

DICER-ASI functions as competing endogenous RNA that targets *CSR1* by sponging miR-650 and suppresses gastric cancer progression

Junyan An¹, Xiaoling Guo² and Bingli Yan¹ 

Abstract

Objective: This study explored the functional interactions between the long non-coding RNA DICER-ASI and the cellular stress response I (*CSR1*) gene in gastric cancer.

Methods: Quantitative polymerase chain reaction (qPCR) and western blotting were used to measure DICER-ASI, *CSR1*, and miR-650 expression levels. Gastric cancer cell line proliferation and migration abilities were analyzed using the MTT and transwell migration and invasion assays, respectively. Bioinformatic analysis and dual luciferase reporter assays were employed to study the functional interactions among miR-650, DICER-ASI, and *CSR1*.

Results: DICER-ASI and *CSR1* expression levels were significantly decreased in gastric cancer tissues compared with normal tissues, and qPCR analysis showed that miR-650 was upregulated in gastric cancer tissues. Bioinformatic analysis and dual luciferase reporter assays revealed that DICER-ASI functioned as a competing endogenous RNA that sponged miR-650, which in turn regulated *CSR1* expression. Importantly, ectopic DICER-ASI and *CSR1* expression inhibited cell proliferation and migration *in vitro* and suppressed xenograft tumorigenicity *in vivo*.

Conclusions: These results suggest that DICER-ASI functions as a competing endogenous RNA that regulates miR-650 to suppress proliferation and migration of gastric cancer cells by targeting *CSR1*. These findings indicate that targeting DICER-ASI and miR-650 could be a novel treatment for gastric cancer.

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Keywords

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Introduction

Gastric cancer is currently the third leading cause of cancer-related deaths worldwide.^{1,2} Despite recent advances in targeted and immuno-therapies, the overall 5-year survival rate of gastric cancer patients is as low as 40%.^{3,4} Therefore, understanding the molecular mechanisms that regulate gastric cancer tumorigenesis and progression is critically important and may provide insights into the formulation of novel therapeutic approaches.

Dysregulation of the nuclear factor kappa B (NF- κ B) signaling pathway has been implicated in the pathogenesis of gastric cancer.⁵ NF- κ B signaling can activate immune responses that are favorable for gastric cancer progression and metastasis.⁵ The NF- κ B pathway is regulated through several genetic and epigenetic mechanisms including long non-coding (lnc)RNAs.⁶ A previous study reported that DICER-AS1, which is a NF- κ B-associated lncRNA, was significantly downregulated in gastric cancer tissues compared with non-cancerous tissues, and that its expression level was correlated with disease stage.⁷ Therefore, modulating DICER-AS1 expression could affect gastric cancer growth.

The cellular stress response 1 (*CSR1*) gene is widely expressed in various cell types and its gene product serves as a cellular stress response protein that protects cells from the oxidative damage induced by reactive oxygen species (ROS).⁸ *CSR1* is a stress response gene located on chromosome 8p21, a region that is frequently deleted in a variety of human malignancies.^{9,10}

Previous studies have demonstrated that *CSR1* is downregulated in prostate cancer.¹¹ A recent study reported that *CSR1* interacts with XIAP, suppressing its anti-apoptotic effects and inducing cell death.¹² However, the expression level and function of *CSR1* in gastric cancer is unclear.

MicroRNAs (miRNA; miR) are endogenous non-coding RNAs of 22 nucleotides in length that regulate gene expression by binding to complementary sequences within the 3'-untranslated regions (3'-UTRs) of target mRNAs.¹³ Dysregulation of miRNAs can either suppress or promote tumorigenesis in various human cell types.^{3,14,15} For example, dysregulation of miR-650 has been widely observed in a variety of cancers including hepatocellular carcinoma,¹⁶ glioma,¹⁷ chronic lymphocytic leukemia,¹⁸ and lung adenocarcinoma.¹⁹ Zhang et al.²⁰ showed that miR-650 is involved in lymphatic and distant metastasis in human gastric cancer and that ectopic miR-650 expression promotes tumorigenesis and the proliferation of gastric cancer cells. However, whether there is a functional relationship between miR-650 and *CSR1* in gastric cancer remains unknown. Herein, we show that both DICER-AS1 and *CSR1* are significantly downregulated in gastric cancer and regulate gastric cancer cell proliferation and migration. Our data show that DICER-AS1 functions as a competing endogenous (ce)RNA that targets miR-650, which negatively regulates *CSR1* expression. These findings elucidate the functional roles of DICER-AS1, miR-650 and *CSR1*

in gastric cancer, and further suggest that targeting DICER-AS1 and miR-650 could be a novel therapeutic intervention for gastric cancer.

Materials and methods

Tissue samples

Surgically resected gastric cancer and adjacent normal tissue samples were collected from 93 patients who were diagnosed with primary gastric cancer at Weifang People's Hospital between 2017 and 2019. The included patients had not undergone prior chemotherapy or radiotherapy. This study was approved by the Ethics Committee of Weifang People's Hospital, Shangdong, China (approval no. WFRM-202-00110, 20 December 2016), and informed consent was obtained from each patient. Tissue samples were snap-frozen in liquid nitrogen after surgery and stored at -80°C for further analysis. We also downloaded gene expression profiles and clinical information of all 619 gastric cancer patients from The Cancer Genome Atlas database, including 408 gastric cancer and 211 normal control cases. Data were analyzed and visualized using the online tool GEPIA (<http://gepia.cancer-pku.cn/>).

