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# SNHG16 lncRNAs are overexpressed and may be oncogenic in human gastric cancer by regulating cell cycle progression

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The small nucleolar RNA host gene 16 (SNHG16) has recently been shown to be a putative oncogene in gastric cancer (GC) and other cancer types, but how its four lncRNA variants are expressed in any physiological and pathological situation remains unknown. To investigate the expression and function of the four lncRNA variants of SNHG16, mainly the variant 1, in GC, we performed quantitative PCR to determine the RNA levels of the four variants in 60 GC tissue samples and several cell lines. We also studied how knocking down of SNHG16 with siRNA affected proliferation, apoptosis, cell cycle progression, as well as migration and invasion of GC cells. Our results showed that variants 1 and 4 were overexpressed in GC tissues compared with adjacent uninvolved tissues. Knockdown of the four variants, mainly the variant 1, enhanced apoptosis and inhibited cell cycle progression of a GC cell line by arresting the cells at the G1 phase. These cellular effects were associated not only with decreased protein levels of c-Myc, PCNA, cyclins D1, E1, A2 and B, as well as CDKs 2 and 6, but also with increased protein levels of the p21, p27 and p53. Knockdown of total SNHG16 lncRNAs also inhibited invasion and migration of the GC cells *in vitro*. These results collectively suggest that SNHG16 may be oncogenic in GC by regulating cell cycle progression and may serve as a GC biomarker.

*Key words: SNHG16, lncRNA variant, gastric cancer, tumor progression, biomarker, oncogenic*

Gastric cancer (GC) has become the third leading cause of cancer-related mortality worldwide [1]. The incidence of GC in China ranks as number one in Asia [2, 3]. Currently, it is still difficult to establish an early diagnosis and good prognostic stratification of GC patients in many countries. Because GC has no characteristic clinical manifestations at its early stage, most patients are diagnosed only at advanced stages and have missed the opportunity for surgical removal of cancer, whereas other therapeutic approaches such as chemo- and radio-therapies have insufficient efficacies in general. For these reasons, GC has high mortality and a poor prognosis [4, 5], making it imperative to identify key factors that mediate the development and progression of GC and to determine their capacity as novel prognostic biomarkers or therapeutic targets.

Long noncoding RNAs (lncRNAs) are generally defined as those RNA transcripts of 200 or more nucleotides in length that do not encode proteins [6, 7], although ample evidence has challenged this definition and many RNA pundits have redefined it as those without an open-reading frame longer than 30 codons [8–10]. Material evidence has demonstrated that lncRNAs regulate a wide range of biological processes, and their dysregulation is involved in a variety of human cancers [11–13]. Because of these properties, some cancer studies consider that various lncRNAs may be used as novel cancer diagnostic markers and as future therapeutic targets

[14]. Many lncRNAs have been characterized in gastrointestinal malignancies, with their biological functions and underlying mechanisms in various stages of carcinogenesis being gradually revealed [15–17].

Small nucleolar RNA host gene 16 (SNHG16) has been suggested to be an oncogene in gastric cancer [16]. A study suggests that its oncogenic role in GC may be mechanistically elicited via sponging mir-135 and promoting the JAK2/STAT3 signaling pathway [18]. However, the NCBI (National Center for Biotechnology Information of the United States) shows that SNHG16 transcripts are alternatively spliced to four lncRNAs, dubbed herein as RNA variants. It is reasonable to assume that these four SNHG16 lncRNAs differ in their regulation and function, and the human body needs different SNHG16 lncRNAs to deal with different developmental, physiological, or pathological situations. Unfortunately, the expression spectrum of these four lncRNAs and the function of each one in different types of tissue or cell as well as in different developmental, physiological, and pathological situations have so far not been studied. These defects may be due partly to technical constraints on distinguishing each specific RNA variant from the others.

Since little information is available on the expression spectrum of the four SNHG16 lncRNAs in GC, we determined their expression levels in GC and adjacent uninvolved tissues from patients and explored their effects on some key behaviors of GC cells. In addition, we knocked down SNGH16 lncRNAs, mainly the variant 1 (V1) with siRNA, to evaluate the effect of low SNHG16 expression levels on proliferation, apoptosis, cell cycle progression, as well as migration and invasion of GC cells.

## Patients and methods

Cell lines and cell culture. Human GC cell lines AGS, SGC-7901, and BGC-823, as well as an immortalized gastric epithelial cell line GES-1, all purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China), were used in this study. All cells were cultured at 37 °C in a humidified atmosphere with 5% $CO_2$, with a culture medium containing 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 100 μg/ml streptomycin.

Patients and tissues. Sixty GC patients were enrolled in this study. All tumors were pathologically diagnosed with the tumor stage stratified based on the criteria of the WHO Classification of Tumors of the Digestive System (2010 edition). GC tissues and the corresponding adjacent uninvolved gastric tissues, which were tissues 5 cm away from the edge of the tumors and macroscopically manifested normal, were collected during surgery, put into an RNAlater solution (Ambion), and stored at –80 °C until use. The patients' clinicopathological data were collected prospectively (Table 1). This study was approved and abided by the institutional research ethics committee of Guizhou Medical University Hospital and was performed in compliance with the principles of the Declaration of Helsinki. All participating patients had signed informed consent. None of the patients' names or other personal information are disclosed.

