

RESEARCH ARTICLE

Modulation of cisplatin resistance in gastric cancer by LncRNA SNHG15

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Abstract

Background: The role of long non-coding RNA (lncRNA) small nucleolar RNA host gene 15 (SNHG15) in cisplatin-resistance was studied in gastric cancer.

Methods: The relative SNHG15 expression and its correlation with survival were assayed on the Cancer Genome Atlas data and confirmed with the clinical samples we collected. Cisplatin-resistant MKN45 cell line (MKN45 CR) was established. SNHG15 plasmid and SNHG15 shRNA plasmid were transfected into MKN45 cells and MKN45 CR cells. Colony formation analysis and cell counting kit-8 assay were utilized to determine cell proliferation and viability. The binding between SNHG15 and zeste 2 polycomb repressive complex 2 subunit (EZH2) was testified with RNA immunoprecipitation (RIP). The promoter activity of phosphatase and tensin homolog (PTEN) was evaluated by luciferase reporter assay.

Results: High SNHG15 expression was correlated with the poor overall survival of gastric cancer patients, and cisplatin treatment can induce time and dose-dependent up-regulation of SNHG15. At the same time, SNHG15 overexpression could promote the cisplatin resistance, while SNHG15 inhibition could diminish the cisplatin resistance. RIP assays confirmed the interaction of SNHG15 with EZH2. More importantly, SNHG15 inhibited PTEN promoter activity by reducing EZH2 recruitment in MKN45 CR cells.

Conclusion: SNHG15 epigenetically prohibits PTEN expression by recruiting EZH2 in gastric cancer cisplatin-resistant cells.

Keywords: *LncRNA SNHG15; cisplatin resistance; PTEN; EZH2*

Received: 29 May 2022; Revised: 6 June 2022; Accepted: 9 June 2022; Published: 2 July 2022

Primary or acquired cisplatin resistance is a general phenomenon that limits therapeutic efficacy, resulting in the relapse and poor survival of gastric cancer (1–3). The genetic and epigenetic dysregulated signaling pathways are reported to mediate cisplatin resistance. More and more long non-coding RNAs (lncRNAs) are testified to regulate gene expression at multiple levels, including epigenetic, transcriptional, and posttranscriptional levels (4, 5).

LncRNAs can exert oncogenic or tumor suppressor function and regulate multiple biological processes. Besides, dysregulated lncRNAs participate in the cisplatin resistance of gastric cancer (6, 7). For

example, as a competitive miR-126 inhibitor, upregulated HOX transcript antisense intergenic RNA (HOTAIR) is detected in cisplatin-resistant gastric cancer tissues and cells (8), regulating phosphoinositol-3 kinase regulatory subunit 2 and vascular endothelial growth factor A to activate the PI3K/AKT/MRP1 signaling. As an oncogene, PVT-1 is upregulated in cisplatin-resistant gastric cancer cells and tissues, increasing the expression of multiple drug transporters, including multidrug resistance mutation one protein (MDR1) and multidrug resistance protein (9). All of these indicate that lncRNAs could function as potential targets in cisplatin resistance.

Upregulation of small nucleolar RNA host gene 15 (SNHG15) is reported in gastric cancer tissue and correlated with poor overall survival (OS) and the disease-free survival (10, 11). In contrast, little research has been performed to testify the association of lncRNA SNHG15 expression with cisplatin resistance and the relevant mechanism. This investigation testifies that SNHG15 can promote cisplatin resistance by epigenetically inhibiting phosphatase and tensin homolog (PTEN) via recruiting enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2).

Methods and materials

Patients

Twenty-two gastric cancer tissues and matched normal tissues, and another 24 gastric cancer tissues without matched normal tissues were retrieved. The informed consent was signed by all the patients enrolled, who were not treated with chemotherapy or radiotherapy before the surgery. The study was approved by People's Hospital of Xinjiang Uygur Autonomous Region.

The Cancer Genome Atlas data analysis

Gene Expression Profiling Interactive Analysis (GEPIA) (12), an online tool based on the expression and survival data derived from the Cancer Genome Atlas (TCGA) (13), was utilized to assay the relative expression of SNHG15 among gastric cancer tissues and matched normal tissues. The correlation analysis between SNHG15 and EZH2, SNHG15 and PTEN, and EZH2 and PTEN, was also performed.