Cell culture and transfection

The human gastric cancer cell lines AGS, HGC-27, MGC-803, SGC-7901, and the normal gastric epithelial cell line GES-1 were purchased from the American Type Culture Collection (Manassas, VA, USA). All cell lines were cultured in Roswell Park Memorial Institute Medium (RPMI 1640; 21875034, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; 26140087, Thermo Fisher Scientific) and 1% penicillin/streptomycin (SV30010, Hyclone, Logan, UT, USA) at 37°C and under 5%

CO_2 . Cells were harvested for experiments in the exponential growth state.

For transfection, SGC-7901 cells were seeded in 12-well plates at 5×10^5 cells/well and transfected with Lipofectamine 2000 (11668030, Invitrogen, Waltham, MA, USA) in Opti-MEM medium (31985062, Gibco, Grand Island, NY, USA) according to the manufacturer's instructions. For CSR1 overexpression experiments, SGC-7901 cells were transiently transfected with pCEFL-ctrl or pCEFL-CSR1/DICER-AS1 plasmids for 24 hours. For DICER-AS1 silencing experiment, cells were transfected with DICER-AS1 small interfering (si)RNAs, siRNA negative control, and miRNA-650 mimic (GenePharma, Shanghai, China) for 48 hours before they were harvested for further analysis.

RNA extraction and quantitative polymerase chain reaction (qPCR)

TRIzol reagent (15596026, Thermo Fisher Scientific) was used to extract RNA from tissues and cells according to the manufacturer's instructions. The extracted total RNA was dissolved in DEPC water, and its concentration was measured with a NanoDrop device (Thermo Fisher Scientific). Then, $1 \mu\text{g}$ of total RNA was reverse-transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Fisher Scientific). For miRNA quantification, $2 \mu\text{g}$ of total RNA was used to synthesize cDNA with the TaqManTM microRNA reverse transcription kit (4366596, Thermo Fisher Scientific). The resulting cDNA was diluted and analyzed in a 7500 Real Time PCR System (Applied Biosystems/Life Technologies, Carlsbad, CA, USA) using the SYBR premix EX TAQ II kit (RR820A, Takara, Dalian, China). Finally, the $-2\Delta\Delta\text{Ct}$ method was used to analyze relative expression levels with U6 small nucleolar (sn)RNA used for

normalization. All primers were purchased from Shanghai Sangon Biotechnology Co., Ltd. (Shanghai, China) and their sequences were as follows:

DICER-AS1 forward: 5'-ACCTGCCTTCTTGACTGAATG-3';

DICER-AS1 reverse: 5'-GCAGTAACCATAGCCAGAAACT-3';

CSR1 forward: 5'-CTCCAGGTCATGGGCTTGC-3';

CSR1 reverse: 5'-GTTCTGGGCGTAGTGGTAT-3';

miRNA-650 forward: 5'-AGGAGGCAGCGCTCTC-3';

U6 snRNA forward: 5'-CGCTTCACGAA TTTGCGTGTCAT-3'; and

miRNA-650 reverse: 5'-GAACATGTCTGCGTATCTC-3';

U6 snRNA: 5'-GCTTCGGCAGCACATA TACTAAAAT-3'.

Dual Luciferase reporter assays

The 3'-UTRs of human DICER-AS1 and CSR1 were subcloned into the pGL3-luciferase reporter plasmid (Promega, Madison, WI, USA). Then pGL3-DICER-AS1-mut and pGL3-CSR1-mut vectors were constructed by mutating the miR-650 targeting sequences of the DICER-AS1 and CSR1 3'-UTRs. Reporter plasmids and Renilla luciferase (hRlucneo) control plasmid were co-transfected into gastric cancer cells with or without miR-650 mimic (GenePharma, Shanghai, China) in 12-well plates (1×10^5 cells/well) using Lipofectamine 2000 (11668030, Invitrogen) in Opti-MEM medium (31985062, Gibco, USA) according to the manufacturer's instructions. At 48 hours post transfection, relative luciferase activities were measured using the Dual-Luciferase Reporter Assay Kit (E1910, Promega) on a luminescence microplate reader (Infinite 200 PRO, Tecan, Männedorf, Switzerland). Relative

firefly luciferase activity from the reporter plasmid was normalized to Renilla luciferase activity from the control plasmid.

Western blotting analysis

Total protein was extracted from tumor tissues and cells using RIPA lysis buffer containing protease inhibitor cocktail (Invitrogen) and was quantified using the BCA Protein assay kit (Solarbio, Beijing, China). Then 10 μ g of total protein was separated by SDS-PAGE electrophoresis. The separated proteins were then transferred onto PVDF membranes (Bio-Rad, Hercules, CA, USA). After blocking with 5% skimmed milk for 1 hour, membranes were incubated with rabbit polyclonal anti-CSR1 primary antibody (Thermo Fisher Scientific) overnight at 4°C. The membrane was washed three times with TBST for 5 minutes each. After washing, the membrane was further incubated with HRP-linked anti-rabbit secondary antibody (1:3000; #7074, Cell Signaling Technology, Danvers, MA, USA) at room temperature for 1 hour. Then the membrane was washed four times with TBST, and immunoreactive bands were visualized using an enhanced chemiluminescence kit (Santa Cruz Biotechnology, Dallas, TX, USA) and imaged on a gel imager system (Bio-Rad).

Cell migration assay

Cell migration was measured using the transwell migration assay as previously described.²¹ Following different treatments, cells were trypsinized and resuspended in serum-free medium. The upper Corning, Inc. transwell chamber (Corning, NY, USA) alone was used for migration assays, while the upper chamber coated with Matrigel (BD Biosciences, Bedford, MA) was used for invasion assays. Cells were inoculated into the upper transwell chamber in serum-free medium and 500 μ L

of 10% serum-containing medium was added to the lower chamber. After 48 hours, culture medium was discarded, and the cells were fixed with 4% paraformaldehyde at room temperature for 10 minutes and stained with 0.5% crystal violet (Sigma-Aldrich, St. Louis, MO, USA) for 20 minutes. Cells were photographed using a Leica AM6000 microscope (Wetzlar, Germany), and the number of invading cells was counted.