Total RNA extraction, reverse transcription, and polymerase chain reaction. Total RNA was extracted from frozen tissues or cultured cells using a TRIZOL reagent (Takara Biotechnology, Dalian, China). Reverse transcription (RT) was carried out to convert the RNA to the first strand of complementary DNA (cDNA), using an RT Kit (Takara Biotechnology, Dalian, China) in compliance with the manufacturer's instruction.

To verify the specificity of each SNHG16 lncRNA, we designed primers, as shown in Table 2, that could specifically amplify the cDNA of each specific SNHG16 RNA variant in polymerase chain reactions (PCR) without cross-amplifying the others (Figures 1A–1C). Routine PCR was performed by an initial denaturation of the first strand of cDNA at 94 °C for 5 min, followed by 35 cycles of 58 °C for 30 s, 72 °C for 40 s, and 95 °C for 30 s, with a final extension at 72 °C for 5 min. The PCR products were fractioned via electrophoresis and visualized with ethidium bromide staining in 1% agarose gel (Figure 1D). Quantitative PCR (qPCR) was performed using a SYBR Premix Ex Taq II (Takara Biotechnology, Dalian, China) in a Light Cycler 480 II (Roche) as per manufacturer's instructions, with the primer pair specific for each SNHG16 RNA variant. RNA of the HPRT1 gene was used as the loading control as often shown by one of us [19–21].

**Small interfering RNA (siRNA) transfection.** We initially designed three siRNA-targeted sequences (synthesized by GenePharma, Shanghai, China) and tested their knockdown efficacy. Unfortunately, the one showing the highest efficacy was the one targeting a region (5'-CCCAGUGUUGACUCACCAATT-3') shared by all four variants. This one, and its control sequence (5'-UUCUCCGAACGUGUCACGUTT-3'), were used in this study. Transfection was performed with the Lipofectamine® RNAiMAX (Invitrogen, USA) as per instructions.

**Cell viability assay.** AGS cells were seeded at the density of 4,000 cells/well in 96-well plates (NEST, Shanghai, China) and, 12 h later, transfected with the siRNA or its control RNA. Using a microplate reader, optical density (OD) value at 450 nm was determined 24, 48, and 72 h after the transfection, with a CCK-8 reagent (Dojindo, Shanghai, China) used according to the manual. Each experiment was performed in triplicate.

**Colony-formation assay.** AGS cells were seeded at a density of 500 cells/well in 6-well plates (NEST, Shanghai, China) and then transfected with the siRNAs for 48 h. Two weeks later the cells were fixed with 4% paraformaldehyde and stained with 0.1% of crystal violet, followed by counting the number of colonies.

**Analysis of cell death.** AGS cells ($3\times10^5$ cells/well) were seeded in 6-well plates and transfected with the siRNAs as described above. At the indicated timepoint, adherent cells were collected, washed twice with ice-cold phosphate-

**Table 1. Correlation between clinicopathological parameters and expression levels of four SNGH16 lncRNAs in GC.**

| Variables | Num | expression of SNHG16-V1 | | | expression of SNHG16-V2 | | | expression of SNHG16-V3 | | | expression of SNHG16-V4 | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | High level | Low level | p-value | High level | Low level | p-value | High level | Low level | p-value | High level | Low level | p-value |
| Gender | | | | | | | | | | | | | |
| Male | 41 | 15 | 26 | 0.242 | 20 | 21 | 0.905 | 13 | 28 | 0.121 | 13 | 28 | 0.432 |
| Female | 19 | 10 | 9 | | 8 | 11 | | 10 | 9 | | 8 | 11 | |
| Age | | | | | | | | | | | | | |
| ≤60 | 30 | 14 | 16 | 0.432 | 15 | 15 | 0.605 | 12 | 18 | 0.791 | 12 | 18 | 0.592 |
| >60 | 30 | 11 | 19 | | 13 | 17 | | 11 | 19 | | 10 | 20 | |
| Differentiation | | | | | | | | | | | | | |
| Moderate/Poor | 17 | 7 | 10 | 0.961 | 5 | 12 | 0.092 | 6 | 11 | 0.761 | 5 | 12 | 0.463 |
| Poor | 43 | 18 | 25 | | 23 | 20 | | 17 | 26 | | 17 | 26 | |
| Invasion depth | | | | | | | | | | | | | |
| T1–T2 | 12 | 9 | 3 | 0.020* | 4 | 8 | 0.245 | 5 | 7 | 0.895 | 6 | 6 | 0.135 |
| T3–T4 | 48 | 18 | 30 | | 25 | 23 | | 19 | 29 | | 16 | 32 | |
| Lymph node invasion | | | | | | | | | | | | | |
| No | 43 | 19 | 24 | 0.529 | 22 | 21 | 0.485 | 18 | 25 | 0.371 | 16 | 27 | 0.89 |
| Yes | 17 | 6 | 11 | | 7 | 10 | | 5 | 12 | | 6 | 11 | |
| TNM stage | | | | | | | | | | | | | |
| I–II | 19 | 10 | 9 | 0.419 | 10 | 9 | 0.781 | 8 | 11 | 0.682 | 7 | 12 | 0.872 |
| III–IV | 41 | 17 | 24 | | 20 | 21 | | 15 | 26 | | 16 | 25 | |
| Distant metastasis | | | | | | | | | | | | | |
| M0 | 57 | 23 | 34 | 0.368 | 25 | 32 | 0.439 | 21 | 36 | 0.902 | 21 | 36 | 0.902 |
| M1 | 3 | 2 | 1 | | 2 | 1 | | 1 | 2 | | 1 | 2 | |

