Long Non-Coding RNA SNHG12 Contributes to Cisplatin Resistance by Mediating WEE1 via miR-503-5p in Cervical Cancer

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Abstract

Background: Emerging evidences have indicated that the aberrant expression of long noncoding RNAs (lncRNAs) was responsible for drug resistance, which represents a major obstacle for chemotherapy failure. Our previous study has showed that small nuclear RNA host gene 12 (SNHG12) was increased and contributed to cell growth and invasion in cervical cancer. In the present study, we aimed to investigate the role of the lncRNA SNHG12 in cisplatin (DDP) resistance and elucidate its underlying mechanisms in cervical cancer.

Methods: The expression and prognosis of SNHG12 in cervical cancer tissues were evaluated based on bioinformatics. MTT, colony formation assay and flow cytometer were performed to detect cell viability. Further, Molecular relationships among CTD-3252C9.4, IRF1 and IFI6 were investigated via luciferase reporter assay, western blot, and qRT-PCR. Finally, subcutaneous xenograft model was established to verify our findings.

Results: In the present study, we evaluated the cell apoptosis and half maximal inhibitory concentration (IC50) of cervical cancer upon DDP treatment. Mechanically, we found that SNHG12 upregulated WEE1 expression to regulate cell and DDP resistance via sponging miR-503-5p. Moreover, SNHG12 silencing inhibited the growth of DDP-resistant cervical cancer tumors in vivo.

Conclusions: Taken together, our findings suggested that a SNHG12/miR-503-5p/ WEE1 axis which modulated the chemoresistance of cervical cancer cell to DDP, and provided promising targets for dealing with the chemoresistance of cervical cancer.

Introduction

Cervical cancer is the second most common malignancy amongst women worldwide [1]. Though great efforts have been made to improve the diagnosis and treatment of cancer in recent years; however, resistance to chemotherapy usually exists in the process of treating cancers [2, 3]. At present, cisplatin (DDP) was considered to be one of the most effective drugs for the clinical treatment of multiple cancers, including cervical carcinoma [3–5]. However, DDP resistance has become a major challenge for cervical carcinoma treatment and the molecular basis for resistance remains unclear.

Long non-coding RNAs (lncRNAs) are a family of non-coding RNAs > 200 nucleotides in length [6, 7]. Increasing evidence indicates that lncRNAs contribute to cancer initiation, progression, as well as chemotherapy resistance [8–10]. Previous studies have suggested that SNHG12 was significantly evaluated in a variety of cancers, including bladder cancer, osteosarcoma, prostate cancer and cervical cancer [11–14]. For example, SNHG12 was significantly increased in bladder cancer tissues compared to adjacent normal tissues and high expression of SNHG12 was associated with shorter recurrence-free survival time [11]. SNHG12 was reported to promote tumorigenesis and metastasis via modulating STAT3 by sponging miR-125b in cervical cancer [15]. SNHG12 was firstly reported to reverse the resistance to cisplatin by SNHG12-miR-181a-MAPK/Slug axis in NSCLC [16]. Subsequent experiments
showed that SNHG12 mediates doxorubicin resistance via miR-320a/MCL1 axis in osteosarcoma [17]. Based on these literatures, we speculated that SNHG12 might play a crucial role in chemoresistance in cervical cancer.

Wee1 is a tyrosine kinase that participates in multiple aspects of tumor biology, including proliferation, migration, invasion, and survival [18, 19]. Wee1 is known to be overexpressed in HPV-positive head and neck squamous cell carcinoma (HNSCC) and inhibition of Wee1 has been shown to sensitize tumor cells to DDP [20]. In the current study, we designed experiments to investigate the role of SNHG12 in cervical cancer resistance to DDP. We proposed that SNHG12 might contribute to CDDP resistance via regulating the miR-503-5p/WEEl axis.

**Materials And Methods**

**Cell culture and construction of the DDP-resistant cervical cancer cell line**

The HEK 293T cells and human cervical cancer cell lines (HeLa and CaSki) were purchased from the Cell Bank of Chinese Academy of Sciences (Shanghai, China) and cultured as our previously described [14]. DDP (Sigma, St. Louis, MO, USA) was dissolved in in 0.9% sodium chloride at a concentration of 50 mM and stored at −20°C for use. DDP-resistant HeLa/DDP and CaSki/DDP cells were established by continuously increasing the concentration of DDP. Eventually, the DDP resistant cervical cancer cell lines were successfully established by culturing the cells in 2 µM DDP.