MTT assay

SGC-7901 cells were transfected with the indicated siRNAs or expression plasmids, and then their proliferation ability was measured using the MTT assay. Briefly, approximately 1×10^3 cells were seeded in each well of 96-well plates, cultured overnight, and then transfected with the indicated siRNAs or expression plasmids. Cells under different treatments were incubated for the indicated duration (0, 24, 48, 72, and 96 hours). For the MTT assay, at each time point, cells were incubated with 0.5 mg/mL MTT reagent (Solarbio) for 3 hours at 37°C. Culture media were removed and 100 μ L of DMSO was added to each well and incubated for 10 minutes. The optical densities of each treatment group were measured at 450 nm using a BioTek ELx800 microplate reader (BioTek, Winooski, VT USA).

Apoptosis assay

Following different treatments, cells were trypsinized, washed twice with PBS, and then resuspended in staining solution. The quantification of apoptotic cells was performed using the apoptosis detection kit (BD Biosciences, San Jose, CA, USA) according to the manufacturer's instructions. Briefly, 5 μ L of Annexin V-FITC and 5 μ L of propidium iodide were added to a 1-mL suspension of 1×10^6 cells and

incubated for 30 minutes in the dark. Stained cells were centrifuged, washed twice with PBS, and resuspended in 400 μ L of PBS. The percentages of apoptotic cells were detected using a BD FACS Canto™ II Flow Cytometer (BD Biosciences).

Tumorigenicity assays in xenograft tumor models

All animal experiments were approved by the Animal Care and Use Committee of Weifang People's Hospital, Shandong, China (20 May 2018). Male athymic BALB/c nude mice (weighing 15–18 g) were purchased from HFKBio (Peking, China) and randomly divided into two groups ($n=5-6$ per group). Then, pCEFL-ctrl- or pCEFL-CSR1-transfected SGC-7901 cells or siDICER-AS1-transfected SGC-7901 cells (0.2 mL cell suspension containing 1×10^7 cells) were subcutaneously injected in the dorsal flank of mice in each group. Tumor sizes were measured every 3 days, and the tumors were removed and weighed 30 days after injection.

Statistical analysis

All statistical analyses were performed using SPSS v20.0 software (IBM Corp., Armonk, NY, USA). All experiments were repeated at least three times. The statistical difference between two groups was compared using the unpaired Student's t-test. Comparisons among multiple groups were analyzed using one-way analysis of variance (ANOVA) with Tukey's post hoc test for pairwise comparisons. Comparisons of data at multiple time points were examined using two-way ANOVA. Data are reported as the mean \pm standard deviation. $P < 0.05$ was considered statistically significant.

Results

DICER-AS1 was downregulated in gastric cancer

DICER-AS1, which is among the NF- κ B-associated lncRNAs, was significantly downregulated in gastric cancer tissues compared with noncancerous tissues (Figure 1a).⁷ We also measured DICER-AS1 mRNA levels in 93-paired human gastric cancer and adjacent noncancerous

tissues by qPCR. This revealed that DICER-AS1 expression level was significantly decreased in gastric cancer tissues (Figure 1b). We next examined DICER-AS1 expression in the human gastric cancer cell lines AGS, HGC-27, MGC-803, SGC-7901, and in the normal human gastric epithelial cell line GES-1. DICER-AS1 expression level was decreased in all gastric cancer lines compared with GES-1 (Figure 1c). Moreover, high DICER-AS1 expression was correlated with an overall

