The long non-coding RNA SNHG3 functions as a competing endogenous RNA to promote malignant development of colorectal cancer

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Abstract. Accumulating evidence has revealed that aberrantly expressed long non-coding transcripts are involved in the development and progression of colorectal cancer (CRC). Small nucleolar RNA host gene 3 (SNHG3) is a newly identified lncRNA, and little is known about its clinical significance and biological functions in the development of CRC. In the present study, we found that the expression of SNHG3 was significantly upregulated in CRC, and upregulation of SNHG3 predicted poor prognosis for patients with CRC as determined through analysis of the data obtained from TCGA database. Gain-of function and loss-of function assays revealed that SNHG3 markedly promoted cellular proliferation of CRC cells. Gene Set Enrichment Analysis (GSEA) suggested that high expression of SNHG3 was positively associated with c-Myc and its targets genes. Furthermore, ectopic overexpression of SNHG3 increased the expression of c-Myc and its target genes, whereas inhibition of SNHG3 had opposite effect on the expression of c-Myc and its targets. Mechanistic investigations demonstrated that SNHG3 functioned as a competing endogenous RNA (ceRNA) to 'sponge' miR-182-5p, thus leading to the release of c-Myc from miR-182-5p and modulating the expression of c-Myc. In conclusion, SNHG3 promoted CRC progression via sponging miR-182-5p and upregulating c-Myc and its target genes.

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Abbreviations: CRC, colorectal cancer; GSEA, gene set enrichment analysis; ceRNA, competing endogenous RNA; lncRNA, long non-coding RNA; mRNA, message RNA; HCC, hepatocellular carcinoma; TICs, liver tumor-initiating cells; SNHG3, small nucleolar RNA host gene 3; ATCC, American Type Culture Collection; CCK-8, Cell Counting Kit-8; siRNA, small interfering RNAs; TCGA, The Cancer Genome Atlas project; GEO, Gene Expression Omnibus

Key words: long non-coding RNA, SNHG3, colorectal cancer, ceRNA

Introduction

Colorectal cancer (CRC) ranks as the third most common malignancy throughout the world, with high incidence and mortality rates (1). Although advanced treatment including resection, chemotherapy, as well as radiation are commonly used to improve the outcomes of patients with CRC, the 5- and 10-year survival rates for CRC patients remain unsatisfactory, and are ~65 and 58%, respectively (2,3). Further clarification of the underlying molecular mechanisms for CRC development and progression are urgently needed (4). Therefore, better understanding of the precise molecular mechanism contributing to the initiation and progression of CRC may provide new diagnostic approaches or therapeutic strategies for patients with CRC.

Long non-coding RNAs (lncRNAs) are types of RNAs which are over 200 nucleotides in length without protein-coding capabilities (5-7). lncRNAs, frequently located in the nucleus, are present with lower abundance, and act in a more disease-specific or tissue-specific manner with poorer interspecies conservation in comparison with message RNAs (mRNAs) (8). Growing evidence demonstrates that lncRNAs are involved in a large range of biological progresses including proliferation, metastasis, differentiation, inflammation, angiogenesis, and metabolism (9-14). Alteration of lncRNAs has been demonstrated to be involved in the onset and development of various cancers. For example, upregulation of lncRNA DSCAM-AS1 in breast cancer mediates tumor progression through direct interaction with hnRNPL (15). LncSox4, highly expressed in hepatocellular carcinoma (HCC) and liver tumor-initiating cells (TICs), is required for TIC self-renewal and tumor initiation via recruitment of Stat3 to the promoter of SOX4 (16). Highly expressed lncRNA SNHG20 facilitates cellular proliferation and predicts poor prognosis for patients with HCC (17). LINC00673, a tumor suppressor, mitigates

SRC-ERK oncogenic signaling through reinforcement of the interaction of PTPN11 and PRPF19, and promotes PTPN11 degradation in the proteasome pathway (18). However, the function of numerous lncRNAs remain largely unclear.

Small nucleolar RNA host gene 3 (SNHG3; GenBank accession no. NR_036473.1), located on 1q35.3, is a newly identified lncRNA. Zhang *et al* found SNHG3 was highly expressed in HCC and significantly associated with malignant status and poor prognosis in HCC (19). However, its functions and the underlying mechanisms in CRC remain to be explored. Herein, we seek to determine the expression level and function of SNHG3, and further explore the potential molecular mechanism of SNHG3 in colorectal carcinogenesis. We found that SNHG3 was highly expressed in CRC tissues and CRC cell lines. Moreover, overexpression of SNHG3 promoted CRC cell proliferation, whereas silencing of SNHG3 impaired cellular proliferation ability. In addition, SNHG3 acted as a competing endogenous RNA (ceRNA) to sponge miR-182-5p, thus leading to the release of c-Myc from miR-182-5p and the regulation of the expression of c-Myc.

Materials and methods

Cell culture. Human CRC cell lines HT29, HCT116, SW480, and LoVo were purchased from the American Type Culture Collection (ATCC; Maryland, MD, USA). The normal colonic epithelial cell line NCM460 was obtained from INCELL (San Antonio, TX, USA). Colorectal cell lines were cultured in RPMI-1640 medium, L-15 or Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, USA) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, and 100 mg/ml streptomycin. All cell lines were incubated in a humidified incubator containing 5% CO_2 at 37˚C.