**Bioinformatics**

Pharmacological screens analysis was performed using online database iGMDR database (https://igmdr.modellab.cn/index.php) to identify potential new treatments and to explore biomarkers of drug sensitivity in cancer cells. GEPIA2 (http://gepia2.cancer-pku.cn/#index) and UALCAN (http://ualcan.path.uab.edu/) was used to the gene expression and patient's clinical data from TCGA. LinkedOmics (http://www.linkedomics.org/) was used to explore the related genes and Gene Set Enrichment Analysis (GSEA).

**RNA isolation and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) analysis**

Total RNA extraction and qRT-PCR were performed as our previously described [14]. The primers used for WEE1 as follows: WEE1 forward: 5’-GACGAAGATGATTGGGCATCC-3’, WEE1 reverse: 5’-TGGACTGGAGATCCTTGTTACA-3’.

**Cell viability and colony formation assays**

Different treatment cells in logarithmic growth phase were seeded into 96-well plates at $8 \times 10^3$ cells per well with 100 µL culture medium. The medium in the administration group was replaced with culture medium containing different DDP concentrations 24 h later. Cells in each well were added with 10 µL CCK-8 (Dojindo, Japan) 48 h later. After 2 h incubation, the optical density (OD) value was measured at a
wavelength of 450 nm using a microplate ELISA reader (Bio-Rad, Hercules, CA). The half inhibitory concentration (IC50) was calculated according to the OD value.

For colony formation assay, 8 × 10^2 cells per well HeLa/DDP and CaSki/DDP cells transduced with or without shSNHG12 were seeded into 6-well plates, the medium was replaced with culture medium containing 8µM DDP 24 h later. 5 × 10^3 cells per well HeLa and CaSki cells transfected with or without SNHG12 were seeded into 6-well plates, the medium was replaced with culture medium containing 200 nM DDP 24 h later. Colonies containing at least 50 cells on the plates were fixed with 4% polyoxymethylene and stained with 1% crystal violet for 15 min 2 weeks later.

Cell apoptosis analysis

Cells were treated as above mention. Then cells were harvested and stained with Annexin V-FITC and propidium iodide (BD Biosciences, NJ, USA) according to the manufacturer’s instructions. The percentage of apoptotic cells was determined on a FACScalibur flow cytometer and analyzed using CellQuestPro software (Becton Dickinson, USA).

Cell transfection

To overexpress SNHG12, the full-length of IncRNA SNHG12 were amplified and cloned into cloned into PCDNA3.1 vector. MiR-503-5p mimics, miRNA negative control mimics (miR-NC), NC inhibitor (anti-NC) and miR-503-5p inhibitor (anti-miR-503-5p) were purchased from GenePharma (Shanghai, China). Cells in the logarithmic growth phase were transfected using HiPerFect Transfection reagent (Qiagen, German) according to the manufacturer’s instructions.

Dual-luciferase reporter gene assay

To construct dual luciferase reporter plasmids, the potential binding sequence of miR-503-5p in SNHG12 or WEE1 and their mutated sequence were separately cloned into pMir-Reporter plasmid (Life Technologies). HEK-293T cells were co-transfected with miR-503-5p mimics or miR-NC, and luciferase reporter constructs using HiPerFect Transfection reagent according to the manufacturer’s protocol. The luciferase activity was then detected using a Dual-Luciferase® Reporter Assay System (Promega, USA) at 24 h post-transfection.

Animal experiments

Female athymic BALB/c nude mice (4 weeks old) were obtained from Shanghai Slac Laboratory Animal Co., Ltd. (Shanghai, China). All of the animal experiments were performed in accordance with the guidelines of the Guide for the Care and Use of Laboratory Animals. This study was approved by the Institutional Animal Care and Use Committee of Obstetrics and Gynecology Hospital of Fudan University. Approximately 1 × 10^7 HeLa/DDP cells transduced with shSNHG12 in 0.2 ml serum-free medium were subcutaneously injected in the axilla of mice. After the tumors grew to approximately 1000 mm^3 in size,
(5 mg/Kg) DDP or saline were administered every 5 days. Mice were sacrificed and the xenograft tissues were harvested and then frozen or paraffin embedded for immunohistochemical detection.

**Statistical analysis**

Data were presented as mean ± standard deviation (SD). Group comparison was performed by Student's t-test or One-Way Analysis of Variance followed by Newman-Keuls post hoc test. The Pearson's correlation analysis was performed to assess the correlation between SNHG12 and miR-03-5p or WEE1 expression. A P value < 0.05 was considered as significant difference.