Note: *p-value less than 0.05 was considered significant

**Table 2. Primers for four putative transcripts of SNHG16.**

| Gene | Forward primer | Reversed primer | Product length(bp) |
|---|---|---|---|
| SNHG16-V1 | 5′-GTGTAAGGATCTTCATGATG-3′ | 5′-GCTGGGAGCTAACTCACATT-3′ | 163 bp |
| SNHG16-V2 | 5′-GTGTAAGGATCTTCATGATG-3′ | 5′-CATCGCTGGGAGCTAACAT-3′ | 163 bp |
| SNHG16-V3 | 5′-GGCCTTTAGTGATGATGGTG-3′ | 5′-GCTGGGAGCTAACTCACATT-3′ | 145 bp |
| SNHG16-V4 | 5′-GGCCTTTAGTGATGATGGTG-3′ | 5′-CATCGCTGGGAGCTAACAT-3′ | 145 bp |
| HPRT | 5′-CCTGGCGTCGTGATTAGTGAT-3′ | 5′-AGACGTTCAGTCCTGTCCATAA-3′ | 131 bp |

buffered saline (PBS), and then stained with 500 μl of a loading buffer (KeyGen Biotechnology, Nanjing, China) containing 5 μl of Annexin V-FITC and 5 μl of propidium iodide (KeyGen Biotechnology, Nanjing, China). The cell suspension was then filtered through a 300-mesh filter and analyzed using a BD FACS Caliber flow cytometer (BD Bioscience, San Diego, CA, USA).

**Wound healing assay.** AGS cells ($3\times10^5$ cells/well) that were seeded in 6-well plates and transfected with the siRNAs, as described above, were allowed to grow for 24 h after the transfection. A wound was then generated using a P200 pipette tip (Axygen, California, USA). Photos of the wound were taken under an inverted microscope (TE2000-U, Nikon, Japan) at the indicated timepoint. The wound area was measured with the ImageJ software (National Institutes of Health, Bethesda, MD, USA) with the wound closure rate calculated. The experiment was performed three times.

**Transwell assay.** For migration assays, $3\times10^5$ cells transfected with the siRNAs were seeded into the upper chamber of a transwell (BD Bioscience). For invasion assays, $3\times10^5$ cells were added into the upper chamber precoated with a Matrigel (BD Bioscience). In both assays, cells were maintained in a medium without FBS in the upper chamber, while a medium containing 10% FBS was added into the lower chamber. Cells that did not migrate or invade through the membrane were carefully wiped out 24 h later. The membranes were then fixed with 4% paraformaldehyde for 30 min, stained with 0.1% crystal violet for 10 min, and air-dried. Three random areas per chamber were photographed and counted under an inverted microscope. Each experiment was conducted three times.

**Analysis of cell cycle distribution.** AGS cells were transfected with the siRNAs as described above. At the indicated timepoint, adherent cells were collected, washed

