

Original Article

lncRNA-SNHG17 promotes colon adenocarcinoma progression and serves as a sponge for miR-375 to regulate CBX3 expression

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Abstract: Long non-coding RNA (lncRNA) has been reported could regulate initiation and progression of colon adenocarcinoma (COAD) tumorigenesis in recent years. Small nucleolar RNA host gene 17 (SNHG17) was found play crucial roles in cancer progression but its role in COAD remains unclear. In this work, qRT-PCR was performed to detect SNHG17 expression level in COAD cell lines. Roles of SNHG17 on COAD cell behaviors were analyzed with gain and loss-of-function experiments. Luciferase activity assay, RNA pull-down assay, and RNA immunoprecipitation assay were performed to analyze the association of SNHG17 or chromobox 3 (CBX3) with microRNA-375 (miR-375). Effects of SNHG17 on miR-375/CBX3 axis were analyzed by rescue experiments. We showed SNHG17 was upregulated expression in COAD tissues and cells. Functionally, SNHG17 could promote COAD cell proliferation, colony formation, migration, and invasion *in vitro*. Further investigations showed SNHG17 serves as competing endogenous RNA (ceRNA) for miR-375 to regulate CBX3 expression. Additionally, we showed the roles of SNHG17 on COAD cell behaviors were exerted via miR-375/CBX3 axis. In conclusion, we demonstrated a novel SNHG17/miR-375/CBX3 triplets that participates in COAD progression, which may provide promising therapeutic targets for COAD.

Keywords: SNHG17, miR-375, CBX3, Colon adenocarcinoma

Introduction

Colon adenocarcinoma (COAD) is the major type of colorectal cancer and can be efficiently controlled if diagnosed at early stages [1, 2]. However, treatment measures are very limited for patients diagnosed at late stages. Hence, it is meaningful to distinguish COAD patients from healthy people at early time.

Roles of non-coding RNAs (ncRNAs) in regulating tumorigenesis have received increasingly attentions [3]. Long ncRNAs are a type of ncRNAs and characterized as nucleotides length above 200 [4]. Small nucleolar RNA host gene 17 (SNHG17) was firstly identified increased expression in colorectal cancer and closely associated with advanced tumor stage and larger tumor size [5]. SNHG17 was also revealed could stimulate tumor growth by downregulating P57 via interacting with enhancer of

zeste homolog 2 [5]. In gastric cancer, SNHG17 was also found upregulated expression in tumor and functional assays showed SNHG17 could promote carcinogenesis by regulating p15 and p57 with enhancer of zeste homolog 2 as a bridge [6]. Besides that, SNHG17 was also found overexpressed in non-small cell lung cancer, and its knockdown could suppress cancer cell proliferation, migration, and promote apoptosis [7].

lncRNA is reported could regulate gene expression through serving as microRNA (miRNA) sponge, which is also called as competitive endogenous RNA (ceRNA) theory [8]. A bioinformatic analysis work based on RNA-sequencing data showed miR-153-3p was a bridge for SNHG17 to regulate Collagen type XI alpha 1/insulin-like growth factor binding protein-3/Krüppel-like factor 6 expression in COAD [9]. miR-375 was found could inhibit esophageal

squamous cell carcinoma cell growth via targeting specificity protein 1 [10]. Moreover, miR-375 was found could be regulated by circular RNA hsa_circ_0008035 and lncRNA ROR1 antisense RNA 1 in cancers and [11, 12]. In addition, miR-375 was revealed decreased expression in colorectal cancer and could be regulated by lncRNA to affect cancer progression [13, 14]. Based on these date, we hypothesized that SNHG17 may also function as a ceRNA for miR-375 to exert its roles in COAD. Besides that, several targets for miR-375 including specificity protein 1 and Wnt5a have been identified in cancers [15, 16]. Hence, we also tried to explore the potential targets of miR-375 in COAD.

The purpose of this work was to analyze the biological roles and associated mechanisms of SNHG17 in COAD. In addition, the detailed mechanisms related to the roles of SNHG17 in COAD were explored.