**Results**

LncRNA SNHG12 is up-regulated in DDP-resistant cervical cancer cell lines

The differential expression of SNHG12 between cervical cancer tissues and normal tissues using GEPIA2 (http://gepia2.cancer-pku.cn/#index) and UALCAN (http://ualcan.path.uab.edu/) based on TCGA database. The results showed that SNHG12 was dramatically higher in cervical cancer patients than in healthy people (Figs. 1A). The upregulation of SNHG12 wasn't found to be associated with the stage (Figs. 1B) and subtype (Figs. 1C) of cervical cancer. Furthermore, it could be observed that the overall survival rate in the high expression group (n = 77) was lower than that in the low expression group (n = 227) from The Cancer Genome Atlas (TCGA) database (http://kmplot.com/analysis/index.php) (Fig. 1D). Our previous studies have shown that SNHG12 was up-regulated in cervical cancer tissues and cell lines [19], then we further explored whether SNHG12 was involved in the DDP resistance of cervical cancer cells. Interestingly. Then DDP-resistant HeLa/DDP and CaSki/DDP cell line were established with gradually increasing DDP concentration. SNHG12 expression was also significantly increased in both HeLa/DDP and CaSki/DDP cells compared with their parental cells by qRT-PCR, respectively (Fig. 1E). Therefore, these prompted us to suspect that SNHG12 might participate in DDP-resistance of cervical cancer.

Inhibition of SNHG12 suppressed the drug resistance of cervical cancer cells to DDP, whereas

To evaluate the role of SNHG12 in the regulation of cervical cancer chemoresistance to DDP, the expression of SNHG12 was knocked down by shRNA in both HeLa/DDP and CaSki/DDP cells. SNHG12 was dramatically decreased after SNHG12 silencing in both HeLa/DDP and CaSki/DDP cells (Fig. 2A). After transduction, the effects of SNHG12 on the proliferation and apoptosis of DDP-resistant cells were assessed. Upon 8 µg/ml DDP treatment, the IC50 value of SNHG12 knockdown was markedly lower than that of SCR group (Fig. 2B). Furthermore, apoptosis assay showed that SNHG12 silencing induced DDP-resistant cells apoptosis (Fig. 2C). Moreover, knockdown of SNHG12 led to a significant repressed anchorage-independent growth upon DDP treatment (Fig. 2D). In contrast, SNHG12 was overexpressed in HeLa and CaSki cells by transfecting SNHG12 and confirmed by qRT-PCR (Fig. 2E). As expected, overexpression of SNHG12 could enhanced the IC50 value (Fig. 2F) and colony formation (Fig. 2G), and
inhibited apoptosis (Fig. 2H) of cervical cancer cells. In summary, these results revealed that SNHG12 played an important role in the chemoresistance of cervical cancer cell to DDP.

SNHG12 served as a molecule sponge for miR-503-5p

It is widely acknowledged that lncRNAs can act as competitive RNA (ceRNA) through sponging with miRNAs in various cancers [7]. To determine the potential mechanism of SNHG12, online software program starbase v2.0 (http://starbase.sysu.edu.cn/miRLncRNA.php) was used to predict the possible miRNAs. To better understand the role of SNHG12 in the development of cervical cancer, LinkedOmics was used to analyze its related genes. The top 50 genes that were significantly positively or negatively correlated with SNHG12 are indicated with a heat map (Fig. 3A). Further, the enrichment functions of GO annotations (Fig. 3B) and KEGG pathways (Fig. 3C) analyzed using GSEA showed that ribosome played a crucial role in biological process of SNHG12. By comparing all of the candidate genes predicted by starbase v2.0, miR-503-5p was selected due to the tumor suppressive roles of miR-503-5p in several cancers (Fig. 3D) [21–23]. To further investigate whether SNHG12 was a functional target of miR-503-5p, luciferase reporter was performed and our results showed that overexpressed miR-503-5p could downregulate the wild-type luciferase activity of SNHG12 but not the mutant of SNHG12 (Fig. 3E). Furthermore, miR-503-5p overexpression resulted in a significant decrease of SNHG12 expression (Fig. 3F), whereas SNHG12 knockdown increased miR-503-5p expression (Fig. 3G). Moreover, miR-503-5p was significantly decreased in cervical cancer tissues compared to the paired adjacent normal tissues (Fig. 3H). A negative correlation between SNHG12 and miR-503-5p expressions was observed in cervical cancer tissues (Fig. 3I). All these findings suggested that SNHG12 was a sponge of miR-503-5p and negatively regulated the expression of miR-503-5p.