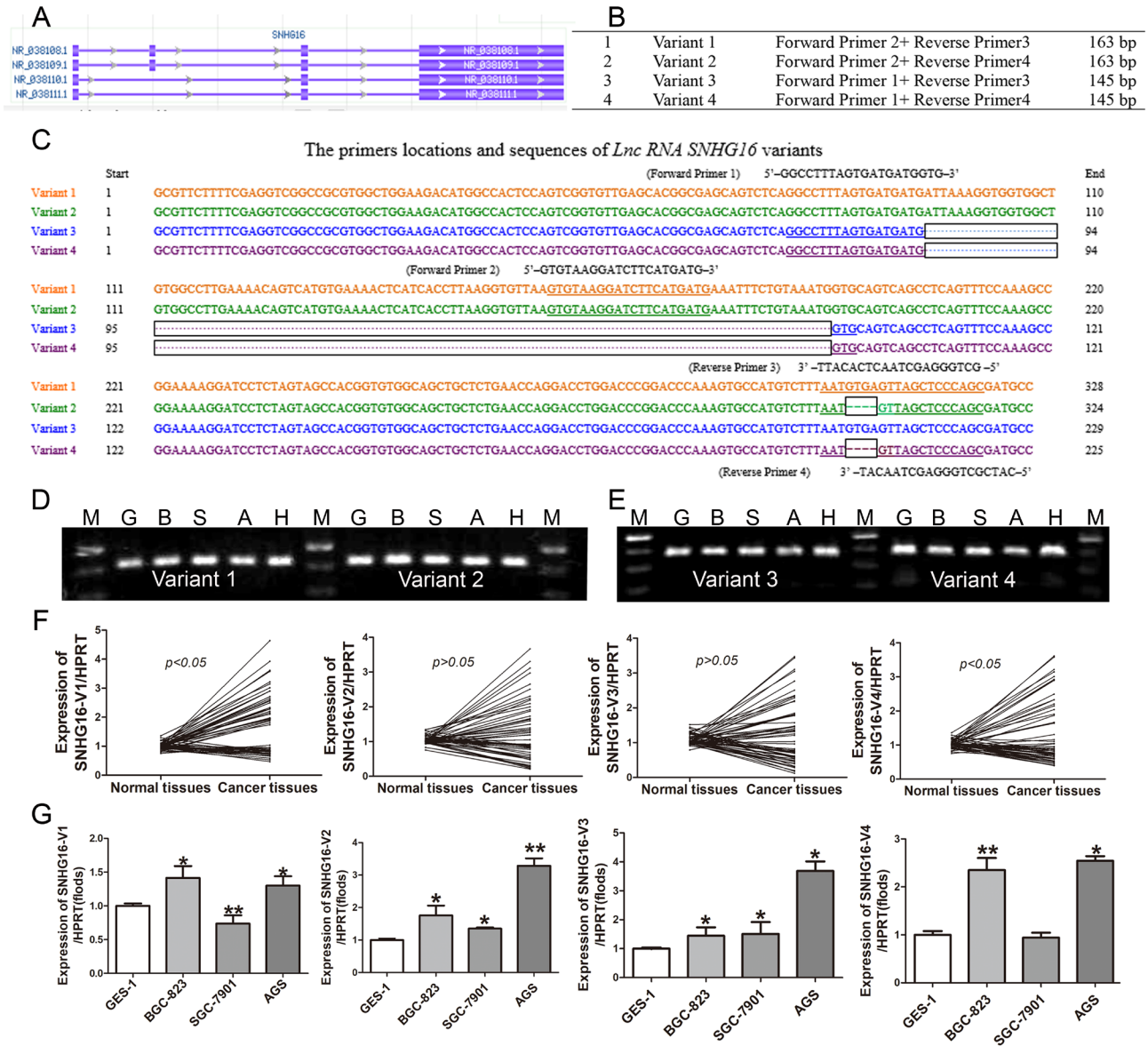


**Figure 1. Depiction of the four SNHG16 RNA variants, our primer design strategy, and the expression level of each variant. A) Illustration of the four RNA variants copied from the NCBI website. B) The primer set used for amplifying each variant and the anticipated size of the corresponding PCR amplicon. C) The sequence region of each variant where the primers are located. D, E) Images of routine RT-PCR showing a single, specific band of each variant, with M indicating a DNA ladder marker while G, B, S, A, and H indicating GES, BGC, SGC, AGS, and HGC cells, respectively. F) Relative expression of each variant in the 60 GC tissues and the adjacent uninvolved tissues, with p-value indicating the result of the statistical comparison between the tumors and the paired uninvolved tissues. G) Relative expression of each variant in four cell lines. The expression level in the immortalized GES-1 cells is arbitrarily set as 100% to serve as the reference for that of the GES-1, SGC-7901, and AGS GC cell lines. (*p<0.05; ** p<0.01, both compared with the GES-1 cells)**

twice with an ice-cold PBS, fixed with ice-cold 70% ethanol at 4 °C for 2 h, and then stained with propidium iodide (KeyGen Biotechnology, Nanjing, China) at a concentration of 50 μg/ml containing 100 μg/ml RNase A (KeyGen Biotechnology, Nanjing, China). The suspension was filtered through a 300-mesh filter and the stained nuclear DNA was analyzed for different cell cycle phases using a FACS Caliber flow cytometer (BD Bioscience, San Diego, CA, USA).

**Western blotting.** Cell lysates were prepared using a lysate buffer (Beyotime Institute of Biotechnology, Shanghai, China) and determined for their protein concentrations using a bicinchoninic acid (BCA; Solarbio, Beijing, China) method. Polyacrylamide gel electrophoresis (PAGE) of the proteins was performed in the presence of sodium dodecyl sulfate (SDS) (Solarbio, Beijing, China), followed by transferring the proteins onto a polyvinylidene difluoride membrane

(EMD Millipore). After blocking with 5% skimmed milk at room temperature for an hour, the membranes were incubated overnight at 4°C with the primary antibody of interest, including antibodies for CDK6 (1:1000, 13331, Cell Signaling Technology, USA), CDK2 (1:1000, ab32147, Abcam, Cambridge, MA, USA), p53 (1:1000, ab32389, Abcam, Cambridge, MA, USA), p21 (1:1000, ab109520, Abcam, Cambridge, MA, USA), p27 (1:1000, ab32034, Abcam, Cambridge, MA, USA), cyclin E1 (1:1000, ab33911, Abcam, Cambridge, MA, USA), cyclin D1 (1:1000, ab134175, Abcam, Cambridge, MA, USA), cyclin A2 (1:10000, ab32386, Abcam, Cambridge, MA, USA), c-Myc (1:1000, ab32072, Abcam, Cambridge, MA, USA), Lamin B (1:500, ab32535, Abcam, Cambridge, MA, USA), and GAPDH (1:10000, ab181602, Abcam, Cambridge, MA, USA), as well as PCNA (1:2000, 10205-2-AP, Proteintech Group Inc., USA). After washing three times with TBST (Tris-HCl, NaCl, and 0.1% Tween 20), the membrane was incubated with a corresponding secondary antibody (1:2000, 7074, Cell Signaling Technology, USA) for 1 h. The density of the specific band on the membrane was determined using the Image-Pro Plus software 6.0 (Rockville, USA). For most genes, the data were normalized to that of GAPDH, but the PCNA and c-Myc data were normalized to the expression of Lamin B.