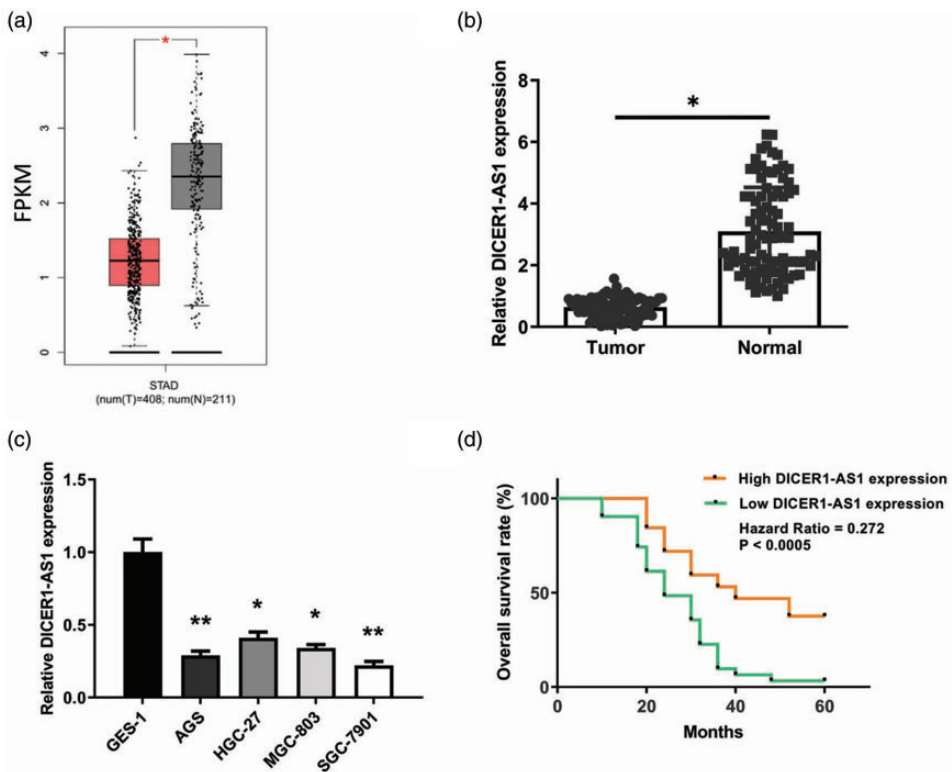


Figure 1. DICER-AS1 expression was decreased in gastric cancer. (a) DICER-AS1 expression in 408 gastric cancer tissues and 211 normal tissues from the TCGA database. Left (T): tumor tissues, right (N) normal tissues. (b) Quantitative PCR (qPCR) analysis showing DICER-AS1 mRNA levels in 93 gastric cancers and adjacent non-tumor tissues. All experiments were repeated three times. Data are summarized as mean \pm standard deviation. * $P < 0.05$ versus non-tumor tissues. (c) DICER-AS1 mRNA level were dramatically decreased in the gastric cancer lines AGS, HGC-27, MGC-803, and SGC-7901 compared with GES-1. All experiments were repeated at least three times. Data are summarized as mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$ versus GES-1 cells. (D) Kaplan-Meier curves showing the effect of DICER-AS1 levels on gastric cancer patient survival.

TCGA, The Cancer Genome Atlas; FPKM, Fragments Per Kilobase of transcript per Million mapped reads.

better prognosis among the 93 gastric patients (Figure 1d).

DICER-AS1 repressed gastric cancer progression *in vitro* and *in vivo*

We next investigated the effects of DICER-AS1 on cell proliferation. We constructed a plasmid expressing the ORF of DICER-AS1 and transfected it into SGC-7901 cells. qPCR confirmed DICER-AS1 overexpression after transfection (Figure 2a). MTT assay results revealed that SGC-7901 cells overexpressing DICER-AS1 had a significantly diminished proliferation capacity (Figure 2b). To further investigate

the role of DICER-AS1 on tumorigenesis, we performed xenograft tumorigenesis experiment by injecting SGC-7901 cells overexpressing DICER-AS1. Consistent with our *in vitro* results, ectopic DICER-AS1 expression significantly attenuated tumor growth *in vivo* (Figure 2c and d). Together, these data suggest that DICER-AS1 functions as tumor suppressor in gastric cancer.

DICER-AS1 targeted miR-650 in human gastric cancer cells

To identify potential targets of DICER-AS1, we used bioinformatic tools

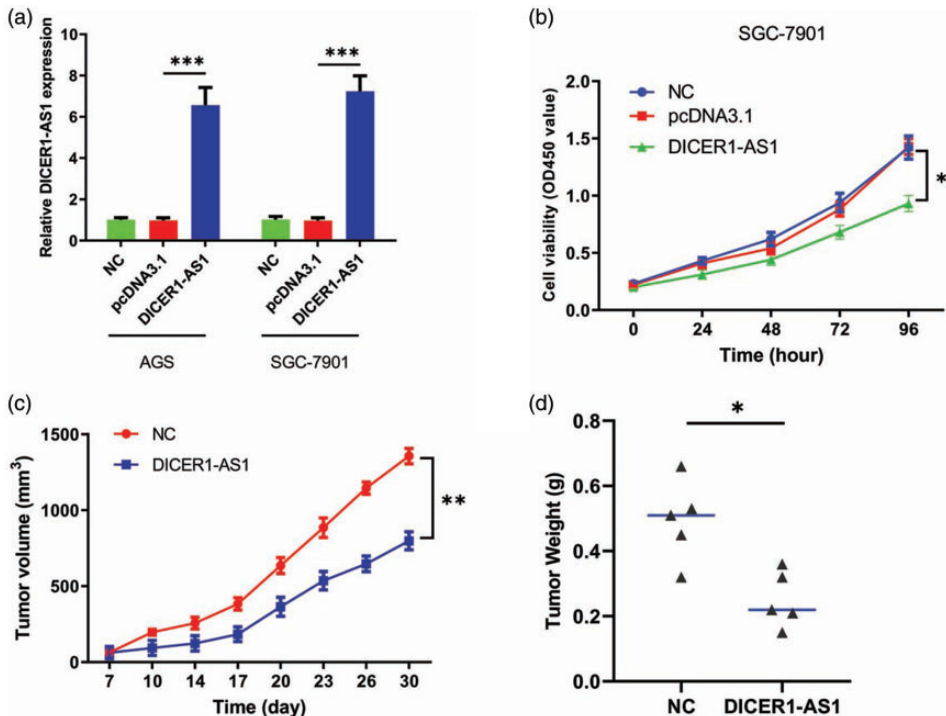


Figure 2. DICER-AS1 suppressed SGC-7901 cell proliferation and tumor formation. The DICER-AS1 plasmid was used to overexpress DICER-AS1. (a) DICER-AS1 mRNA levels after plasmid transfection as detected by quantitative PCR (qPCR). (b) The MTT assay was performed to evaluate cell proliferation after DICER-AS1 overexpression. All experiments were performed three times independently. (c, d) DICER-AS1 overexpression suppressed tumor growth *in vivo*. Nude mice were injected with SGC-7901 cells overexpressing DICER-AS1 or control cells. Tumor size was monitored every 3 days. Data are summarized as mean \pm standard deviation. * $P < 0.05$ versus control or the negative control (NC) group.