MiR-503-5p was required for SNHG12-mediated drug sensitivity of DDP-tolerated cervical cancer cells

To determine the functional significance of miR-503-5p in the SNHG12-induced phenotype, a series of restoration assays were performed in DDP resistance of cervical cancer cells. Anti-miR-503-5p was used to inhibit miR-503-5p expression in SNHG12-depleted DDP-tolerant cervical cancer cells and miR-503-5p was significantly decreased after miR-503-5p inhibitor transfection (Fig. 4A). As illustrated in Fig. 4B and 4C, decreased IC50 value of SNHG12-depleted DDP-tolerant cervical cancer cells was increased by miR-503-5p inhibitor transfection. Furthermore, inhibition of miR-503-5p could significantly abrogate the effect of SNHG12 on DDP-tolerant cervical cancer cells. Colony formation assay could not be performed because of the few numbers of colonies after SNHG12 silencing. Taken together, these results implied that miR-503-5p was required for the biological functions of SNHG12 in cervical cancer cells.

WEE1 was directly targeted by miR-503-5p and partly controlled by SNHG12

Using online software program, TargetScan, starbase v2.0 and miRDB, a total of 145 genes were identified to potentially regulate by miR-503-5p (Fig. 5A). Investigating genetic model of drug response (iGMDR) (https://igmdr.modellab.cn) was used to assess the drug sensitivity of WEE1 in cervical cancer [24]. The results showed the related genes (Fig. 5B) and pathways (Fig. 5C) involved in drug response of WEE1. By
comparing all candidate genes predicted by the programs, WEE1 was selected for further experiments due to the ontogenetic role of WEE1 in cervical cancer (Fig. 5D). Luciferase reporter assays were performed and the results revealed that miR-503-5p could dramatically suppress the luciferase activity of the reporter gene in recombinant plasmids containing the wild-type 3'UTRs of WEE1 in HEK-293T cells but did not affect the activities of mutant (Fig. 5E). In concordance with these results, the mRNA and protein expression of WEE1 was significantly decreased in miR-503-5p-overexpressed cervical cancer cells (Fig. 5F). Similar results were observed in SNHG12-depleted DDP-tolerant cervical cancer cells (Fig. 5G). Moreover, introduction of anti-miR-503-5p partly abrogated the downregulation of WEE1 induced by SNHG12 silencing in both HeLa/DDP and CaSki/DDP cells (Fig. 5H). In addition, the mRNA level of WEE1 was upregulated in cervical cancer tissues (Fig. 5I). A dramatically negative correlation was noted between miR-503-5p and WEE1 expression (Fig. 5J) and a positive correlation was also noted between SNHG12 and WEE1 expression in cervical cancer tissues (Fig. 5K). Taken together, these data indicated that the miR-503-5p/WEE1 axis mediated the effect of SNHG12 on cervical cancer cells DDP resistance.

SNHG12 silencing decreases cervical cancer cell resistance to DDP therapy in vivo

To investigate the in vivo effect of SNHG12 on cervical cancer DDP resistance, we generated xenografts using SNHG12 stale knockdown HeLa/DDP cells. Once the tumors reached a diameter of 1.0 cm, mice was divided into two groups and treated with either DDP or saline solution, respectively (Fig. 6A). DDP treatment led to a significant reduction of tumor volume compared to that of the group without DDP treatment (Fig. 6B). Furthermore, the growth curse and weight of the tumors with DDP treatment were decreased compared with those in the group without DDP treatment (Fig. 6C and D). In addition, the expression of c-Myc was remarkably decreased in SNHG12 silencing xenografts tissues compared to the PBS-treated xenografts sections (Fig. 6E), which further confirmed that SNHG12 has a positive effect on both the growth and drug resistance in vitro and in vivo.

Discussion

Chemotherapy is regarded as a standard strategy for patients with advanced or recurrent cervical cancer and DDP was the first-line treatment in chemotherapy of cervical cancer [25, 26]. The anti-tumor effects of DDP has shown to promote DNA damage by binding to DNA and crosslinking the DNA strands, which resulted in cell death [27–29]. However, DDP resistance became a major obstacle for cancer therapy and compromised the efficacy of DDP to treat advanced or recurrent cervical cancer. Our previous study has shown that SNHG12 is significantly upregulated in cervical tissues and knockdown of SNHG12 expression suppressed cell proliferation and invasion by modulating miR-424-5p expression [14].