**Statistical analysis.** Data were presented as the mean ± standard deviation (SD) and were analyzed using the SPSS 19.0 software for t-test and $\chi^2$-test. A p-value <0.05 is considered statistically significant.

## Results

**SNHG16 lncRNA variant 1 was elevated in GC and correlated with the invasion depth.** Results from routine RT-PCR confirmed that the primers we designed were able to specifically amplify each SNHG16 lncRNA variant without cross-amplifying another (Figures 1D, 1E). The results of qRT-PCR with these primers showed that, of the four variants, V1 and V4 manifested a significantly higher level in the GC tissues than in the paired adjacent uninvolved tissues. The expression level of V1 was the highest among the four variants in the GC tissues, while the levels of V2 and V3 showed a trend of elevation without reaching a statistical significance (Figure 1F). The expression of V4 in cell lines was higher than that of V1. Of the four cell lines studied, GC cell lines AGS and BGC-823 manifested higher levels of the four variants than the other two, generally speaking (Figure 1G).

A higher expression of V1 was positively correlated with invasion depth T1–T2 in our cohort, while the other three variants did not show a significant correlation. We also analyzed the relationships of all variants to gender, age, tumor differentiation, invasion depth, lymph node involvement, TNM stage, and distant metastasis, but none of these parameters showed a statistical correlation with the level of any of the variants (Table 1).

**Knockdown of SNHG16 enhanced apoptosis and inhibited the proliferation of GC cells *in vitro*.** Since the sequence similarity among the four variants refrained us from specifically knocking down each specific variant, we used an siRNA, which showed the highest knockdown efficacy among the siRNAs we tested (data not shown) but unfortunately targeted all four variants, to knock down SNHG16 in the AGS cells that expressed much higher levels of all four variants than the other cell lines we studied. qRT-PCR results showed that all four variants were significantly knocked down, with V1 decreasing most dramatically (Figures 2A, 2B). CCK-8 results revealed that transient transfection with the siRNA decreased the cell viability, but the effect was transient since no difference in cell viability was discerned between 48 and 72 h post siRNA transfection (Figures 2C, 2D). Results of flow cytometry revealed an increase in the early and late apoptotic fractions in the siRNA-treated cells, compared with the vector-treated controls (Figures 2E, 2F), suggesting that the decrease in the viability may mainly be attributed to the enhanced apoptosis. Colony formation assay showed that, while the number of colonies was decreased by the siRNA transfection, the sizes of the colonies formed by the siRNA-treated cells were similar or even slightly larger than those formed by the vector-treated counterparts (Figures 2G, 2H). These data agreed with the above results and suggest again that the effects of the transient siRNA transfection on inhibiting cell growth and promoting cell death may be transient and may not be long-lasting.

Cell cycle analysis showed that siRNA-treated cells manifested an increased G1 fraction but a decreased S fraction, suggesting that the siRNA inhibits cell proliferation by arresting the cells at the G1 phase (Figures 3A, 3B). Indeed, western blotting results also showed decreased protein levels of cyclins D1, A2 and E1, as well as CDKs 2 and 6, but increased levels of p21, p27 and p53 (Figures 3C and 3D). In line with these results, the PCNA and c-Myc levels were also decreased (Figures 3E and 3F)

**Knockdown of SNHG16 inhibited migration and invasion of GC cells *in vitro*.** Both wound-healing and Transwell assays revealed a marked decrease in invasion and migration of the AGS cells transfected with the siRNA when compared with the vector-treated counterparts (Figures 4A–4D).

## Discussion

Initiation of human cancer is an extremely complex stepwise process in which regulation of gene expression may be a critical aspect [22]. Alternative splicing is one of the post-transcriptional regulatory mechanisms, enabling a single gene to produce multiple mature RNAs. These same-gene-derived RNAs, with or without different protein isoforms as their end products, may have similar, partially different, or even completely opposite functions [23–27].