(TargetScan: <http://www.targetscan.org/> and miRanda: <http://www.microrna.org/microrna>) to survey downstream miRNAs with potential binding capacity for DICER-AS1. We identified that miR-650 could bind to the 3'-UTR of DICER-AS1 mRNA (Figure 3a). Moreover, we validated the functional interaction between miR-650 and DICER-AS1 *via* the dual luciferase reporter assay. When we co-transfected cells with luciferase reporter plasmids containing wild-type (WT) DICER-AS1 3'-UTR or mutated (MUT) DICER-AS1 3'-UTR together with miR-650 mimic, miR-650 mimic significantly inhibited luciferase activity of the WT reporter but not of the MUT reporter (Figure 3b). We also performed qPCR to measure miR-650 expression in human gastric cancer and paired adjacent noncancerous tissues. As shown in Figure 3c, miR-650 expression was significantly higher in gastric cancer tissues. Pearson's correlation coefficient analysis further demonstrated that miR-650 expression was inversely correlated with DICER-AS1 in gastric cancer tissues; however, there was no clear relationship in noncancerous tissues (Figure 3d and e). These results revealed that miR-650 interacts with the 3'-UTR of DICER-AS1, negatively regulating its expression.

miR-650 suppressed the antitumor activity of DICER-AS1 in SGC-7901 cells

We next investigated the effects miR-650 on cell proliferation and migration. SGC-7901 cells transfected with miR-650 mimic showed enhanced proliferation (Figure 4a). Additionally, the inhibitory effect of DICER-AS1 on gastric cancer cell proliferation was partially rescued by miR-650 mimic (Figure 4a). Annexin V and 7-AAD staining apoptosis assays showed that cells overexpressing DICER-AS1 has an increased percentage of apoptotic cells,

which could also be partially rescued by miR-650 mimic (Figure 4b). Furthermore, overexpressing DICER-AS1 inhibited the migration and invasion of SGC-7901 cells, while miR-650 mimic promoted cell migration and invasion (Figure 4c). Cells overexpressing both miR-650 and DICER-AS1 showed decreased cell migration and invasion compared with cells transfected with miR-650 alone (Figure 4c). Together, these data suggest that miR-650 targeted DICER-AS1 and functions as an oncogene in gastric cancer.

miR-650 was upregulated in human gastric cancer and targeted CSR1

Previous studies have shown that miR-650 is significantly upregulated in gastric cancer (20). To investigate potential targets of miR-650, we used publicly available bioinformatic tools (TargetScan: <http://www.targetscan.org/> and miRanda: <http://www.microrna.org/microrna>) to identify potential targets. This analysis revealed that miR-650 could bind to the 3'-UTR of CSR1 mRNA (Figure 5a). Investigating the functional interaction between miR-650 and CSR1 by the dual luciferase assay showed that miR-650 mimic specifically targeted and inhibited the WT CSR1 reporter (Figure 5a). We further evaluated the biological effect of miR-650 on CSR1 expression in SGC-7901 cells. Overexpressing miR-650 significantly decreased the mRNA and protein levels of CSR1 (Figure 5b and c). These results revealed that miR-650 could bind to the 3'-UTR of CSR1, which negatively regulated its expression.

CSR1 and DICER-AS1 were functionally implicated in SGC-7901 cell proliferation and tumor formation