Cell lines

Human gastric cancer cell lines (MKN28, MGC803, MKN45, NCI-N87, HGC27, and SGC7901) and GSE-1 (immortalized gastric epithelial cell line) were pursued from ATCC. A cisplatin-resistant MKN45 subline (MKN45 CR) was constructed by stepwise cisplatin exposure (starting from 0.1 to 1 µg/mL), which was further maintained in the cisplatin medium (10 µM) for more than 10 months to establish a stable MKN45 CR. Cisplatin was ordered from sigma, which was utilized to further treat gastric cancer cells as indicated concentration.

Cell Counting Kit-8 (CCK-8) assay

Gastric cancer cells of 1×10^5 were seeded into 96-well plates. When the cells reached the logarithmic growth phase, CCK-8 (10 µL) was added and incubated for 2 h. The absorption was monitored at 450 nm by a Spectra-Max M5 Microplate Reader.

Colony formation assay

Gastric cancer cells of 1×10^3 were inoculated into the 6-well plates and cultured for 14 days. Then, the cells were

fixed with 4% paraformaldehyde and stained with 0.5% crystal violet, and the number of the colony was counted.

Reverse transcription polymerase chain reaction (RT-PCR)

Trizol reagent was utilized to extract total RNAs from the specimens following the instruction, and cDNA was synthesized using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). The amplification was detected with the Power SYBR Green Master mix (Applied Biosystems). The relative expression was normalized to *GAPDH* using the $2^{-\Delta\Delta C_t}$ method. Primer sequences were listed: *GAPDH*, forward: 5'-GTCTCCTCTGACTTCAACAGCG-3', reverse: 5'-ACCACCCTGTTGCTGTAGCCAA-3'; *SNHG15*, forward: 5'-GCATCTCTTCCCCTACTATCTGC-3', reverse: 5'-TGGTTTCAT CTCCCAGCAC-3'; *PTEN*, forward: 5'-TGAGTTCCCTCAGCCGTTACCT-3', reverse: 5'-GAGGTTTCCTCTGGTCTGGTA-3'.

RNA immunoprecipitation

The chromatin was cross-linked and sheared into 200–1,000 fragments, and RNA immunoprecipitation (RIP) was performed using the Millipore immunoprecipitation kit to capture relevant RNA with antibodies against EZH2 (Cell Signaling Technology). The relative immunoprecipitated RNA was determined by RT-PCR analysis.

Western blot

Radioimmunoprecipitation assay lysis buffer was utilized to extract total protein, which was gel electrophoresis with 10% sodium dodecyl sulfate-polyacrylamide and transferred onto polyvinylidene difluoride (PVDF) membranes. The PVDF membranes were incubated with EZH2 primary antibody, and a secondary antibody conjugated with horseradish peroxidase (Abcam, Cambridge, MA). An enhanced chemiluminescence detection reagent (Thermo Fisher) was used to get the signal. The relative intensity of the bands was calculated by correcting for *GAPDH*.

Luciferase reporter assay

The SNHG15 expression plasmid, SNHG15 shRNA plasmid, or PTEN expression plasmid was transfected into MKN45 cells or MKN45 CR cells. The PTEN promoter-reporter (Genechem, Shanghai, China) was cotransfected with SNHG15 expression plasmid or SNHG15 shRNA plasmid into MKN45/MKN45 CR cells, and the luciferase activity was detected with Luciferase Reporter assay (Promega).

Statistics

All data were shown as mean \pm SD. The ANOVA test (one- and two-way) and Student's *t*-test were utilized to analyze the data using GraphPad 6. Statistical significance was determined when *P* values were less than 0.05.

Pearson correlation analysis was performed to assay the expression correlation between interest genes.

Results

Upregulated SNHG1556 correlates with the prognosis of gastric cancer

Upregulated SNHG15 was found in gastric cancer samples compared with matched normal tissue according to TCGA datasets analyzed by GEPIA (Fig. 1a), and upregulated SNHG15 was also confirmed in the gastric cancer samples obtained by ourselves (Fig. 1b). It was further revealed that MKN28, MKN45, MGC803, HGC27, NCI-N87, and SGC7901 cells also showed upregulated SNHG15 expression when compared with immortalized gastric GES-1 cells (Fig. 1c). Kaplan–Meier analysis showed that patients with high SNHG15 expression had worse OS compared with patients who had low SNHG15 expression ($P = 0.0148$, Fig. 1d). All of these indicated

that upregulated SNHG1556 correlated with the poor prognosis.