Recently, a growing body of reports has demonstrated that IncRNAs played functional roles in DDP resistance of cancer cells [30, 31]. SNHG12 was been reported to increase in Temozolomid(TMZ)-resistant glioblastoma cells and enforcing SNHG12 expression led to the development of acquired TMZ resistance [32]. This study focuses on the importance of further understanding the role of SNHG12 in drug resistance in cervical cancer. In this study, we confirmed the expression of SNHG12 upregulated in
DDP-resistant cervical cancer tissues and DDP-resistant cells. Further investigation demonstrated that SNHG12 knockdown decreased the IC50 value of DDP-resistant cervical cancer cells and clone formation, and promoted cell apoptosis, whereas overexpression of SNHG12 had an opposite effects in the presence of DDP. These results indicated that SNHG12 may be a promising new treatment intervention for the patients with cervical cancer.

Numerous reports have suggested that IncRNAs harbored the recognition sequence of many miRNAs and SNHG12 has been predicted to function as ceRNA of several miRNAs, including miR-320, miR-125b and miR-181, miR-195-5p, miR-129-5p [7, 12, 13, 15–17]. In our research, we found that SNHG12 functions as a ceRNA to repress miR-503-5p expression. MiR-503-5p was reported to be downregulated in several cancers, including lung cancer, gastric cancer, ovarian cancer, and correlates with tumor progression and clinical prognosis [22, 33, 34]. In addition, downregulation of miR-503-5p contribute to cell survival and chemoresistance in ovarian cancer and colorectal carcinoma [22, 23]. Consisted with these data, the inhibitory phenomenon of SNHG12 silencing could partially was blocked by miR-503-5p inhibitor in DDP resistance cervical cancer cells. Subsequent study confirmed that WEE1 was a direct target of miR-503-5p and indirectly regulated by SNHG12. WEE1, a known oncogene, has been reported to increase in many cancers and involved in DNA damage and drug resistance [35, 36].

**Conclusion**

In conclusion, our data proved that SNHG12 was upregulated in cervical cancer tissues from patients who did not respond to DDP treatment than those from patients who experienced response to chemotherapy. *In vitro* experiments demonstrated that overexpression or inhibition of SNHG12 would alter DDP resistance through the miR-503-5p/WEE1 axis. There are some limits in our present study: 1, the effects of SNHG12 on DNA damage; 2, WEE1 inhibitors could effectively overcome the DDP resistance patients, especially high SNHG12 expression; 3, more patients were needed to confirm our data.

**Declarations**

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None

**Author information**

Author notes

Jing Dong and Qing Cong contributed equally to this work.

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Ethics declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author Contributions
DJ, CQ and XF performed the experiments and analysed the data. ZXJ and DJ designed the research study and write this manuscript. All authors reviewed and approved the manuscript.

Availability of data and materials
Not applicable.

References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin. 2015;65(2):87–108.

2. Kondagunta GV, Sheinfeld J, Mazumdar M, Mariani TV, Bajorin D, Bacik J, Bosl GJ, Motzer RJ. Relapse-free and overall survival in patients with pathologic stage II nonseminomatous germ cell cancer treated with etoposide and cisplatin adjuvant chemotherapy. J CLIN ONCOL. 2004;22(3):464–7.

3. Siddik ZH. Cisplatin: mode of cytotoxic action and molecular basis of resistance. ONCOGENE. 2003;22(47):7265–79.

4. Guo L, Zhang C, Zhu J, Yang Y, Lan J, Su G, Xie X. Proteomic identification of predictive tissue biomarkers of sensitive to neoadjuvant chemotherapy in squamous cervical cancer. LIFE SCI. 2016;151:102–8.

5. Wang X, Xu Z, Sun J, Lv H, Wang Y, Ni Y, Chen S, Hu C, Wang L, Chen W, et al. Cisplatin resistance in gastric cancer cells is involved with GPR30-mediated epithelial-mesenchymal transition. J CELL MOL MED. 2020;24(6):3625–33.

6. Lin C, Yang L. Long Noncoding RNA in Cancer: Wiring Signaling Circuitry. TRENDS CELL BIOL. 2018;28(4):287–301.

7. Nagano T, Fraser P. No-nonsense functions for long noncoding RNAs. CELL. 2011;145(2):178–81.
8. Shin VY, Chen J, Cheuk IW, Siu MT, Ho CW, Wang X, Jin H, Kwong A. Long non-coding RNA NEAT1 confers oncogenic role in triple-negative breast cancer through modulating chemoresistance and cancer stemness. CELL DEATH DIS. 2019;10(4):270.