We further investigated the functional interaction between CSR1 and DICER-AS1 on

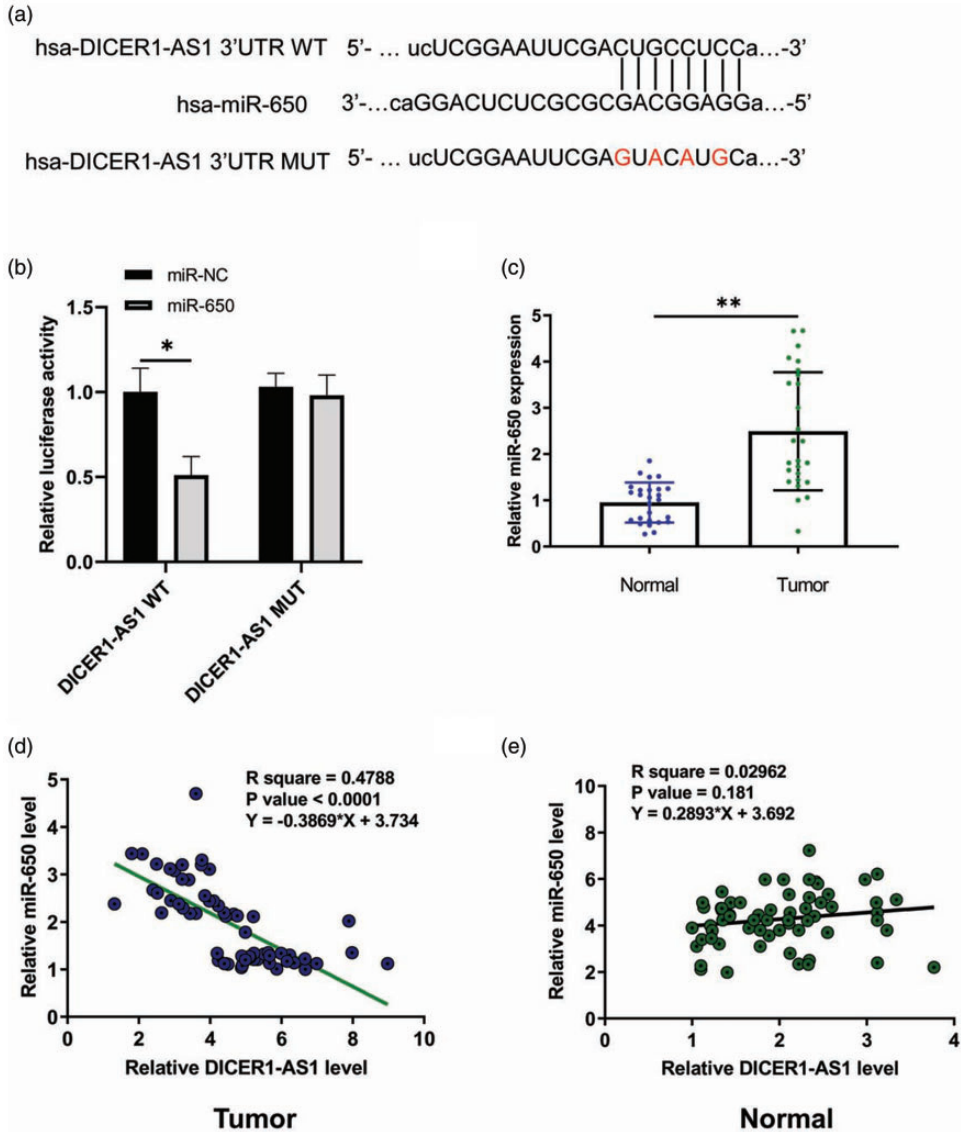


Figure 3. miR-650 targeted the 3'-untranslated region (3'-UTR) of DICER-AS1. (a) Bioinformatics tools predicted miR-650 as a potential micro (mi)RNA targeting DICER-AS1. (b) Dual Luciferase reporter assays using SGC-7901 cells transfected with luciferase reporter plasmids containing the wild-type (WT) or mutated (MUT) DICER-AS1 3'-UTR. * $P < 0.05$ compared with control. (c) qPCR analysis showed the miR-650 expression level in gastric cancer and adjacent non-tumor tissues. All experiments were performed three times independently. Data are summarized as mean \pm standard deviation. ** $P < 0.01$ versus non-tumor tissues. (d, e) Correlation analysis of miR-650 and DICER-AS1 expression levels in gastric cancer and adjacent non-tumor tissues. Data are expressed using the r -value from Pearson's correlation.

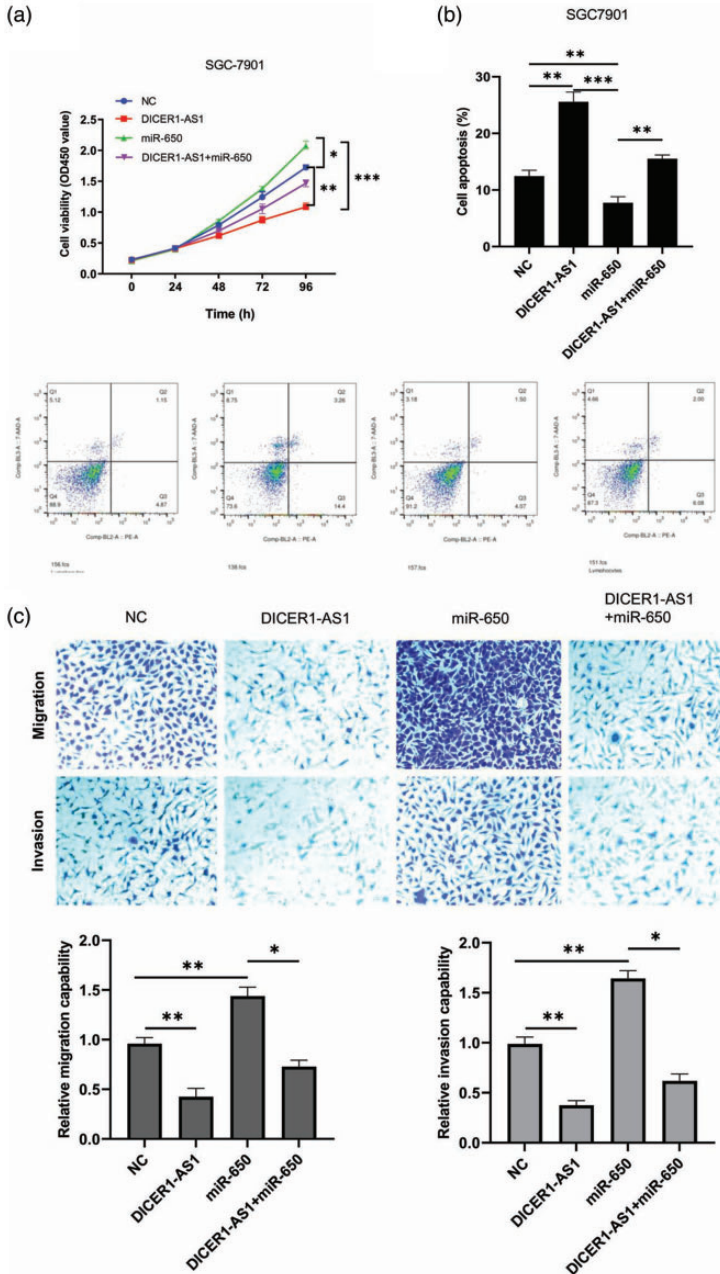


Figure 4. miR-650 suppressed the anti-tumor function of DICER-AS1 in SGC-7901 cells. The DICER-AS1 plasmid was used to overexpress DICER-AS1 in SGC-7901 cells transfected with miR-650 mimic or DICER-AS1 plasmid or both. (a) The MTT assay was used to examine the proliferation ability of SGC-7901 cells under different conditions. (b) Flow cytometry analysis of apoptotic cells under different conditions. (c) Cell migration and invasion capabilities under different conditions. All experiments were performed three times independently. Data are summarized as mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$ versus the control and negative control (NC) groups.