SNHG15 promotes gastric cancer proliferation and colony formation

To explore the function of SNHG15 in gastric cancer, SNHG15 plasmid was transfected into MKN45, and the success of transfection was testified by upregulated SNHG15 in MKN45 cells (Fig. 2a). The SNHG15 transfection could promote the proliferation (Fig. 2b) and colony formation (Fig. 2c) in MKN45 cells. SNHG15 shRNA was transfected into HGC27 to establish the SNHG15 inhibition model (Fig. 2d). And the results indicated that SNHG15 shRNA could inhibit the proliferation and colony formation ability (Fig. 2e, f).

SNHG15 promotes gastric cancer cisplatin resistance

MKN45 CR was constructed, and the relative improved viability of MKN45 CR treated with 10 μ M of cisplatin

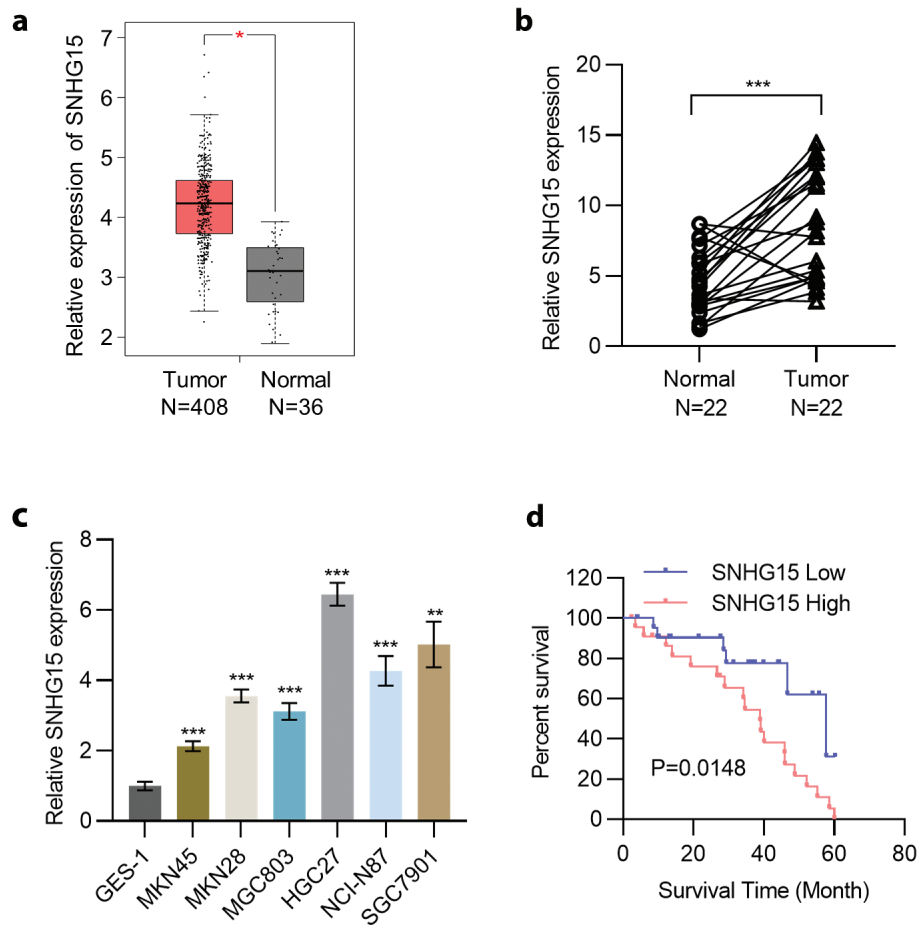


Fig. 1. SNHG15 overexpressed in gastric cancer tissues and cells and correlated with poor survival. (a) The relative SNHG15 expression in stomach adenocarcinoma was analyzed in GEPIA database. Student's t test. (b) SNHG15 expression in 22 gastric cancer samples and matched normal tissues. Student's t test. (c) The relative SNHG15 expression in gastric cancer cells and normal GES-1 cell. One-way ANOVA analysis. (d) The correlation of SNHG15 expression with the OS of gastric cancer patients. Data were shown as mean \pm SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