9. Shi W, Zhang C, Ning Z, Hua Y, Li Y, Chen L, Liu L, Chen Z, Meng Z. Long non-coding RNA LINC00346 promotes pancreatic cancer growth and gemcitabine resistance by sponging miR-188-3p to derepress BRD4 expression. J Exp Clin Cancer Res. 2019;38(1):60.

10. Sui C, Dong Z, Yang C, Zhang M, Dai B, Geng L, Lu J, Yang J, Xu M. LncRNA FOXD2-AS1 as a competitive endogenous RNA against miR-150-5p reverses resistance to sorafenib in hepatocellular carcinoma. J CELL MOL MED. 2019;23(9):6024–33.

11. Jiang B, Hailong S, Yuan J, Zhao H, Xia W, Zha Z, Bin W, Liu Z. Identification of oncogenic long noncoding RNA SNHG12 and DUXAP8 in human bladder cancer through a comprehensive profiling analysis. BIOMED PHARMACOTHER. 2018;108:500–7.

12. Zhou S, Yu L, Xiong M, Dai G. LncRNA SNHG12 promotes tumorigenesis and metastasis in osteosarcoma by upregulating Notch2 by sponging miR-195-5p. Biochem Biophys Res Commun. 2018;495(2):1822–32.

13. Song J, Wu X, Ma R, Miao L, Xiong L, Zhao W. Long noncoding RNA SNHG12 promotes cell proliferation and activates Wnt/beta-catenin signaling in prostate cancer through sponging microRNA-195. J CELL BIOCHEM. 2019;120(8):13066–75.

14. Dong J, Wang Q, Li L, Xiao-Jin Z. Upregulation of Long Non-Coding RNA Small Nucleolar RNA Host Gene 12 Contributes to Cell Growth and Invasion in Cervical Cancer by Acting as a Sponge for MiR-424-5p. CELL PHYSIOL BIOCHEM. 2018;45(5):2086–94.

15. Jin XJ, Chen XJ, Zhang ZF, Hu WS, Ou RY, Li S, Xue JS, Chen LL, Hu Y, Zhu H. Long noncoding RNA SNHG12 promotes the progression of cervical cancer via modulating miR-125b/STAT3 axis. J CELL PHYSIOI. 2019;234(5):6624–32.

16. Wang P, Chen D, Ma H, Li Y. LncRNA SNHG12 contributes to multidrug resistance through activating the MAPK/Slug pathway by sponging miR-181a in non-small cell lung cancer. Oncotarget. 2017;8(48):84086–101.

17. Zhou B, Li L, Li Y, Sun H, Zeng C: Long noncoding RNA SNHG12 mediates doxorubicin resistance of osteosarcoma via miR-320a/MCL1 axis. BIOMED PHARMACOTHER 2018, 106:850–857.

18. Richer AL, Cala JM, O'Brien K, Carson VM, Inge LJ, Whitsett TG. WEE1 Kinase Inhibitor AZD1775 Has Preclinical Efficacy in LKB1-Deficient Non-Small Cell Lung Cancer. CANCER RES. 2017;77(17):4663–72.

19. Leijen S, van Geel RM, Sonke GS, de Jong D, Rosenberg EH, Marchetti S, Pluim D, van Werkhoven E, Rose S, Lee MA, et al. Phase II Study of WEE1 Inhibitor AZD1775 Plus Carboplatin in Patients With TP53-Mutated Ovarian Cancer Refractory or Resistant to First-Line Therapy Within 3 Months. J CLIN ONCOL. 2016;34(36):4354–61.

20. Mendez E, Rodriguez CP, Kao MC, Raju S, Diab A, Harbison RA, Konnick EQ, Mugundu GM, Santana-Davila R, Martins R, et al. A Phase I Clinical Trial of AZD1775 in Combination with Neoadjuvant
21. Li T, Li Y, Gan Y, Tian R, Wu Q, Shu G, Yin G: **Methylation-mediated repression of MiR-424/503 cluster promotes proliferation and migration of ovarian cancer cells through targeting the hub gene KIF23.** *CELL CYCLE* 2019, **18**(14):1601–1618.

22. Park GB, Kim D. MicroRNA-503-5p Inhibits the CD97-Mediated JAK2/STAT3 Pathway in Metastatic or Paclitaxel-Resistant Ovarian Cancer Cells. *NEOPLASIA.* 2019;21(2):206–15.