RNA extraction, reverse transcription, and qRT-PCR. The total RNA of the CRC cell lines was extracted using TRIzol® reagent (Invitrogen, Carlsbad, CA, USA). After quantitating the amount of total RNA, 500 ng RNA from each sample was converted to cDNA using a Reverse Transcription kit (Takara Biotechnology, Dalian, China). Real-time PCR was performed using the SYBR-Green PCR kit purchased from Takara Biotechnology, and the data collection was carried out on an ABI Prism 7500 Sequence detection system (Applied Biosystems, Foster City, CA, USA). Primer sequences used in this study were synthesized by Invitrogen (Shanghai, China) and the sequences were as follows: SNHG3 forward, 5'-TTCA AGCGATTCTCGTGCC-3' and reverse, 5'-AAGATTGTCAA ACCCTCCCTGT-3'; c-Myc forward, 5'-TACAACACCCGAG CAAGGAC-3' and reverse, 5'-GAGGCTGCTGGTTTTCC ACT-3'; CCNB1 forward, 5'-CAACTTGAGGAAGAGCAA GCA-3' and reverse, 5'-AGCATCTTCTTGGGCACACA-3'; CCND2 forward, 5'-CAGCTGTCACTCCTCATGACT-3' and reverse, 5'-TTGAGACAATCCACGTCTGTGTT-3'; CDK4 forward, 5'-TACAACACCCGAGCAAGGAC-3' and reverse, 5'-GAGGCTGCTGGTTTTCCACT-3'; E2F1 forward, 5'-GAG GAGACCGTAGGTGGGAT-3' and reverse, 5'-GGACAACA GCGGTTCTTGC-3'; GAPDH forward, 5'-CGCTCTCTGCT CCTCCTGTTC-3' and reverse, 5'-ATCCGTTGACTCCGAC CTTCAC-3'; U6 forward, 5'-CTCGCTTCGGCAGCACA-3' and reverse, 5'-AACGCTTCACGAATTTGCGT-3' and miR-183-5p forward, 5'-ACACTCCAGCTGGGTTTGGCAATGG TAGAACT-3' and reverse, 5'-TGGTGTCGTGGAGTCG-3'. Fold change was calculated using the $2^{-\Delta\Delta Ct}$ method. U6 and GAPDH were used as internal controls.

CCK-8 assay. The cell proliferation ability was assessed using Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) following the manufacturer's instructions. Cancer cells ($2x10^3$) were seeded into 96-well plates 12 h before transfection. Ten microliters of CCK-8 reagent was added into each well of the 96-well plates every 24 h, mixed gently and cultured at 37˚C for 2 h. Finally, the absorption was assessed at a wavelength of 450 nm.

Small interfering RNA, miRNA mimics, and inhibitor of transfection. The small interfering RNAs (siRNAs) specifically targeting SNHG3, miR-182-5p mimics and miR-182-5p inhibitor were commercially designed and synthesized by Shanghai GenePharma (Shanghai, China). Cells were transfected with siRNAs, miR-182-5p mimics, or miR-182-5p inhibitor with a cell density of 60% using Lipofectamine 3000 (Invitrogen, USA). The cells were then harvested for qRT-PCR, western blot analyses, or for further studies 48 h after transfection.

Lentivirus production and construction of stable cell lines. A lentivirus harboring full length SNHG3 was constructed by Shanghai Jikai (Shanghai, China). SW480 cells were infected with a lentivirus containing full length SNHG3 using the recombinant lentivirus-transducing units plus 8 mg/ml Polybrene obtained from Jikai. Three days later, the cells transfected with the lentivirus were subjected to FACS analysis for GFP to obtain cells stably overexpressing SNHG3.

Luciferase reporter assay. Wild-type c-Myc 3'-UTR SNHG3 sequences and mutant c-Myc 3'-UTR SNHG3 sequences were cloned and inserted into psiCHEK2.0 vector (Promega, Madison, WI, USA) to construct the psiCHEK-c-Myc-wt, psiCHEK-c-Myc-mut, psiCHEK-SNHG3-wt, and psiCHEK-SNHG3-mut plasmids, respectively. SW480 cells were co-transfected with 1,000 ng of luciferase construct vectors along with miR-182-5p mimics, miR-182-5p inhibitor, or a plasmid harboring SNHG3. Luciferase activities were assessed using the dual-luciferase reporter assay system 48 h after transfection.

RIP assay. RIP assay was performed according to the instructions of the Magna RIP RNA-Binding Protein Immunoprecipitation kit (Millipore, Bedford, MA, USA). SW480 cells transfected with MS2-SNHG3-wt and MS2-SNHG3-mut were lysed in lysis buffer containing a protease inhibitor cocktail and an RNase inhibitor. Magnetic beads were incubated with anti-GFP antibody or anti-rabbit IgG for 30-60 min at room temperature. The lysates were immunoprecipitated with beads at 4˚C overnight. The RNA-protein complex was eluted from the beads. Purified RNA from the RNA-protein complex was analyzed using qRT-PCR.

Animal studies. The animal experiments were approved by the Institutional Animal Care and Use Committee of the