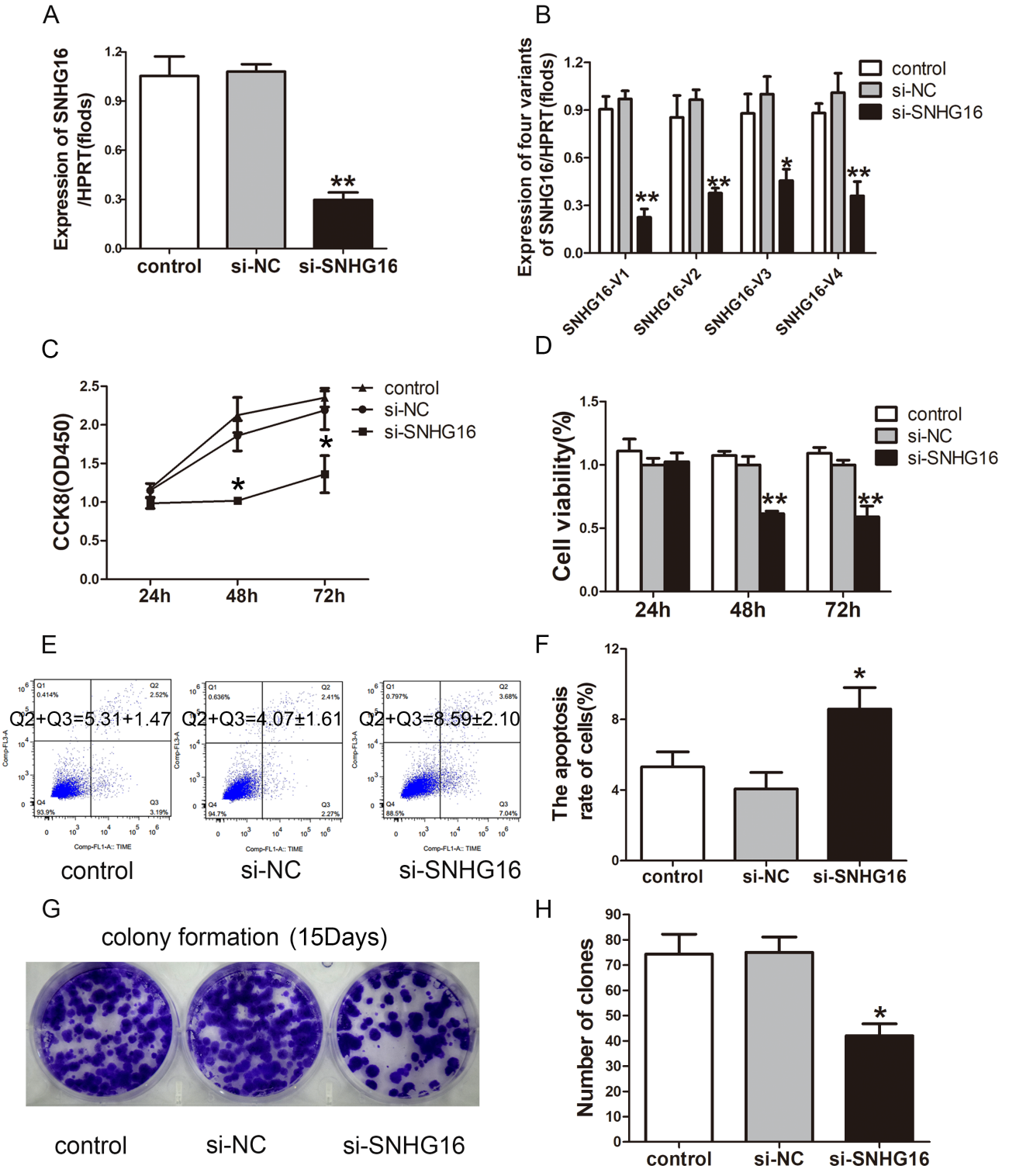


**Figure 2. Effects of the SNHG16 siRNA on the AGS cell viability. A) Total SNHG16 RNA level, detected with qRT-PCR, in AGS cells transfected with the siRNA or control siRNA (NC), with the level in the untransfected cells set as 100% as the reference. B) Expression level of each variant relative to the ratio of total SNGH16 to HPRT1. C, D) Cell viability determined with a CCK-8 assay, shown as the OD value. E, F) Flow cytometry assay revealing an increase of early and late apoptotic fractions in the siRNA. G, H) Colonies formed by untransfected AGS cells or by the cells transfected with the SNGH16 siRNA or control siRNA. (*p<0.05; **p<0.01, both compared with si-NC)**