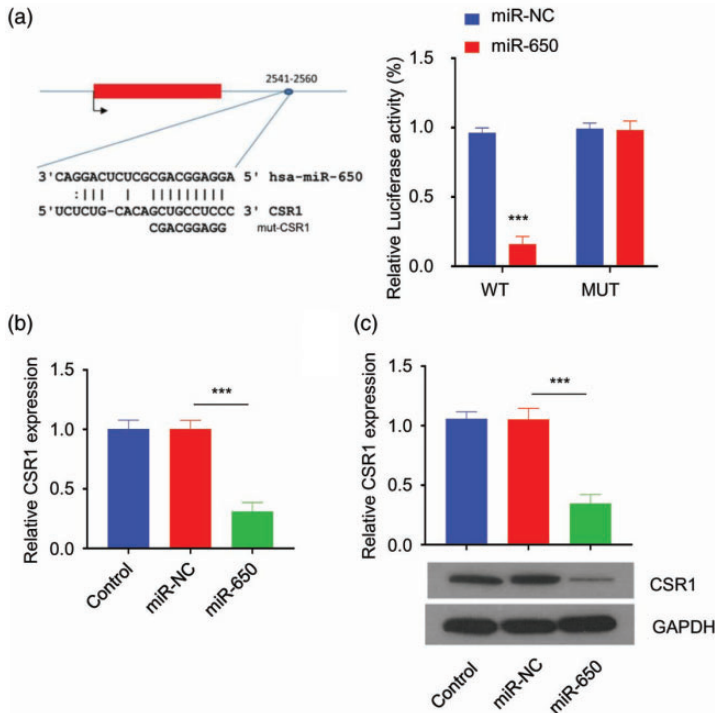


Figure 5. miR-650 is upregulated in human gastric cancer tissues and targets the 3'-untranslated region (3'-UTR) of cellular stress response 1 (*CSR1*) gene. (a) Bioinformatics tools predicted miR-650 as a potential miRNA targeting *CSR1*. Dual Luciferase reporter assays were performed using reporter plasmids containing wild-type (WT) or mutated (MUT) *CSR1* 3'-untranslated region (3'-UTR). * $P < 0.01$ compared with control. All experiments were performed three times independently. Data are summarized as mean \pm standard deviation, $n = 3$. *** $P < 0.001$ versus non-tumor tissues. (b) Relative *CSR1* mRNA levels in SGC-7901 cells after transfecting miR-650 mimic. (c) Western blot quantification of *CSR1* protein levels in SGC-7901 cells after transfecting miR-650 mimic. All experiments were performed three times independently. *** $P < 0.001$ versus miRNA negative control (NC) groups.

cell proliferation and migration. We constructed a plasmid expressing the *CSR1* ORF and applied siDICER-AS1 to silence the lncRNA. The plasmids were transfected into SGC-7901 cells, and then qPCR and western blot were used to confirm the transfection efficiency (Figure 6a and b). As shown in Figure 6, the mRNA and protein levels of *CSR1* were increased after transfecting *CSR1*-pcDNA3.1 (Figure 6a and b), and DICER-AS1 expression was significantly reduced after transfecting siDICER-AS1 (Figure 6c).

Then we found that SGC-7901 cells transfected with siDICER-AS1 showed increased proliferation, while overexpressing *CSR1* significantly decreased proliferation (Figure 6d). *CSR1* overexpression rescued the level of cell proliferation in siDICER-AS1-transfected cells. Similarly, overexpressing *CSR1* decreased the migration and invasion ability of SGC-7901 cells induced by siDICER-AS1 (Figure 6e). To further evaluate the effect of *CSR1* on tumorigenesis, we performed xenograft tumorigenesis experiments by injecting