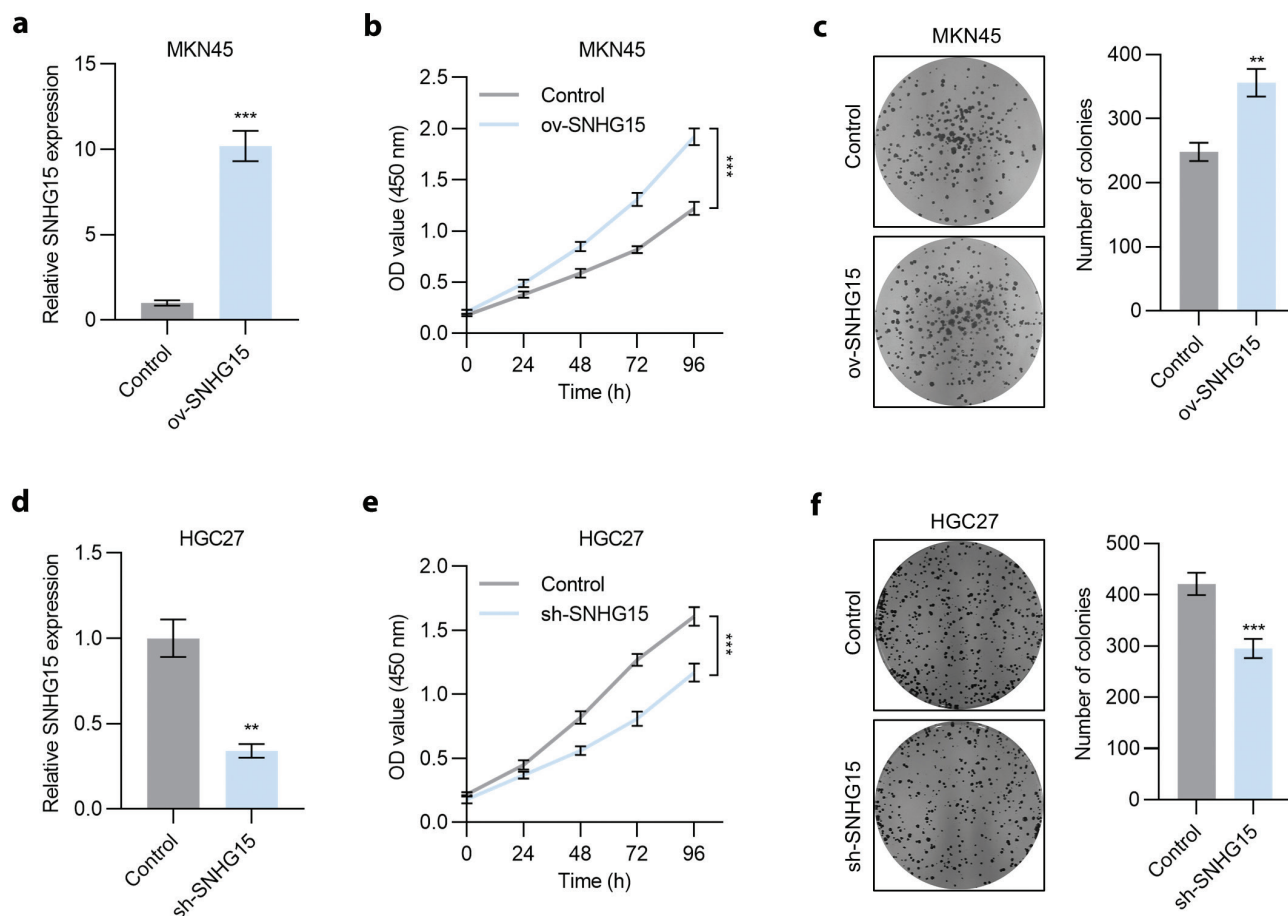


Fig. 2. SNHG15 promoted gastric cancer proliferation. (a) The relative SNHG15 expression in MKN45 cells transfected with SNHG15 expression plasmid. Student's *t* test. (b) Cell viability of MKN45 cells transfected with SNHG15 expression plasmid. Two-way ANOVA analysis. (c) Cell proliferation of MKN45 cells transfected with SNHG15 expression plasmid was evaluated by colony formation assays. Student's *t* test. (d) The relative SNHG15 expression in HGC27 cells transfected with vector or SNHG15 shRNA was determined by qPCR. Student's *t* test. (e) Cell viability of HGC27 cells transfected with vector or SNHG15 shRNA was determined by CCK-8 assays. Two-way ANOVA analysis. (f) Cell proliferation of HGC27 cells transfected with SNHG15 shRNA was evaluated by colony formation assays. Student's *t* test. Data were shown as mean \pm SD. ** $P < 0.01$; *** $P < 0.001$.