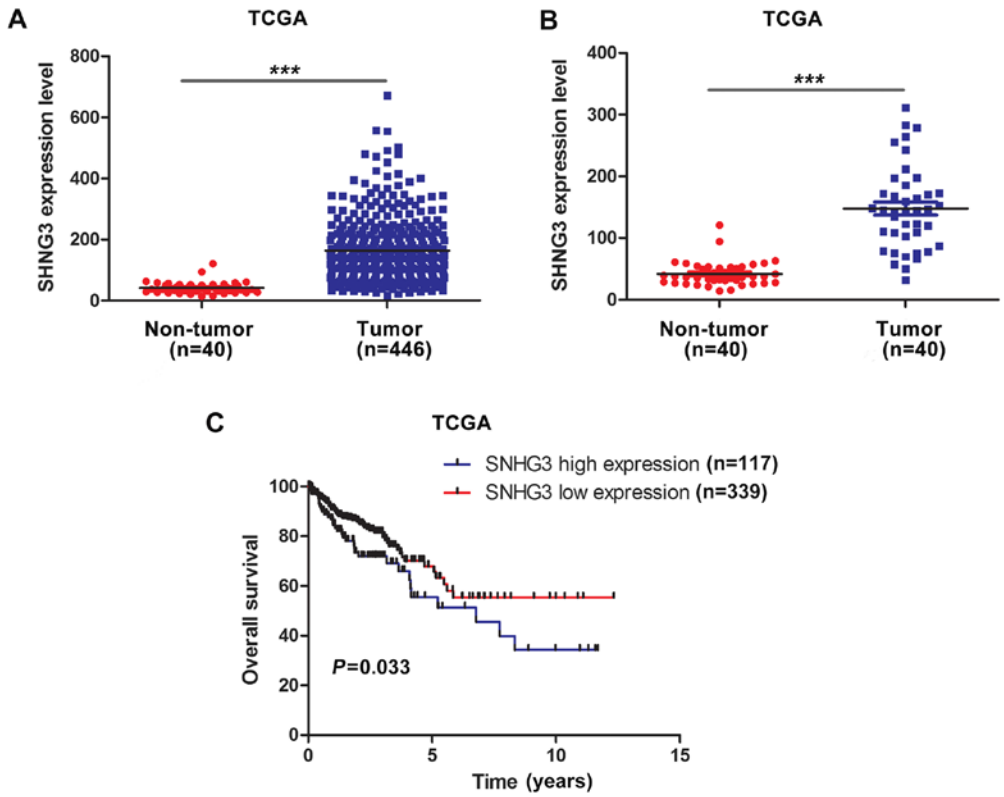

Figure 1. SNHG3 is upregulated in CRC and high expression of SNHG3 predicts poor prognosis for CRC patients. (A) Analysis of SNHG3 expression in CRC patients from TCGA database (n=456, ***P<0.001). (B) Re-analysis of the expression of SNHG3 in 40 paired CRC samples and adjacent non-tumor tissues from TCGA database (n=40, ***P<0.001). (C) Kaplan-Meier survival analysis of the overall survival of CRC patients in TCGA database (log-rank, P=0.033). SNHG3, small nucleolar RNA host gene 3; CRC, colorectal cancer; TCGA, The Cancer Genome Atlas project.

Huizhou Municipal Central Hospital. Male BALA/c nude mice (4-6 weeks old) were used to detect the effect of SNHG3 on the tumor growth *in vivo*. Briefly, $1x10^7$ SW480 cells with SNHG3 overexpression (right side) or control vector (left side) were injected into the bilateral flanks of nude mice. Six weeks later, the tumors were removed and subjected to further experiments.

Western blot analysis. Western blot analysis was used to assess the expression level of c-Myc, as previously described (20).

Expression datasets. To explore the expression level of SNHG3 and miR-182-5p in CRC tissues, we analyzed the data from the Cancer Genome Atlas project (TCGA; https://tcga-data.nci.nih.gov/tcga/) and Gene Expression Omnibus (GEO, accession number GSE54632, platform: GPL8786, [miRNA-1_0] Affymetrix miRNA Array), respectively. In general, a total of 456 CRC patients with mRNA expression data from TCGA were enrolled in the current study. Among these patients, 40 patients had SNHG3 expression data from both CRC tumor tissues and non-tumor tissues, while miRNA expression data from 5 patients in the GSE54632 dataset were used to compare the expression level of miR-182-5p in CRC tumor tissues and non-tumor tissues.

Gene Set Enrichment Analysis (GSEA). GSEA is a method used to analyze and interpret microarray data through biological technology, which has been previously described (21). The data is analyzed in terms based on their differential enrichment in a predefined co-expression or biochemical pathway (gene set) in a previous experiment. If the majority of a gene set had high expression accompanied with a high risk score, this gene set would have a positive enrichment score and would be termed 'enriched'. GSEA was carried out by the JAVA program (http://www.broadinstitute.org/gsea) using GSEA version 2.0. Data from TCGA dataset was subjected to GSEA analysis. Parameters used in the current study were as follows: 1,000 random sample permutations were used to calculate the p-value. An FDR <25% and a nominal p-value <0.05 were considered as significant.

Statistical analysis. Statistical analysis was performed by SPSS 20.0 (Abbott Laboratories, North Chicago, IL, USA). All data were expressed as the mean ± standard error of mean (SEM) from at least three independent experiments. The t-test was used to analyze data between two groups and a parametric generalized linear model with random effects was used for cell proliferation analysis. All statistical tests were two-sided and a P<0.05 was regarded as statistically significant.

Results

Upregulation of SNHG3 is correlated with poor prognosis in patients with CRC. To determine the expression of SNHG3 in CRC, we analyzed the data of 456 colon adenocarcinoma patients from TCGA dataset. As shown in Fig. 1A, the expression of SNHG3 was significantly overexpressed in CRC tumor tissues in comparison with non-tumor tissues (P<0.001). To eliminate the possibility that the difference of expression