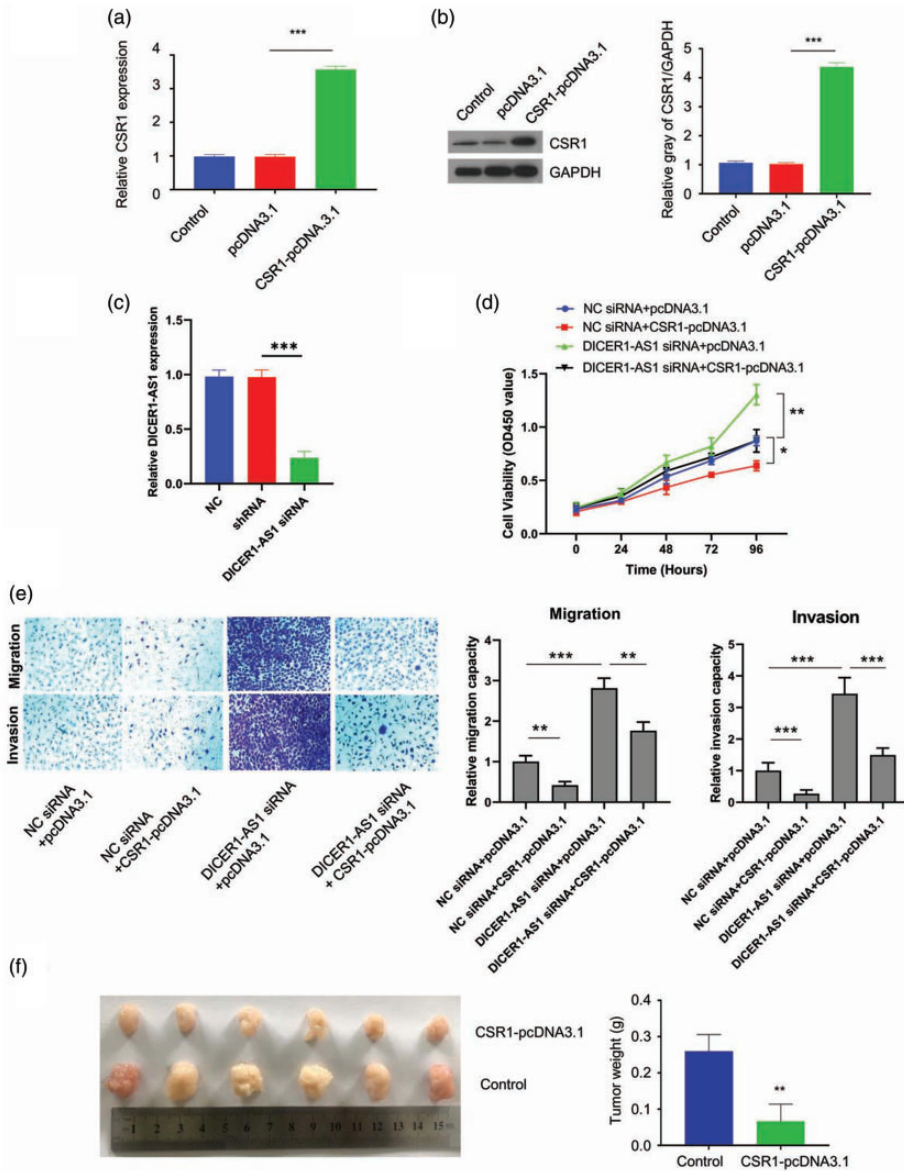


Figure 6. Cellular stress response I (CSR1) and DICER-AS1 affected the proliferation and tumor formation ability of SGC-7901 cells. The CSR1-pcDNA3.1 plasmid was used to overexpress CSR1; siDICER1-AS1 was applied to silence DICER1-AS1. pcDNA3.1 and siNC were controls for overexpression and small interfering (si)RNA silencing, respectively. CSR1 mRNA (a) and protein (b) levels were detected by qPCR and western blot, respectively. (c) DICER-AS1 levels were detected by qPCR in SGC-7901 cells after being transfected with siRNAs. (d, e) MTT (d) and transwell migration/invasion (e) cells in SGC-7901 cells transfected with CSR1 plasmid, DICER-AS1 siRNA, or both. All experiments were performed three times independently. (f) Overexpressing CSR1 suppressed tumor growth *in vivo*. Nude mice were injected with SGC-7901 cells overexpressing CSR1 or control cells, and then tumors were resected and weight on day 30. Data are summarized as mean \pm standard deviation. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus the control and negative control (NC) groups.

SGC-7901 cells with/out CSR1 overexpression. Ectopic CSR1 expression significantly attenuated tumor growth *in vivo* (Figure 6f). Collectively, these data suggest that CSR1 functions as a tumor suppressor downstream of DICER-AS1 in gastric cancer.

Discussion

Accumulating evidence has revealed that various genes and non-coding RNAs are implicated in tumorigenesis and malignant progression by regulating cell proliferation and invasion. Therefore, finding new therapeutic targets requires insight into the molecular mechanisms underlying carcinogenesis. A growing body of evidence has also highlighted the role of lncRNAs, miRNAs, and their target genes as novel therapeutic targets in different malignancies.

Among NF- κ B-associated genes, lncRNA DICER-AS1 was differentially expressed between gastric cancer and non-cancerous tissues. It has been reported that DICER-AS1 is downregulated in breast cancer and gastric cancer.^{7,22} The relative expression level of DICER1-AS1 in cancer tissues was also associated with histological grade.⁷ Our findings are consistent with the previously reported role of DICER1-AS1 as a tumor suppressor in gastric cancer that suppresses cell proliferation and migration.

miRNAs regulate gene expression post-transcriptionally by controlling mRNA stability and have been reported to be useful biomarkers for cancer diagnosis. A number of miRNAs, including miR-1271,²³ miR-543,²⁴ miR-181a,²⁵ and miR-363,²⁶ have been implicated in gastric cancer tumorigenesis, invasion, and metastasis. A recent study reported that miR-650 promotes the tumorigenesis of gastric cancer cells.²⁰ In our study, we found that miR-650 could bind to the 3'-UTR of DICER1-AS1, which acts as a sponge for miR-650. This

suggests that DICER1-AS1 inhibits tumorigenesis by negatively regulating miR-650 activity.

The *CSR1* gene is located at chromosome 8p21, a region that is frequently deleted in various human malignancies.^{9,10} Previous studies have shown that CSR1 functions as a cellular stress response protein that is widely expressed in various tissues, where it plays important roles in protecting cells from ROS-induced damage.⁸ Recently, CSR1 was found to be dysregulated in several cancers, where it is thought to regulate tumorigenesis. For example, Yu et al.¹¹ showed that CSR1 was downregulated in prostate cancer. In this study, we found that miR-650 targets CSR1, negatively regulating CSR1 expression in gastric cancer cells. Exogenous expression of miR-650 alone promoted cell migration, which may be because miR-650 negatively targets CSR1, relieving its inhibitory effect. Consistent with previous finding, our study suggests that CSR1 is a tumor suppressor that may mediate the effect of DICER1-AS1. Our results showed that DICER1-AS1 could sponge miR-650, which targets and downregulates CSR1. Moreover, the inhibition of cell proliferation and migration caused by silencing DICER1-AS1 was rescued by overexpressing CSR1. These findings indicate that CSR1 is a downstream effector of miR-650 that controls the tumor suppressor effect of DICER1-AS1 in gastric cancer.

Although our study revealed novel roles of the DICER1-AS1/miR-650/CSR1 axis in gastric cancer cells, further clinical investigation is needed to confirm that miR-650 and CSR1 are associated with the progression and prognosis of gastric cancer patients. Additionally, further experiments will be required to show the detailed mechanism through which CSR1 inhibits the proliferation and migration of gastric cancer cells.

In conclusion, our results suggest that DICER1-AS1 sponges miR-650, which regulates CSR1 expression, allowing CSR1 to inhibit the proliferation and migration of gastric cancer cells. This study describes a novel DICER1-AS1/miR-650/CSR1 regulatory axis that could provide potential targets for therapeutic intervention in gastric cancer.


Declaration of conflicting interest

The authors declare that there is no conflict of interest.

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