when compared with parental cells indicated the success of the establishment of MKN45 CR (Fig. 3a). When compared with the parental cells, MKN45 CR showed upregulated SNHG15 expression (Fig. 3b). It was further revealed that 10 μ M of cisplatin incubation could upregulate SNHG15 expression in MKN45 cells at the processing time (Fig. 3c), and the increased dose of cisplatin could also upregulate the expression of SNHG15 after 24 h of observation (Fig. 3d). All of these indicated that the SNHG15 expression was correlated with cisplatin resistance with time and dose manner. It was further demonstrated that when compared with MKN45 cells, MKN45 cells transfected with SNHG15 expressing plasmid will show increased cell viability (Fig. 3e), and the SNHG15 shRNA transfected MKN45 CR cells will show decreased cell viability (Fig. 3f) when treated with different dose of cisplatin. All of these testified that SNHG15 could promote cisplatin resistance of gastric cancer.

SNHG15 epigenetically inhibits PTEN through binding with EZH2 in gastric cancer

The relative SNHG15 and EZH2 expressions in gastric cancer samples derived from the TCGA analysis showed a significant positive correlation (Fig. 4a, $R = 0.35$, $P < 0.001$). A previous report indicated that EZH2 could enrich H3K27me3 on the promoter region to suppress the relative expression of PTEN (14, 15). RNA pull-down assay (Fig. 4b) and RIP assay (Fig. 4c) indicated that SNHG15 could bind directly with EZH2. Correlation analysis also testified such regulation, as indicated by the negative correlation between EZH2 and PTEN ($R = -0.13$, $P = 0.0066$, Fig. 4d), and SNHG15 and PTEN ($R = -0.21$, $P < 0.001$, Fig. 4e). Biologically, SNHG15 transfection diminished the relative expression of PTEN (Fig. 4f) and the promoter activity of PTEN assayed by luciferase reporter assay, while SNHG15 inhibition could upregulate the promoter activity of PTEN (Fig. 4g).