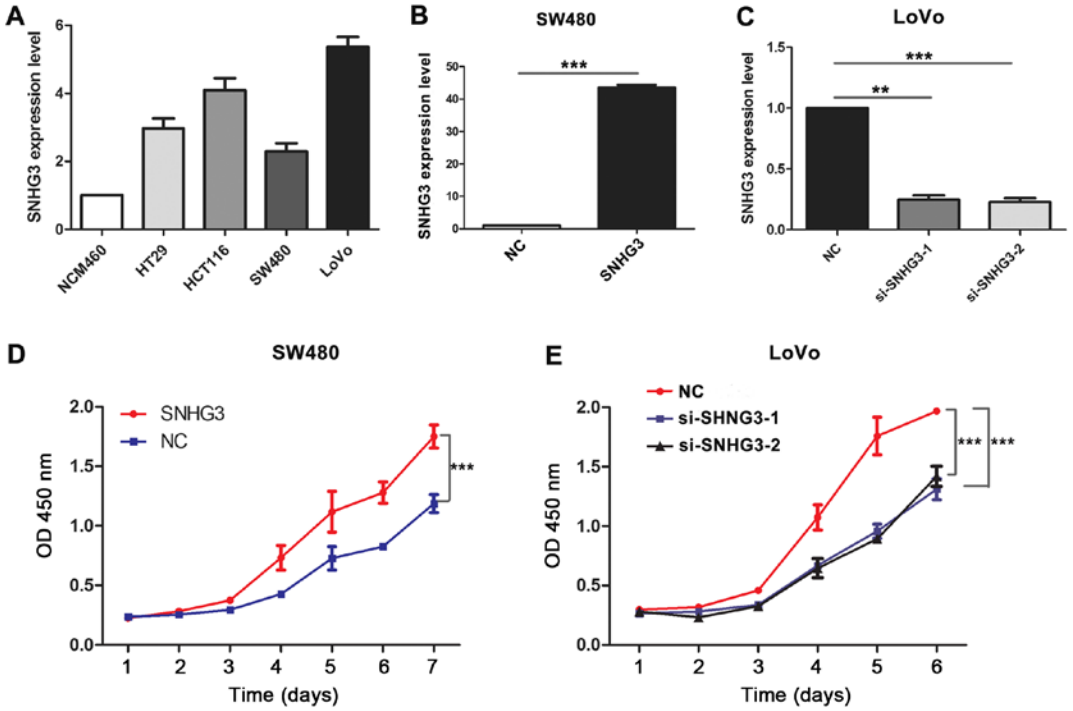

Figure 2. SNHG3 overexpression promotes CRC cell proliferation. (A) Analysis of the expression of SNHG3 in the normal colonic epithelial cell line (NCM460) and CRC cell lines (HT29, HCT116, SW480, LoVo) using qRT-PCR. (B) Detection of the expression level of SNHG3 in SW480 cells infected with lentiviral vectors harboring SNHG3 determined by qRT-PCR (***P<0.001). (C) The knockdown efficiency of SNHG3 was determined using qRT-PCR in LoVo cells (***P<0.001, **P<0.01). (D) CCK-8 assays indicated that overexpression of SNHG3 promoted the cellular proliferation ability of SW480 cells (***P<0.001). (E) LoVo cell growth was suppressed after silencing of SNHG3 (***P<0.001). SNHG3, small nucleolar RNA host gene 3; CRC, colorectal cancer; CCCK-8, Cell Counting Kit-8.

of SNHG3 between the CRC tumor tissues and non-tumor tissues was caused by the imbalance of the sample size, we reanalyzed the expression of SNHG3 in 40 paired CRC tumor tissues and adjacent non-tumor tissues. The results confirmed that SNHG3 was markedly upregulated in CRC (Fig. 1B, P<0.001). Notably, Kaplan-Meier analysis and log-rank test demonstrated that patients with overexpression of SNHG3 had poorer overall survival time than those with low expression of SNHG3 (Fig. 1C, P=0.033).

SNHG3 promotes CRC cell proliferation. To explore the biological functions of SNHG3 in CRC, we first analyzed the expression of SNHG3 in CRC cell lines using qRT-PCR. The results revealed that the expression of SNHG3 was significantly upregulated in the CRC cell lines compared with the normal colonic epithelial cells (NCM460, Fig. 2A). Moreover, the expression level of SNHG3 was the highest in the CRC cell line with the more malignant phenotype (LoVo), whereas the expression of SNHG3 was the lowest in the SW480 cells. These results revealed that SNHG3 may be associated with the progression of CRC. Lentiviral vectors harboring SNHG3 were introduced into SW480 cells, whereas the endogenous SNHG3 was knocked down using siRNAs in LoVo cells. As shown in Fig. 2B, the expression of SNHG3 was markedly increased in SW480 cells transfected with lentiviral vectors harboring SNHG3 (P<0.0001). Meanwhile, siRNAs specifically targeting SNHG3 decreased the expression of SNHG3 in LoVo cells (Fig. 2C, P<0.001). CCK-8 assays revealed that overexpression of SNHG3 visibly increased the proliferation abilities of SW480 cells (Fig. 2D, P<0.001). Consistently, silencing of SNHG3 in LoVo cells significantly impaired the cellular proliferation abilities of the LoVo cells (Fig. 2E, P<0.001).

Overexpression of SNHG3 correlates with c-Myc and its target genes. To investigate the biological pathway and progression involved in CRC pathogenesis through SNHG3, GSEA analysis was performed on the CRC tumor samples in TCGA database. An FDR <25% and a nominal p-value <0.05 were considered as significant. GSEA analysis indicated that high expression of SNHG3 was associated with c-Myc and its target genes including cell cycle-related genes like CCNB1, CCND2, CDK4, and E2F1 (Fig. 3A), which suggested that SNHG3 may regulate CRC cell proliferation through the c-Myc pathway. To find out whether SNHG3 regulated c-Myc and its target genes, expression of c-Myc and its target genes were evaluated after overexpression or silencing of SNHG3 in SW480 and LoVo cells, respectively. Notably, upregulation of SNHG3 significantly increased the expression of c-Myc and its target genes related to the cell cycle including CCNB1, CCND2, CDK4 and E2F1 (Fig. 3B). Consistently, knockdown of SNHG3 obviously decreased the expression of c-Myc and its target genes (Fig. 3C). We further explored the correlation between SNHG3 and the mRNA levels of c-Myc and its target genes in 456 CRC tumor tissues from TCGA dataset. Positive correlations between SNHG3 and c-Myc, CCNB1, CCND2, CDK4, and E2F1 were found in CRC tumor tissues (Fig. 3D).

c-Myc is the target of miR-182-5p. It has been reported that the activities of lncRNAs depend on their subcellular