23. Xu K, Chen G, Qiu Y, Yuan Z, Li H, Yuan X, Sun J, Xu J, Liang X, Yin P. miR-503-5p confers drug resistance by targeting PUMA in colorectal carcinoma. *Oncotarget.* 2017;8(13):21719–32.

24. Chen X, Guo Y, Chen X. iGMDR: Integrated Pharmacogenetic Resource Guide to Cancer Therapy and Research. *Genomics Proteomics Bioinformatics.* 2020;18(2):150–60.

25. He L, Wu L, Su G, Wei W, Liang L, Han L, Kebría M, Liu P, Chen C, Yu Y, et al. The efficacy of neoadjuvant chemotherapy in different histological types of cervical cancer. *GYNECOL ONCOL.* 2014;134(2):419–25.

26. Zhu H, Luo H, Zhang W, Shen Z, Hu X, Zhu X. Molecular mechanisms of cisplatin resistance in cervical cancer. *Drug Des Devel Ther.* 2016;10:1885–95.

27. Todd RC, Lovejoy KS, Lippard SJ. Understanding the effect of carbonate ion on cisplatin binding to DNA. *J AM CHEM SOC.* 2007;129(20):6370–1.

28. Dijt FJ, Fichtinger-Schepman AM, Berends F, Reedijk J. Formation and repair of cisplatin-induced adducts to DNA in cultured normal and repair-deficient human fibroblasts. *CANCER RES.* 1988;48(21):6058–62.

29. Schweizer U, Hey T, Lipps G, Krauss G. Photocrosslinking locates a binding site for the large subunit of human replication protein A to the damaged strand of cisplatin-modified DNA. *NUCLEIC ACIDS RES.* 1999;27(15):3183–9.

30. Qu W, Huang W, Yang F, Ju H, Zhu G: **Long noncoding RNA LINC00461 mediates cisplatin resistance of rectal cancer via miR-593-5p/CCND1 axis.** *BIOMED PHARMACOTHER* 2020, **124**:109740.

31. Wang Z, Wang Q, Xu G, Meng N, Huang X, Jiang Z, Chen C, Zhang Y, Chen J, Li A, et al: **The long noncoding RNA CRAL reverses cisplatin resistance via the miR-505/CYLD/AKT axis in human gastric cancer cells.** *RNA BIOL* 2020:1–14.

32. Lu C, Wei Y, Wang X, Zhang Z, Yin J, Li W, Chen L, Lyu X, Shi Z, Yan W, et al. DNA-methylation-mediated activating of IncRNA SNHG12 promotes temozolomide resistance in glioblastoma. *MOL CANCER.* 2020;19(1):28.

33. Park GB, Kim D. MicroRNA-503-5p Inhibits the CD97-Mediated JAK2/STAT3 Pathway in Metastatic or Paclitaxel-Resistant Ovarian Cancer Cells. *NEOPLASIA.* 2019;21(2):206–15.

34. Cai X, Nie J, Chen L, Yu F. **Circ_0000267 promotes gastric cancer progression via sponging MiR-503-5p and regulating HMGA2 expression.** *Mol Genet Genomic Med.* 2020;8(2):e1093.
35. Sand A, Piacsek M, Donohoe DL, Duffin AT, Riddell GT, Sun C, Tang M, Rovin RA, Tjoe JA, Yin J. WEE1 inhibitor, AZD1775, overcomes trastuzumab resistance by targeting cancer stem-like properties in HER2-positive breast cancer. CANCER LETT. 2020;472:119–31.

36. Lee YY, Cho YJ, Shin SW, Choi C, Ryu JY, Jeon HK, Choi JJ, Hwang JR, Choi CH, Kim TJ, et al. Anti-Tumor Effects of Wee1 Kinase Inhibitor with Radiotherapy in Human Cervical Cancer. Sci Rep. 2019;9(1):15394.

Figures
SNHG12 is upregulated in DDP-resistant cells. (A) The expression of SNHG12 between cervical cancer tissues and normal tissues was analyzed by public databases. Upregulation of SNHG12 correlated with disease stage (B) and (C) tumor metastasis. (D) Kaplan-Meier survival analysis of cervical cancer patients based on SNHG12 expression levels from TCGA database. **P < 0.01. (E) qRT-PCR analysis of SNHG12 expression levels in HeLa/DDP and CaSki/DDP and their parent cells.