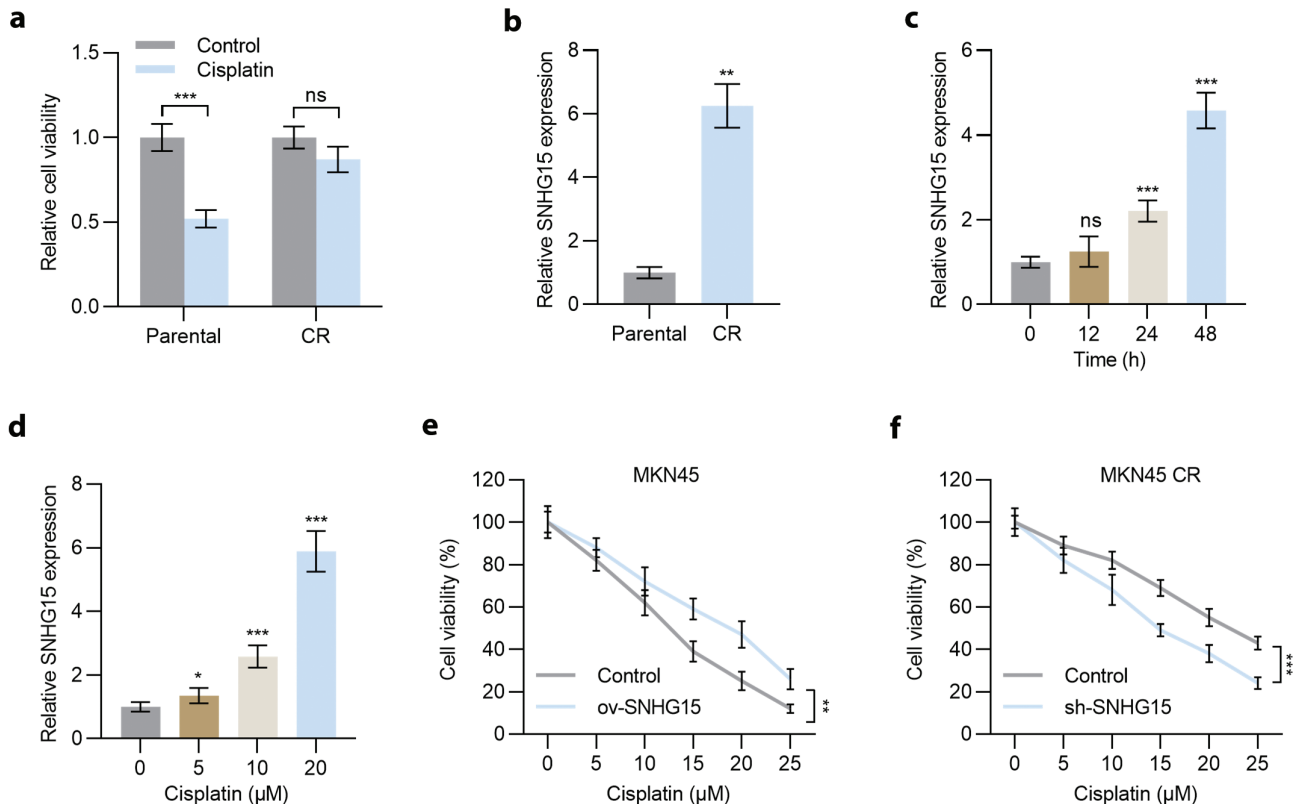


Fig. 3. SNHG15 promoted gastric cancer cisplatin resistance. (a) The cellular viability of MKN45 CR cells or the parental cells treated with 10 μ M of cisplatin. Two-way ANOVA analysis. (b) The mRNA expression of SNHG15 in MKN45 CR cells or the parental cells. Student's *t* test. (c) The relative SNHG15 expression in MKN45 cells treated with 10 μ M cisplatin for the indicated times. One-way ANOVA analysis. (d) The relative SNHG15 expression in MKN45 cells treated with a series dose of cisplatin for 24 h. One-way ANOVA analysis. (e) Cell viability of MKN45 cells transfected with or without SNHG15 expressing plasmid and treated with a series dose of cisplatin. Two-way ANOVA analysis. (f) Cell viability of MKN45 CR cells transfected with or without SNHG15 shRNA and treated with a series dose of cisplatin. Two-way ANOVA analysis. Data were shown as mean \pm SD. ns, not significant; ** $P < 0.01$; *** $P < 0.001$.

SNHG15 promotes gastric cancer proliferation and cisplatin resistance by inhibiting PTEN

PTEN-overexpressed MKN45 cell line was established (Fig. 5a), and it revealed that the promotion of MKN45 proliferation induced by SNHG15 overexpression could be reversed by the PTEN transfection (Fig. 5b). Furthermore, SNHG15 overexpression-induced colony formation (Fig. 5c) and cell resistance to cisplatin (Fig. 5d) could also be reversed by PTEN transfection. All of these indicated that SNHG15 induced gastric cancer proliferation, and cisplatin resistance is mediated by the inhibition of PTEN.

Discussion

Increased SNHG15 expression suggests advanced lymph node metastasis and tumor node metastasis stage in 33 types of malignancies detected in the GEPIA cohort, including gastric cancer. It is further revealed that upregulated SNHG15 could induce matrix metalloproteinase 2 and MMP9 expression to increase gastric cancer cell invasion and proliferation (16). Furthermore, SNHG15 could improve the relative PD-L1 expression, which may

lead to the resistance to the immune responses (17). It is worth noting that SNHG15 is testified to be a short-lived non-coding transcript with a relatively short half-life ($t_{1/2} < 4$ h), and the elevation and enrichment of SNHG15 in tissue are attributed to the prolonged decay rates and/or the interruption of the RNA degradation process (18). Whether novel mechanism is involved in gastric cancer-related cisplatin resistance needs further detailed analysis. In summary, SNHG15 might be considered as a novel treatment target in gastric cancer.

PTEN functions as a tumor suppressor in many malignancies (19, 20). Likewise, PTEN over-expression will promote apoptosis and improve cisplatin sensitivity in gastric cancer, as previously reported (6, 21). Consistently, our investigation also testifies that PTEN is involved in gastric cancer cisplatin resistance. EZH2 is reported to recruit H3K27me3 to the PTEN promoter and inhibit the relative expression of PTEN, which can further activate the AKT/mTOR signaling pathway (22). In this study, such regulation is also indicated. All these data demonstrate that the knockdown of SNHG15 resensitizes