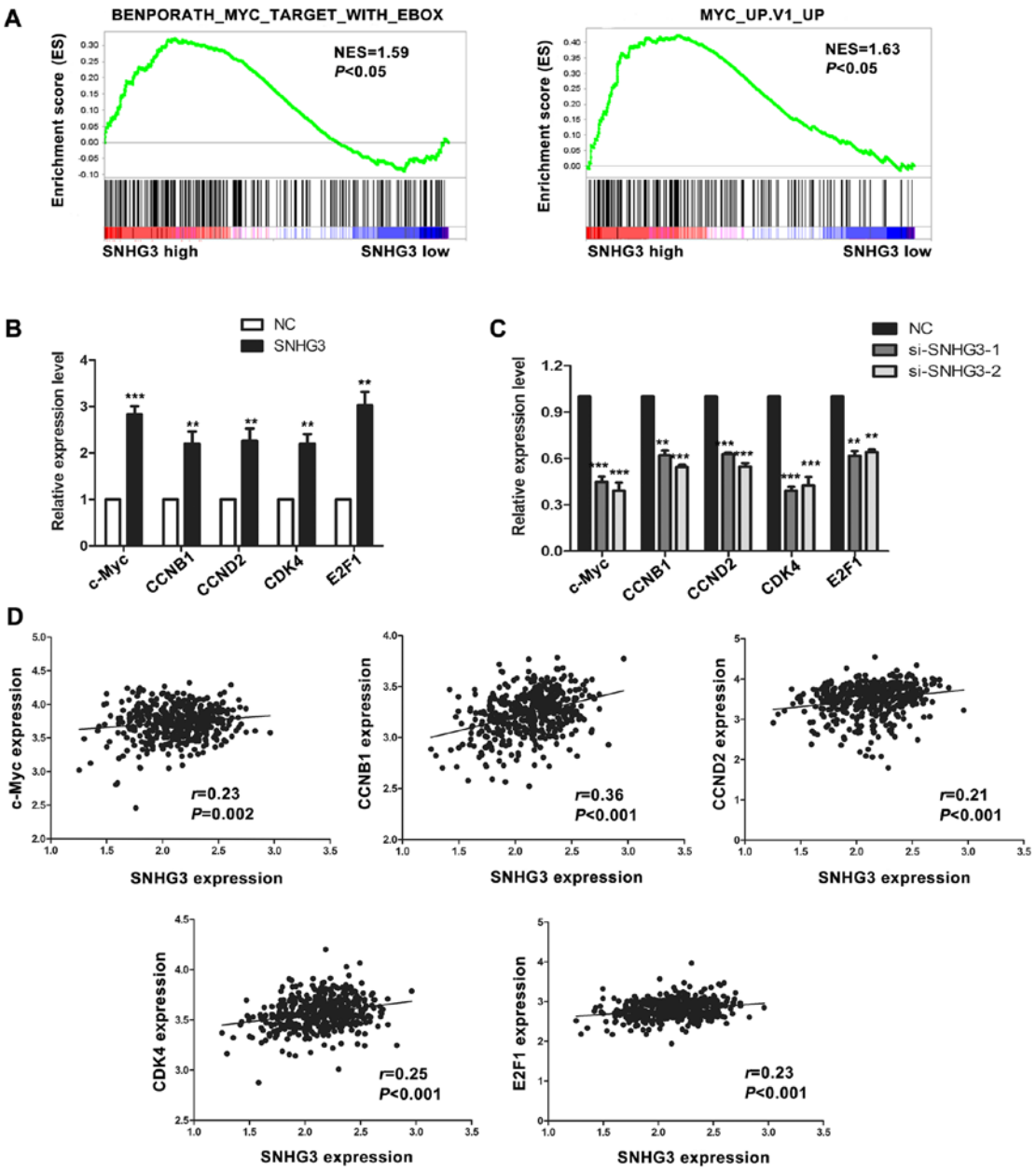

Figure 3. SNHG3 is correlated with c-Myc and its target genes. (A) GSEA plot indicated a significant correlation between high expression of SNHG3 and c-Myc as well as its target genes. (B) Overexpression of SNHG3 increased the mRNA expression level of c-Myc and its target genes related to the cell cycle (CCNB1, CCND2, CDK4, E2F1) in SW480 cells as detected by qRT-PCR (***P<0.001, **P<0.01). (C) Silencing of SNHG3 decreased the mRNA expression of c-Myc and its targets in LoVo cells by qRT-PCR (***P<0.001, **P<0.01). (D) Correlation between SNHG3 and c-Myc, CCNB1, CCND2, CDK4, and E2F1 in CRC samples from TCGA database. SNHG3, small nucleolar RNA host gene 3; CRC, colorectal cancer; GSEA, Gene Set Enrichment Analysis.

localization (22). Thus to gain further insight into the mechanism by which SNHG3 promoted the malignant phenotypes of CRC cells, subcellular distribution of SNHG3 was detected using cytoplasmic and nuclear RNA fractions from CRC cells. We found that similar to GAPDH, SNHG3 was mainly located in the cytoplasm (Fig. 4A). Growing evidence suggests that cytoplasmic distribution of lncRNAs may function through interaction with mRNAs and proteins (23). Recently, lncRNA has been reported to act as a ceRNA via sponging miRNA and releasing the target mRNA from specific miRNA (24). It was important to determine whether SNHG3 upregulated c-Myc in a ceRNA dependent manner, thus bioinformatic analysis was performed to investigate the potential miRNAs that can target both SNHG3 and c-Myc. Using starBase2.0, 20 potential miRNAs were predicted to target c-Myc, and 40 miRNAs were predicted to target SNHG3. After overlapping these 60 miRNAs, we found 4 potential miRNAs (miR-186-5p, miR-135b-5p, miR-135a-5p, miR-182-5p) which can target both SNHG3 and c-Myc. To further narrow the range, we overlapped these 4 miRNAs with 922 miRNAs downregulated in CRC from the GSE54632 database. Finally, miR-182-5p was the only candidate obtained through this process. MiR-182-5p was significantly downregulated in CRC (Fig. 4C, P<0.001). To further investigate whether c-Myc is the target of miR-182-5p, miR-182-5p mimics and miR-182-5p inbihitor were introduced into SW480 and LoVo cells, respectively (Fig. 4D). qRT-PCR and western blot assays revealed that the miR-182-5p mimic decreased c-Myc both in mRNA and protein

