic effect contributing to significant enhancement in cell apoptosis in MCF-7/DDP and MDA-MB-231/DDP cells (Figure 2G and H). Collectively, SNHG15 knockdown facilitated cisplatin sensitivity in breast cancer cells.

**SNHG15 Acted As A miR-381 Sponge In Breast Cancer Cells**

Starbase2.0 predicts that the sequence of SNHG15 harbors a binding site of miR-381 (Figure 3A). Moreover, subcellular fraction assay revealed that SNHG15 was mainly distributed in the cytoplasm of MCF-7/DDP and MDA-MB-231/DDP cells (Figure 3B and C). Hence, we further investigated whether SNHG15 functioned as a miR-381 sponge in DDP-resistant breast cancer cells. Luciferase reporter assay indicated that miR-381 overexpression obviously reduced the relative luciferase activity of SNHG15-WT but not SNHG15-MUT, which suggests SNHG15 could interact with miR-381 (Figure 3D and E). RNA RIP assay confirmed that SNHG15 and miR-381 were greatly enriched in the Ago2 antibody-treated group compared with the IgG antibody-treated group (Figure 3F and G). To further explore the regulatory role of SNHG15 in miR-381 expression, MCF-7/DDP and MDA-MB-231/DDP cells were transfected with si-SNHG15 or si-con, Vector, SNHG15 or SNHG15-MUT. In both MCF-7/DDP and MDA-MB-231/DDP cells, knockdown of SNHG15 markedly elevated miR-381 expression. Oppositely, the expression level of miR-381 was remarkably decreased by SNHG15 up-regulation. However, transfection with SNHG15-MUT has no effect on miR-381 expression (Figure 3H and I). Especially, miR-381 expression was dramatically reduced in breast cancer tissues (Figure 3J). miR-381 expression was lower in DDP-resistant breast cancer tissues than that in DDP-sensitive ones (Figure 3K). Moreover, SNHG15 is negatively correlated with miR-381 expression in breast cancer tissues (Figure 3L). All these data evidenced that SNHG15 sponges miR-381 in breast cancer cells.

**miR-381 Overexpression Enhanced Cisplatin Sensitivity Of Breast Cancer Cells**

To further study the effect of miR-381 on DDP-resistant breast cancer cells, MCF-7/DDP and MDA-MB-231/DDP
cells were transfected with miR-381 mimics or miR-con. qRT-PCR analysis revealed that miR-381 expression was strangely increased in miR-381 transfecting MCF-7/DDP and MDA-MB-231/DDP cells (Figure 4A and B). Moreover, overexpression of miR-381 enhanced DDP sensitivity in MCF-7/DDP and MDA-MB-231/DDP cells (Figure 4C and D). Additionally, flow cytometry analysis showed that miR-381 overexpression strangely enhanced DDP-induced apoptosis in MCF-7/DDP and MDA-MB-231/DDP cells (Figure 4E and F). Together, miR-381 overexpression facilitated cisplatin sensitivity in breast cancer cells.
SNHG15 Knockdown Improved Cisplatin Sensitivity Of Breast Cancer Cells Through Sponging miR-381

To further investigate whether SNHG15 contributed to cisplatin resistance in breast cancer through sponging miR-381 expression, MCF-7/DDP and MDA-MB-231/DDP cells were transfected with si-con, si-SNHG15, si-SNHG15+anti-miR-con or si-SNHG15+anti-miR-381. Transfection of si-SNHG15 elevated miR-381 expression, which was extremely reversed by miR-381 inhibition (Figure 5A and B). MTT assay revealed that down-regulation of SNHG15 facilitated cisplatin sensitivity of MCF-7/DDP and MDA-MB-231/DDP cells, however, SNHG15 inhibition-mediated enhancement of cisplatin sensitivity in MCF-7/DDP and MDA-MB-231/DDP cells was patently abolished by miR-381 down-regulation (Figure 5C and D). Furthermore, introduction of anti-miR-381 extremely demolished the inductive effect of silenced SNHG15 on apoptosis in MCF-7/DDP and MDA-MB-231/
DDP cells (Figure 5E and F). Taken together, these results demonstrated that SNHG15 led to cisplatin resistance of breast cancer cells through sponging miR-381.