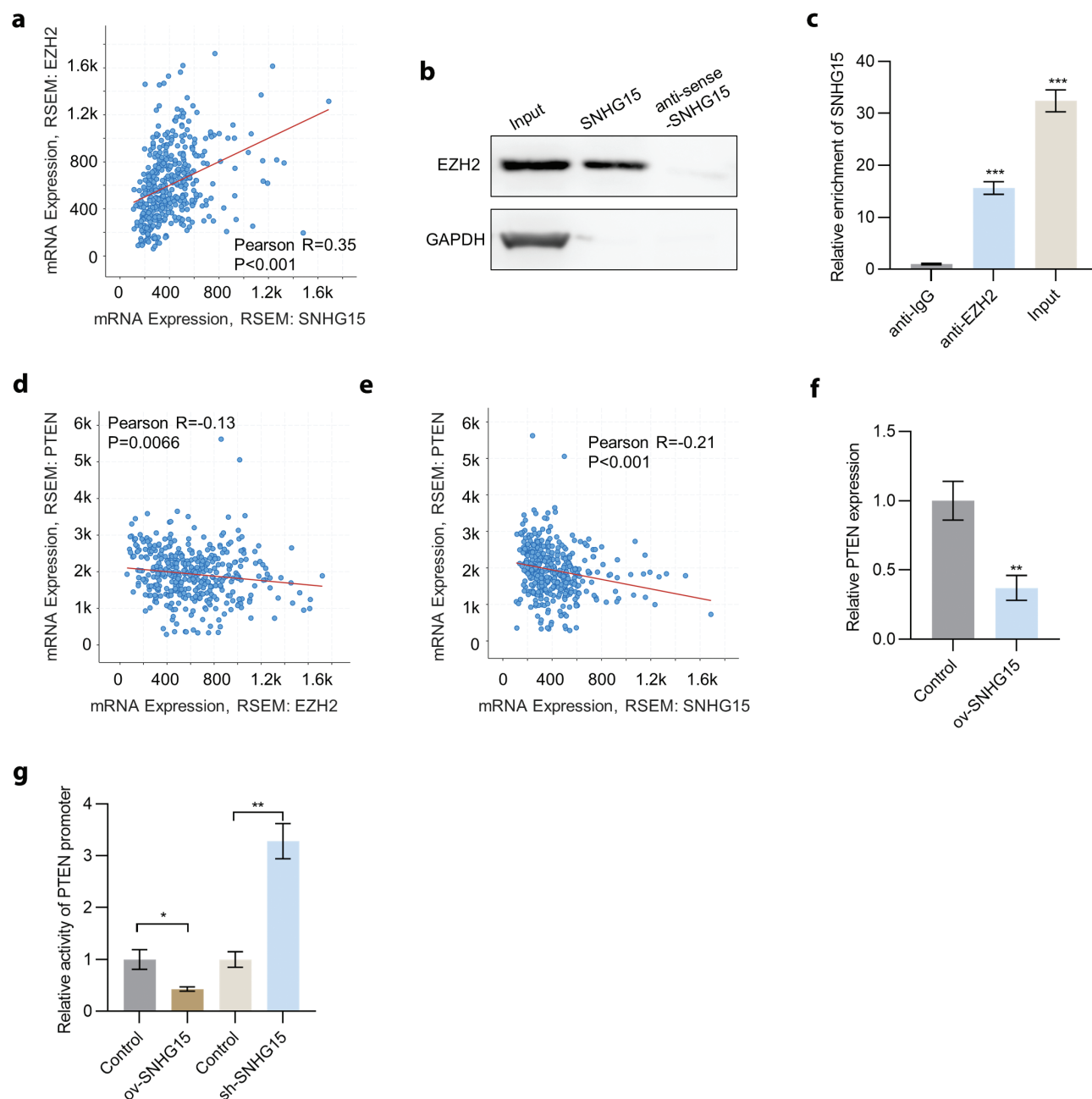


Fig. 4. SNHG15 inhibited PTEN expression by binding with EZH2 in gastric cancer. (a) SNHG15 and EZH2 correlations in stomach adenocarcinoma samples derived from TCGA (PanCancer Atlas). The interaction between SNHG15 and EZH2 was testified using RNA pull-down assay (b) and RIP assay (c). One-way ANOVA analysis. (d) The expression correlation between EZH2 and PTEN in stomach adenocarcinoma samples derived from TCGA (PanCancer Atlas). (e) The expression correlation between SNHG15 and PTEN in stomach adenocarcinoma samples from TCGA (PanCancer Atlas). (f) The relative PTEN expression in MKN45 cells transfected with SNHG15 expression plasmid. Student's *t* test. (g) The promoter activity of PTEN in MKN45 cells transfected with SNHG15 expression plasmid or SNHG15 shRNA plasmid. One-way ANOVA analysis. Data were shown as mean \pm SD. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

cisplatin-resistant gastric cancer to cisplatin by epigenetically suppressing PTEN expression.