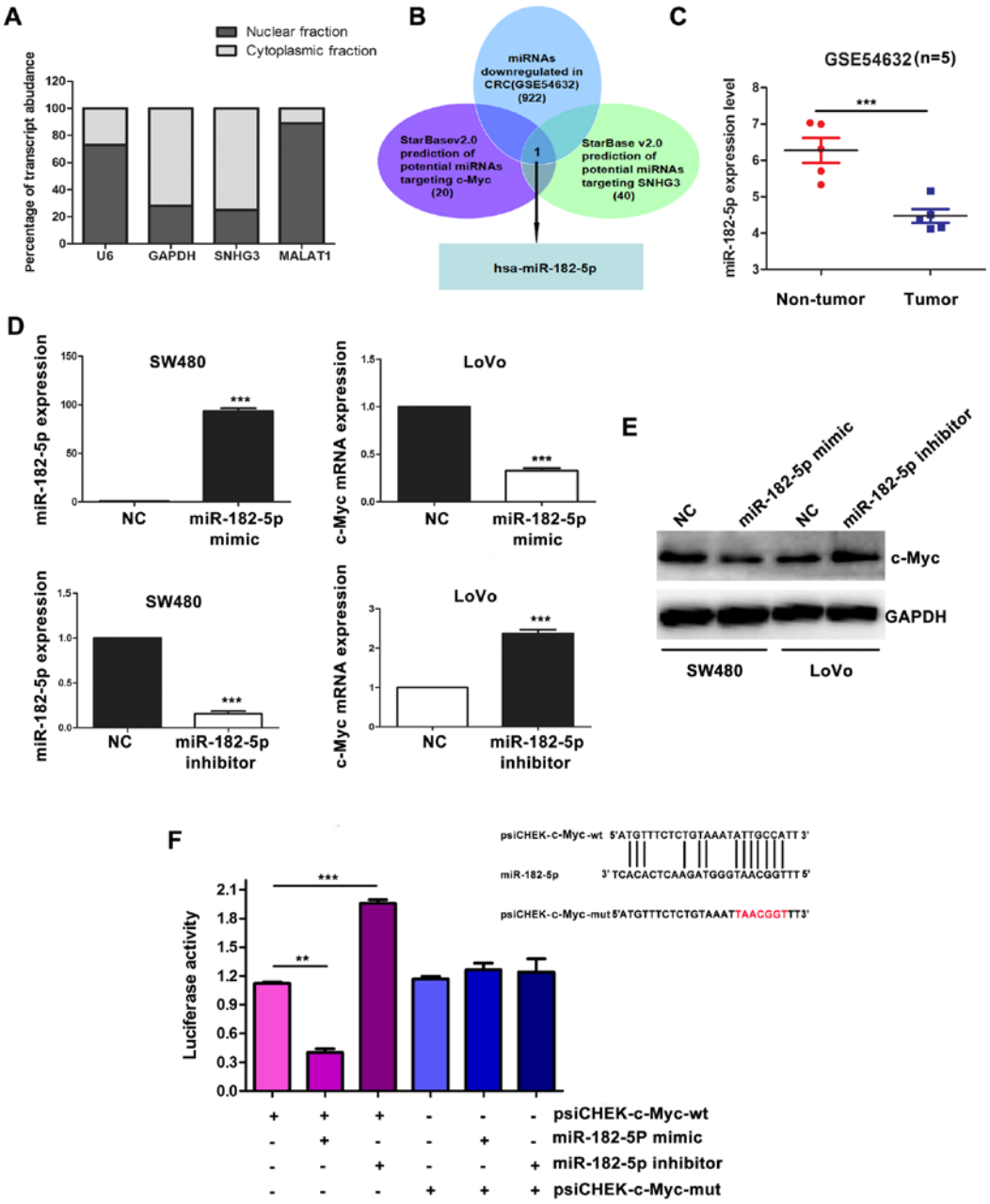

Figure 4. c-Myc is the target of miR-182-5p. (A) Cytoplasmic and nuclear RNA fractions were isolated from SW480 cells. SNHG3 was mainly located in the cytoplasm as detected by qRT-PCR. (B) A schematic diagram of the protocol used to search for candidate miRNAs targeting both SNHG3 and c-Myc. (C) Analysis of the expression level of miR-182-5p in CRC samples and paired non-tumor tissues in the GSE54632 dataset (n=5, ***P<0.001). (D) The overexpression or knockdown efficiency of miR-182-5p in the indicated cells detected by qRT-PCR (upper panel). Overexpression of miR-182-5p using miR-182-5p mimic decreased mRNA expression of c-Myc, while inhibition of miR-182-5p increased mRNA expression of c-Myc (**P<0.01). (E) Western bloting detected the expression level of c-Myc in the indicated cells with overexpression or inhibition of miR-182-5p. (F) Upper: diagram of the putative binding site of miR-182-5p on the 3'-UTR of c-Myc predicted by Starbase V2.0 and the mutant sequences of the binding site is shown in red. Lower: relative luciferase reporter activities with wild-type c-Myc 3'-UTR or mutant c-Myc 3'-UTR after transfection with the miR-182-5p mimic and inhibitor in SW480 cells (***P<0.001, **P<0.01). SNHG3, small nucleolar RNA host gene 3; CRC, colorectal cancer.

levels (Fig. 4D and E). Conversely, inhibition of miR-182-5p increased the expression of c-Myc (Fig. D and E). In addition, plasmids harboring wild-type c-Myc 3'-UTR (psiCHEK-c-Myc-wt) and mutant c-Myc 3'-UTR (psiCHEK-c-Myc-mut) were transiently introduced into SW480 cells. miR-182-5p mimic decreased the luciferase activity of the psiCHEK-c-Myc-wt luciferase reporter, while inhibition of miR-182-5p increased the psiCHEK-c-Myc-wt luciferase reporter activity. However, neither miR-182-5p mimic nor miR-182-5p inhibition had an effect on the psiCHEK-c-Myc-mut luciferase reporter activity. These results indicated c-Myc was the direct target of miR-182-5p.

SNHG3 functions as a sponge for miR-182-5p in CRC cells. Bioinformatics prediction suggested that both SNHG3 and c-Myc are targets of miR-182-5p. Furthermore, SNHG3 upregulated c-Myc expression level in CRC cells. Based on these results, we speculated that SNHG3 may function as a miR-182-5p sponge in CRC. To determine whether miR-182-5p recognized the predicted target site within SNHG3, luciferase