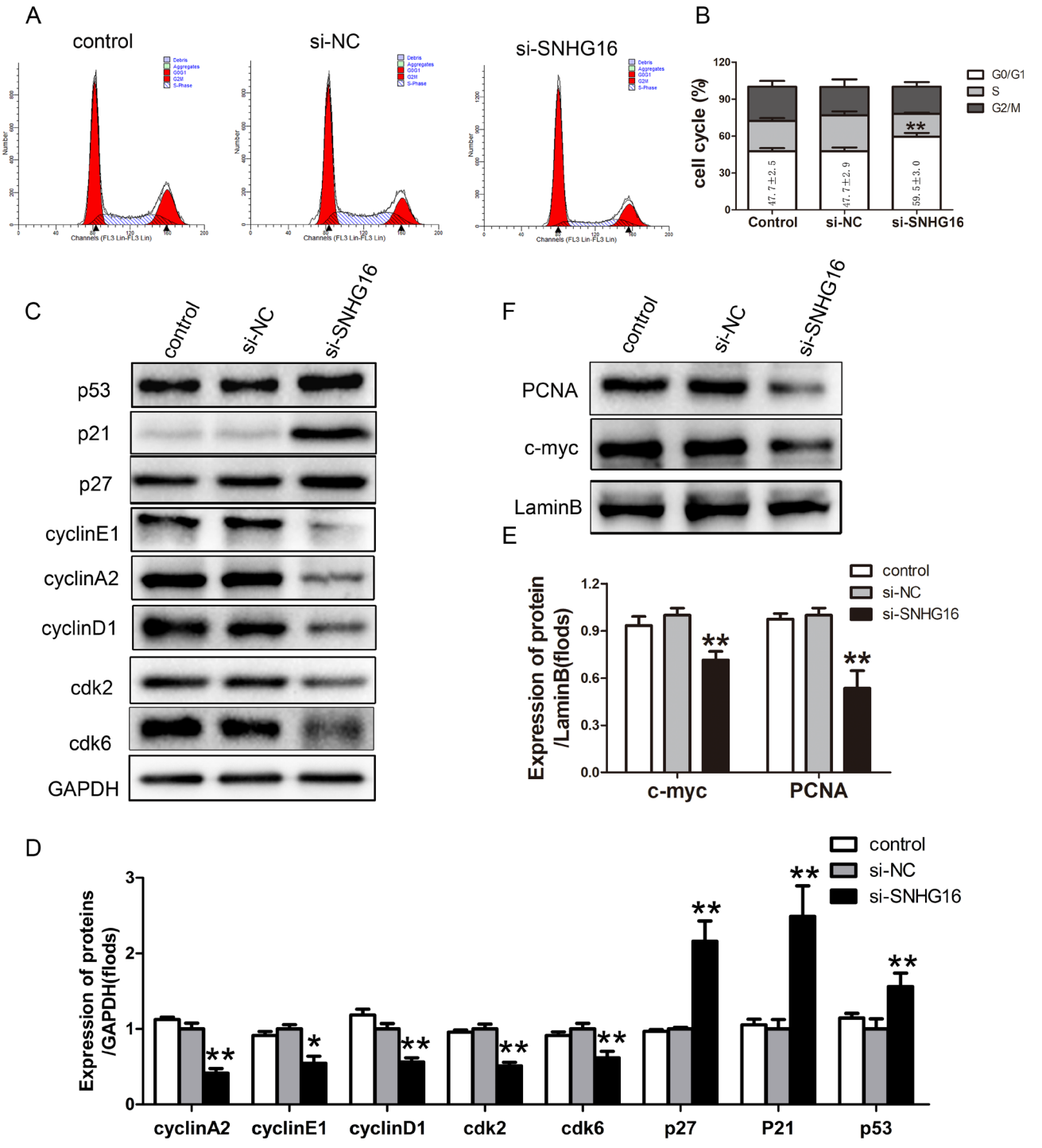


**Figure 3. Effects of the SNHG16 siRNA on cell cycle progression, on the levels of P53, c-Myc, and other cell cycle regulators, and on the cell cycle progression. A, B) Fractions of cells at different cell cycle stages detected with FACS. C–F) Protein levels of different cell cycle regulators detected with western blotting, with their ratios to the corresponding gene (GAPDH or Lamin B) used as the loading control. AGS cells were evaluated by flow cytometry 48 hours after transfection with the si-SNHG16. (*p<0.05; **p<0.01, both compared with si-NC)**

LncRNAs have recently been shown to have many functions in multiple biologic processes. However, although there have been numerous studies of lncRNAs in cancer, few reports have addressed alternative splicing of lncRNA and have evaluated the expression spectrum of different RNA variants, especially in cancer [28, 29].

SNHG16 has been shown to be commonly expressed in human tissues and cancer cells and has been suggested to be an oncogene [30, 31]. However, expression of any of its four lncRNAs has never been studied in any normal or abnormal situation, including cancer, and thus the functions and underlying mechanisms of each variant remain completely unknown, including their regulation of the cell cycle progression and cell behaviors. These defects are, presumably, in part because few SNHG16 researchers realize that its transcript may undergo alternative splicing. Our present study reports, for the very first time, a strategy and method to detect the level of each specific SNHG16 lncRNA variant, which should lend peers a means to study each RNA variant of the SNHG16 gene in particular and other lncRNA-encoding genes in general, in variegated developmental, physiological, and pathological situations. We also present quantitative data of each SNHG16 RNA variant in GC and adjacent uninvolved tissues from patients. Although we are not able to specifically manipulate the expression, and thus are unable to determine the function of each variant due to the technical constraints, our data, notwithstanding, dovetail with the recent report by Wang et al. that SNGH16 lncRNAs may play an oncogenic role in GC [18].

It is generally accepted that CDKs regulate cell cycle progression at several irreversible transition points with the formation of different cyclin/CDK/CKI (cyclin kinase inhibitor) complexes involved at different cell cycle stages [32, 33]. p27 and p21 belong to the CKI family and bind to such cyclin/CDK complexes as cyclin D1/CDK6, cyclin E1/CDK2, and cyclin A2/CDK2, c-Myc, and P53 also exhibit indispensable effects on the proliferation, transformation, cellular death, and cell cycle progression of mammalian cells [34], and their dysregulation or mutation may convert them into pure oncogenes to promote development and progression of cancer [35, 36]. In this study, we show for the first time that knockdown of SNHG16 lncRNAs, mainly the V1, signifi-