Emerging evidence indicates that lncRNAs may serve mainly as miRNA sponges to implement posttranscriptional regulation, which might be more effective than the traditional anti-miRNA approach (23, 24). All of these

indicate that SNHG15 could be considered as a potential treatment target in clinical practice.

Conclusion

SNHG15 can mediate cisplatin resistance by epigenetically inhibiting PTEN via recruiting EZH2.

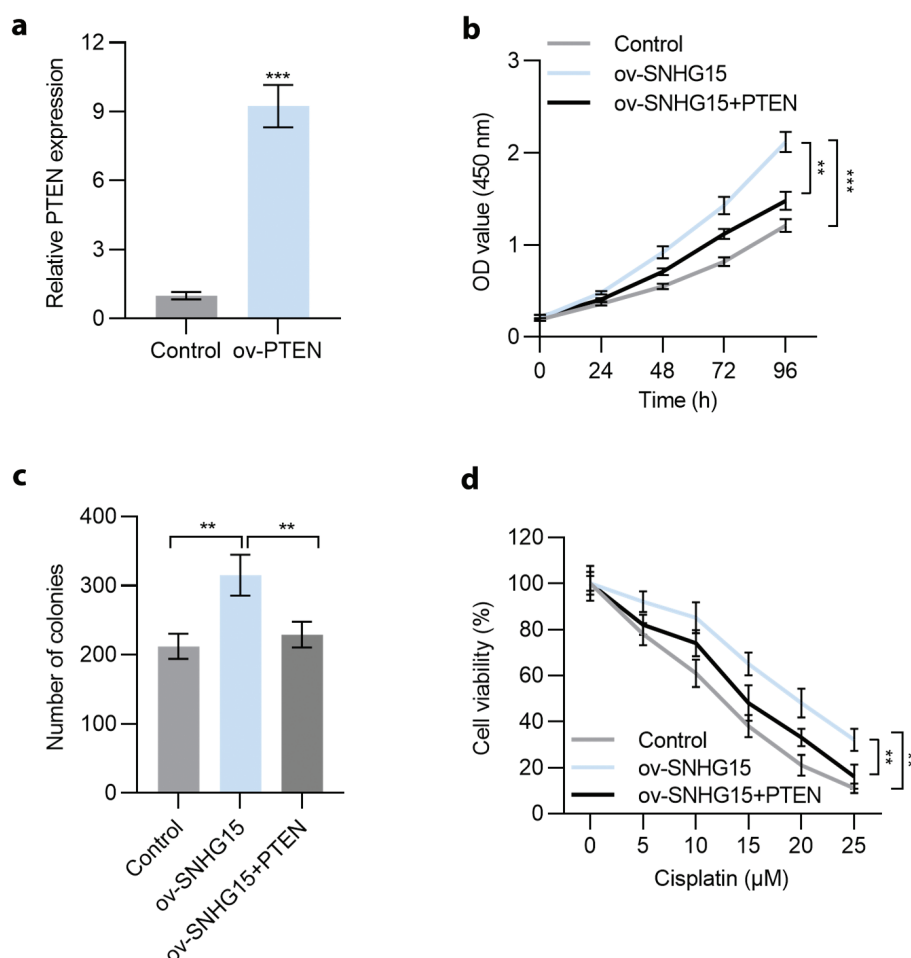


Fig. 5. SNHG15 promoted gastric cancer proliferation and cisplatin resistance by inhibiting PTEN. (a) The relative PTEN expression in MKN45 cells transfected with PTEN expression plasmid. Student's *t* test. (b) Cell viability detection of MKN45 cells transfected with SNHG15 expression plasmid and/or PTEN expression plasmid. Two-way ANOVA analysis. (c) Cell proliferation of MKN45 cells transfected with SNHG15 expression plasmid and/or PTEN expression plasmid. One-way ANOVA analysis. (d) Cell viability of MKN45 cells transfected with SNHG15 expression plasmid and/or PTEN expression plasmid treating with a series dose of cisplatin. Two-way ANOVA analysis. Data were shown as mean \pm SD. ***P* < 0.01; ****P* < 0.001.

Acknowledgments

None.

Conflict of interest and funding

The authors declare that they have no conflict of interest. This study was supported by the Science Funds of Xinjiang Uygur Autonomous Region (2019D01C110).

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