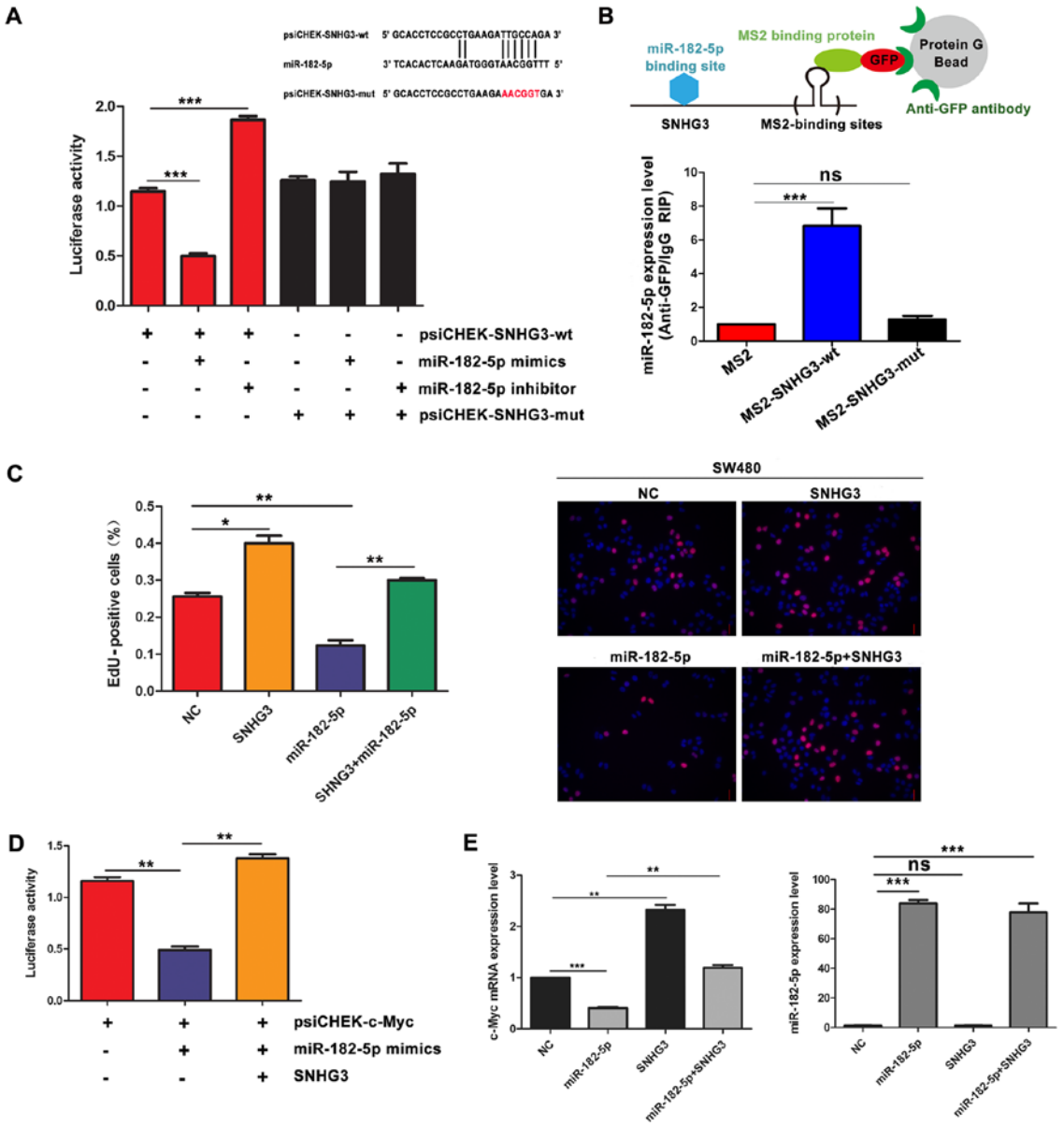

Figure 5. SNHG3 acts as a sponge for miR-182-5p. (A) Wild-type and mutant SNHG3 sequences were inserted into psiCHEK vectors and co-transfected with miR-182-5p mimic and miR-182-5p inhibitor in SW480 cells. Reporter activities were assessed using dual-luciferase assay (***P<0.001, **P<0.01). (B) MS2-RIP followed by qRT-PCR to detect miR-182-5p endogenously associated with SNHG3 in SW480 cells. (C) EdU assays indicated that overexpression of SNHG3 abolished the inhibition of proliferation induced by miR-182-5p (***P<0.001, **P<0.01, *P<0.05). (D) The 3'-UTR of c-Myc was inserted into psiCHEK vectors and co-transfected with miR-182-5p mimics and the SNHG3 plasmid into SW480 cells. Reporter activities were assessed using dual-luciferase assay (**P<0.01). (E) Analysis c-Myc and miR-182-5p expression level after transfection with miR-182-5p and SNHG3 alone or co-transfection with miR-182-5p and SNHG3 (***P<0.001, **P<0.01). SNHG3, small nucleolar RNA host gene 3.

vectors containing wild-type and mutant SNHG3 (the binding motif for miR-182-5p was mutated) was constructed. The dual-luciferase assays indicated that miR-182-5p significantly decreased the wild-type SNHG3 (psiCHEK-SNHG3-wt), but not the mutant SNHG3 (psiCHEK-SNHG3-mut) luciferase activities (Fig. 5A). Consistently, inhibition of miR-182-5p significantly increased the psiCHEK-SNHG3-wt luciferase reporter activity. However, miR-182-5p inhibitor did not have an effect on the psiCHEK-SNHG3-mut luciferase reporter activity (Fig. 5A). Moreover, MS2-RIP assay was performed to demonstrate the direct interaction between SNHG3 and miR-182-5p. The results revealed that wild-type SNHG3 (SNHG3-wt) rather than SNHG3 with the mutated miR-182-5p binding site (SNHG3-mut) could be immunoprecipitated with miR-182-5p, indicating that miR-182-5p can bind directly to SNHG3 through a miRNA recognition site (Fig. 5B). Notably, EdU assays were performed to determine whether SNHG3 abolished the function of miR-182-5p on CRC cell proliferation. As shown in Fig. 5C, overexpression of SNHG3 prevented the inhibition of proliferation of miR-182-5p overexpression in SW480 cells. Subsequently, the psiCHEK-c-Myc plasmid was co-transfected with the SNHG3 plasmid and miR-182-5p mimic to conduct another dual-luciferase assay. Overexpression of SNHG3 totally abolished the decrease of the luciferase activity induced by miR-182-5p overexpression (Fig. 5D). Furthermore, overexpression of SNHG3 completely abrogated the decrease of c-Myc expression caused by miR-182-5p overexpression without altering the expression