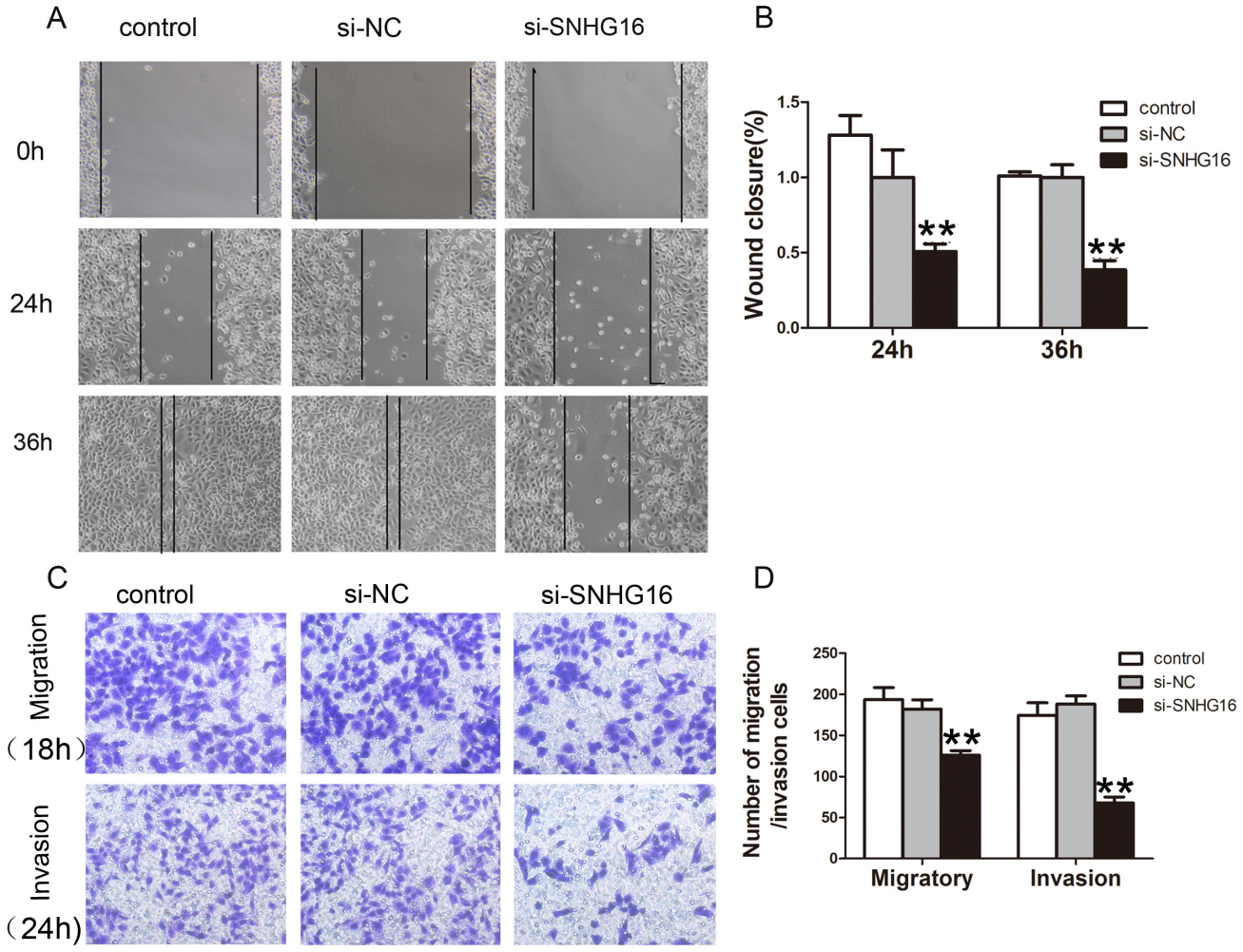


**Figure 4. Effects of the SNHG16 siRNA on invasion and migration of the AGS cells. A–D) Wound-healing assay showing an inhibition of the cell migration by the siRNA. A, B) Transwell assay showing an inhibition of *in vitro* invasion by the siRNA. C, D). (*p<0.05; **p<0.01, both compared with si-NC)**

cantly decreases the c-Myc expression and affects the formation of the p27/cyclin D1/CDK6, p53/cyclin E1, and cyclin A2/CDK2 complexes, collectively leading to the arrest of cells in the G1 phase. These alterations are likely to be important parts of the molecular and cellular mechanism for the aforementioned cellular effects, i.e., promotion of apoptosis and inhibition of proliferation, invasion, and migration of GC cells. These cellular effects are shown, also for the first time, to be attributed at least in part to the effects of SNGH16 lncRNAs on some key regulators of the G1 and S phases of the cell cycle, including P53 and c-Myc.

In conclusion, our data show that V1 is the major one overexpressed in GC tissue and that knockdown of SNGH16 lncRNAs, mainly the V1, inhibits proliferation, invasion, and migration of GC cells *in vitro*. These findings are associated with a decrease in several key G1 and S phase cyclin-dependent kinases (CDKs) and an increase in several CDK inhibitors. Collectively, these results suggest that SNHG16 lncRNAs, mainly the V1, may be oncogenic in GC and may serve as a good biomarker for GC.

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