Figure 1
SNHG12 was essential for DDP resistance of cervical cancer cells. (A) The silencing efficacy was evaluated after transduction of shRNA targeting SNHG12 in HeLa/DDP and CaSki/DDP cells by qRT-PCR. (B) The IC50 value of DDP was detected for both sensitive and resistant cells by CCK8 assay. (C) Colony formation assay analysis of anchorage-independent growth in HeLa/DDP and CaSki/DDP cells by DDP treatment. (D) Cell apoptosis was assessed by DDP treatment in both DDP-resistant cell lines by flow
cytometry analysis. (E) Relative expression of SNHG12 in HeLa and CaSki cell lines after SNHG12 transfection by qRT-PCR. (F) The IC50 value of DDP was detected in HeLa and CaSki cell lines by CCK8 assay. (G) Colony formation assay analysis of anchorage-independent growth in HeLa and CaSki cell lines by DDP treatment. (H) Cell apoptosis was assessed by DDP treatment in both HeLa and CaSki cell lines by flow cytometry analysis. **P < 0.01.

Figure 3

Reciprocal repression between SNHG12 and miR-503-5p. (A) Heat map of the top 50 positively and negatively correlated significant genes of SNHG12 in cervical cancer tissues. (B) Bar chart of Biological Process, Molecular Function, and Bar chart of Cellular Component. (C) KEGG pathway of SNHG12 analyses by LinkedOmics using GSEA methods in cervical cancer tissues. (D) Schematic representation of the predicted binding sites between miR-503-5p and SNHG12, and the MUT design for the reporter assays. (E) Luciferase reporter assay was performed by co-transfecting with the reporter plasmid (or the corresponding mutant reporter) and the indicated miRNAs in HEK293T cells. (F) Effects of miR-503-5p mimics on SNHG12 expression in HeLa/DDP and CaSki/DDP cells. (G) The expression of miR-503-5p after SNHG12 silencing. (H) Relative miR-503-5p expression in 46 paired cervical cancer tissues and normal non-cancer tissues. (I) An invert relationship was observed in cervical cancer tissues. Pearson correlation coefficients (r) and P values are shown. **P < 0.01.
Figure 4

Inhibition of miR-503-5p reversed malignant phenotypes inhibition of cervical cancer cells induced by SNHG12 silencing. (A) miR-503-5p inhibitors effectively reversed miR-503-5p expression inhibition induced by SNHG12 silencing. (B) Cell survival capacity was determined by CCK-8 assays in HeLa/DDP and CaSki/DDP cells transfected with or without miR-503-5p inhibitors at the indicated DDP treatment. (C) Cell apoptosis was determined by flow cytometry analysis in HeLa/DDP and CaSki/DDP cells transfected with or without miR-211-5p inhibitors at the indicated DDP treatment. **P < 0.01.
Figure 5

SNHG12 regulated WEE1 expression by sponging miR-503-5p. (A) Venn diagram of the potential targets of miR-503-5p overlapping. (B) SNHG12-related model genes that interact with other anticancer drugs and the drug-gene network. (C) Major oncogenic pathways were associated with SNHG12. (D) The putative miR-503-5p binding sequence in the WEE1 3'UTR. A mutation was generated as indicated. (E) Luciferase assays were performed by transfected with wild-type or mutant and miR-503-5p mimics or...
miR-503-5p inhibitors in HEK 293T cells. (F) The mRNA and protein levels of WEE1 were assessed after transfection of miR-503-5p mimics by qRT-PCR and western blotting, respectively. (G) The mRNA and protein levels of WEE1 were assessed in SNHG12-deleted HeLa/DDP and CaSki/DDP cells. (H) The protein levels of WEE1 in SNHG12-deleted HeLa/DDP and CaSki/DDP cells were transfected with miR-503-5p inhibitors or without miR-503-5p inhibitors. (I) The mRNA of WEE1 was frequently increased in 46 paired cervical cancer tissues and normal non-cancer tissues. (J) Correlation analysis between miR-503-5p and WEE1 expression. (K) Correlation analysis between SNHG12 and WEE1 expression. **P < 0.01.
Figure 6

Effect of SNHG12 on DDP drug resistance of HeLa/DDP xenografts in nude mice. (A) Schematic description of the experimental design in animal model. (B) Reprehensive images of xenografts. (C) Alterations of tumor growth curve after DDP treatment. (D) Weight measurements of the tumors described above. (E) The protein levels of c-Myc in each group were determined by immunostaining. ** p < 0.01.