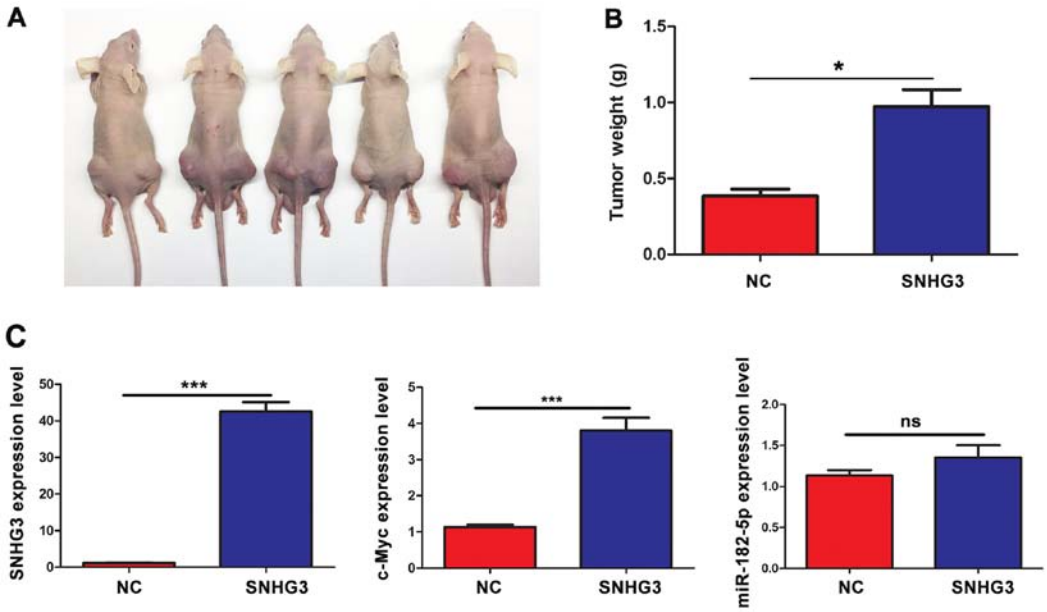

Figure 6. SNHG3 promotes tumor growth *in vivo*. (A) Effect of SNHG3 on tumor growth *in vivo*. Images of tumor formation in nude mice (n=5) injected subcutaneously with SW480 cells overexpressing SNHG3 (right side) or vector (left side). (B) The tumor weight was detected in the indicated group (*P<0.01). (C) The expression of SNHG3, c-Myc and miR-182-5p was detected in the subcutaneously tumors in the indicated group (***P<0.001). SNHG3, small nucleolar RNA host gene 3.

of miR-182-5p (Fig. 5E). These results implied that SNHG3 sponged miR-182-5p and released c-Myc from miR-182-5p.

SNHG3 promotes tumor growth in vivo. To further confirm whether SNHG3 promoted tumor growth and the expression level of c-Myc *in vivo*, SW480 cells with SNHG3 overexpressing or control vector were injected subcutaneously into the bilateral flanks of nude mice (n=5). As shown in Fig. 6A and B, the tumor burden and tumor weight in cells overexpressing SNHG3 were significantly increased than those of the control cells. Furthermore, SNHG3 markedly increased the expression of c-Myc without altering the expression of miR-182-5p *in vivo* (Fig. 6C).

Discussion

The results of the present study revealed that SNHG3 was significantly upregulated in CRC tissues and its high expression was closely associated with the poor overall survival of CRC patients. Functional investigation demonstrated that SNHG3 strongly promoted cellular proliferation ability. Mechanistically, SNHG3 functioned as a ceRNA to sponge miR-182-5p and decrease the binding of miR-182-5p to c-Myc, thereby contributing to the increase of c-Myc.

Recently, Tay *et al* proposed a new regulatory mechanism in which most RNA transcripts harbored miRNA-binding sites capable of communicating and regulating each other's expression level by competing for binding to shared miRNAs. These RNA transcripts acted as ceRNAs (25). It's worthwhile to systematically investigate whether lncRNAs function as ceRNA for the following reasons: firstly, a large amount of human RNA transcripts are lncRNAs; secondly, lncRNAs frequently express and function in more disease-specific, stage-specific, and tissue-specific manners; thirdly, more and more lncRNAs are found to be located in the cytoplasm and involved in post-transcriptional gene regulation. Additionally, growing evidence has proven that lncRNAs communicate with miRNAs through a ceRNA crosstalk. For example, lncRNA Unigene56159 directly bound to miR-140-5p and acted as a ceRNA to regain the expression of Slug, a target gene of miR-140-5p (26). lncRNA MALAT1 functioned as a ceRNA to upregulate the expression of ANKXA2 and KRAS, and to drive gallbladder cancer development (27). Increasing evidence suggests that a new post-transcriptional regulation model has emerged in which lncRNA transcripts control miRNA availability.

Based on our study, we suggested a ceRNA regulation model including SNHG3, miR-182-5p, and c-Myc in CRC. In our study, we demonstrated that SNHG3, sharing a miR-182-5p response element with c-Myc, regulated the expression of c-Myc in both mRNA and protein levels. c-Myc is a basic-helix-loop-helix-leucine zipper protein which regulates abundant genes involved in the cell cycle, protein synthesis, cell migration and adhesion, inflammation, and DNA damage (28-30). GSEA analysis determined a significant correlation between high expression of SNHG3 and c-Myc gene signatures. Ectopic expression of SNHG3 increased the expression of c-Myc and its targets including CCNB1, CCND2, CDK4, and E2F1, whereas knockdown of SNHG3 had opposite effect on c-Myc. Notably, by examining the expression data from TCGA database, we found that SNHG3 was positively correlated with c-Myc, CCNB1, CCND2, CDK4, and E2F1. Dual-luciferase reporter assays confirmed that miR-182-5p bound to both SNHG3 and c-Myc, notably, SNHG3 overexpression decreased the interaction between miR-182-5p and c-Myc. These results indicated that SNHG3 and c-Myc shared the same miRNA-responsive element with miR-182-5p and promoted CRC progression in a ceRNA manner.

In conclusion, high expression of SNHG3 predicted poor prognosis for patients with CRC. SNHG3 acted as a ceRNA

to upregulate c-Myc by sponging miR-182-5p. Hence, SNHG3 may serve as a novel prognostic approach or therapeutic target for CRC.

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