SNHG15 Knockdown Enhanced Cisplatin Sensitivity In Tumors In Vivo

To validate whether SNHG15 contributed to cisplatin resistance in vivo, a mouse breast cancer xenograft model was created. We made an sh-SNHG15 stably expressing MCF-7/DDP cell line and inoculated them subcutaneously into nude mice. Thenceforth, mice were injected intraperitoneally with PBS or CDDP, and tumor size was evaluated every 7 days. SNHG15 silencing evidently suppressed tumor growth. Cisplatin treatment also inhibited the growth of tumors. Moreover, SNHG15 silencing in combination with cisplatin administration displayed slower tumor growth (Figure 6A). At the 35th day post treatment, mice were euthanized, and xenograft tumors were excised and weighed. The tumor weight was reduced after SNHG15 knockdown or cisplatin exposure, especially when SNHG15 knockdown and cisplatin treatment simultaneously (Figure 6B). In addition, SNHG15 silencing or cisplatin treatment inhibited the expression of SNHG15 while up-regulated the expression of miR-381, and this effect could be strengthened by combination of SNHG15 silencing and cisplatin exposure (Figure 6C). All these data demonstrated that knockdown of SNHG15 improved cisplatin sensitivity in breast cancer cells in vivo.

Discussion

Therapeutic outcome has been restricted by chemoresistance severely for breast cancer patients. Consequently, to reveal the underlying mechanism and uncover novel therapeutic strategies for chemoresistance is not dispensable. In this study, we found that SNHG15 expression was significantly increased in DDP-resistant breast cancer tissues and cells. Moreover, knockdown of SNHG15 re-sensitized DDP-resistant breast cancer cells to DDP. Prominently, SNHG15 silencing improved the response of breast cancer cells to cisplatin through elevating miR-381 expression. Therefore, SNHG15 is a positive regulator in breast cancer cisplatin resistance and targeting SNHG15 may be an effective scheme for cisplatin chemoresistance in breast cancer.

Elucidating the molecular mechanism underlying DDP resistance was helpful to discover reasonable and effective targeted therapeutic strategies to overwhelmed DDP resistance. Our study found that SNHG15 expression was increased in DDP-resistant breast cancer tissues and cells, and SNHG15 silencing overcame DDP resistance in DDP-resistant breast cancer cells. In line with our findings, increasing evidence demonstrated that dysregulated...
SNHG15 was implicated with chemoresistance in various malignancies. For instance, knockdown of SNHG15 could suppress cell proliferation and invasion, and sensitize colorectal cancer cells to 5-FU.\textsuperscript{13} Moreover, SNHG15 was overexpressed in epithelial ovarian cancer, and contributed to the proliferation, migration, invasion, and

Figure 5 miR-381 knockdown reversed the enhancive effect of down-regulated SNHG15 on cisplatin sensitivity of breast cancer cells. MCF-7/DDP and MDA-MB-231/DDP cells were transfected with si-con, si-SNHG15, si-SNHG15+anti-miR-con or si-SNHG15+anti-miR-381, followed by determination of miR-381 expression by qRT-PCR analysis (A and B), IC50 of cisplatin by MTT assay (C and D), and cell apoptotic rate by flow cytometry analysis (E and F). *P < 0.05.
chemoresistance of epithelial ovarian cancer cells towards cisplatin. All these findings demonstrated that targeted inhibiting SNHG15 maybe a hopeful therapeutic strategy for CDDP chemoresistance.

How elevated SNHG15 contributes to cisplatin resistance in breast cancer remains elusive. Hence, the operative mechanism of SNHG15 was further explored in this work. Emerging evidence suggested that SNHG15 inhibited the expression of miRNAs through functioning as miRNA sponges. However, whether SNHG15 could regulate cisplatin resistance in breast cancer through controlling miRNA expression was still indescribable. In our study, Starbase2.0 database prediction and luciferase reporter assays confirmed that SNHG15 could bind with miR-381 in MCF-7/DDP and MDA-MB-231/DDP cells. Mounting evidence have indicated that miR-381 played a tumor suppressor role in tumorigenesis. Moreover, down-regulated miR-381 was implicated with chemoresistance in many cancers. Prominently, miR-381 could enhance cisplatin sensitivity of breast cancer cells through targeting MDR1. Correspondingly, our found indicated that overexpression of miR-381 overcame cisplatin resistance in MCF-7/DDP and MDA-MB-231/DDP cells. Additionally, miR-381 inhibition reversed SNHG15 silencing-mediated enhancement of DDP sensitivity in MCF-7/DDP and MDA-MB-231/DDP cells. All these data demonstrated that SNHG15 inhibition overcame DDP resistance in breast cancer cells through sponging miR-381, providing a promising therapeutic target for breast cancer DDP resistance.

**Ethics Approval And Consent To Participate**

Written informed consent was obtained from all participants. This study had acquired the approval of the ethics committee of the First Affiliated Hospital of Zhengzhou University. The animal experiments were conducted in strict accordance with the guiding principles of the institutional ethics committee and approved by the institutional research committee of the First Affiliated Hospital of Zhengzhou University.

**Disclosure**

The authors have no conflicts of interest to declare regarding this work.

**References**