Materials and methods

Cell culture

Normal colon epithelial cell line (NCM460) and COAD cells (HT29 and T84) were obtained from ATCC were incubated at DMEM in supplement with 10% fetal bovine serum (Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin and 100 mg/ml streptomycin in a moist incubator at 37°C with 5% CO₂ and 95% air.

Tissues collection

COAD tissues and adjacent normal tissues collected from 45 COAD patients who received treatment at Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin, Tianjin's Clinical Research Center for Cancer were used to detect gene expression in this work. These patients did not receive any anti-cancer treatment before enrollment. All these tissues were frozen in liquid nitrogen and stored at -80°C for further usage. Study protocol was approved by the ethic committee of our hospital. Informed written consent was obtained from all patients. Experiments were conducted in accordance with Declaration of Helsinki.

Cell transfection

SNHG17 sequence was inserted into pcDNA3.1 (pSNHG17, Invitrogen). miR-375 mimic (5'-UU-

UGUUCGUUCGGCUCGCGUGA-3') and negative control (NC-miR, 5'-GCGUGCUUCCGAUUGUUCUGUG-3') were obtained at RiboBio (Guangzhou, China). Small interfering RNA against SNHG17 (si-SNHG17, 5'-GAUUGUCAGCUGACCUUCGUCCUGU-3') or chromobox 3 (CBX3, si-CBX3, 5'-GCGUUUCUUAACUCUCAGAAA-3') and negative controls (NC-siR, 5'-UUCUCCGAACGUUCAGGU-3') were purchased from GenePharm (Shanghai, China). Lipo 2000 (Invitrogen) was used for cell transfection in accordance with the manufacturer's instructions.

Real-time quantitative PCR (RT-qPCR) analysis

RNA in tissues and cells was isolated with Trizol reagent (Invitrogen) based on the manufacturer's instructions and treated with RT-PCR kit (Takara, Dalian, Liaoning, China) to obtain complementary DNA (cDNA). Then, cDNA was subjected to RT-qPCR analysis using SYBR Green (Takara) at ABI7500 system (Applied Biosystems, Foster City, CA, USA). Primers were synthesized by GenePharm and listed as follows: SNHG17: 5'-GTTCTGGGGCTTGGATGAT-3' (F), 5'-GATCTAAGGCTGAGACCCACG-3' (R); CBX3: 5'-TAGATCGACGTGAGTGAATGGG-3' (F), 5'-TGTCTGTGGCACCAATTATTCTT-3' (R); glyceraldehyde-3-phosphate dehydrogenase (GAPDH): 5'-GTCTCCTCTGACTTCAACAGCG-3' (F), 5'-ACCACCCCTGTTGCTGTAGCCAA-3' (R); miR-375-3p: 5'-ACACTCCAGCTGGTTGTCGGCTC-3' (F), 5'-CTCAACTGGTGTGAGTCGGCAATTCAAGTTGAGTCACGCGA-3' (R); U6 small nuclear RNA (U6 snRNA): 5'-CTCGCTTCGGCAGCACA-3' (F), 5'-AACGCTTCACGAATTGCGT-3' (R). RT-qPCR conditions were as follows: 95°C for 5 min, followed by 30 cycles at 95°C for 10 s and 60°C for 30 s. Relative gene expression level was calculated using 2-ΔΔCq method.

Western blot

Protein sample was extracted, separated using 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and transferred to polyvinyl difluoride membrane. The membrane was blocked by fat-free milk, and incubated with primary antibodies (anti-E-cadherin: ab15148, 1:1,000; anti-N-cadherin: ab20-2030, 1:1,000; anti-Vimentin: ab8978, 1:800; anti-GAPDH: ab181602, 1:1,000) overnight at 4°C. Then, membranes were incubated with horseradish peroxidase-conjugated secondary antibody (ab6721, 1:5,000) 1 h at room temperature. Band signal was visualized using

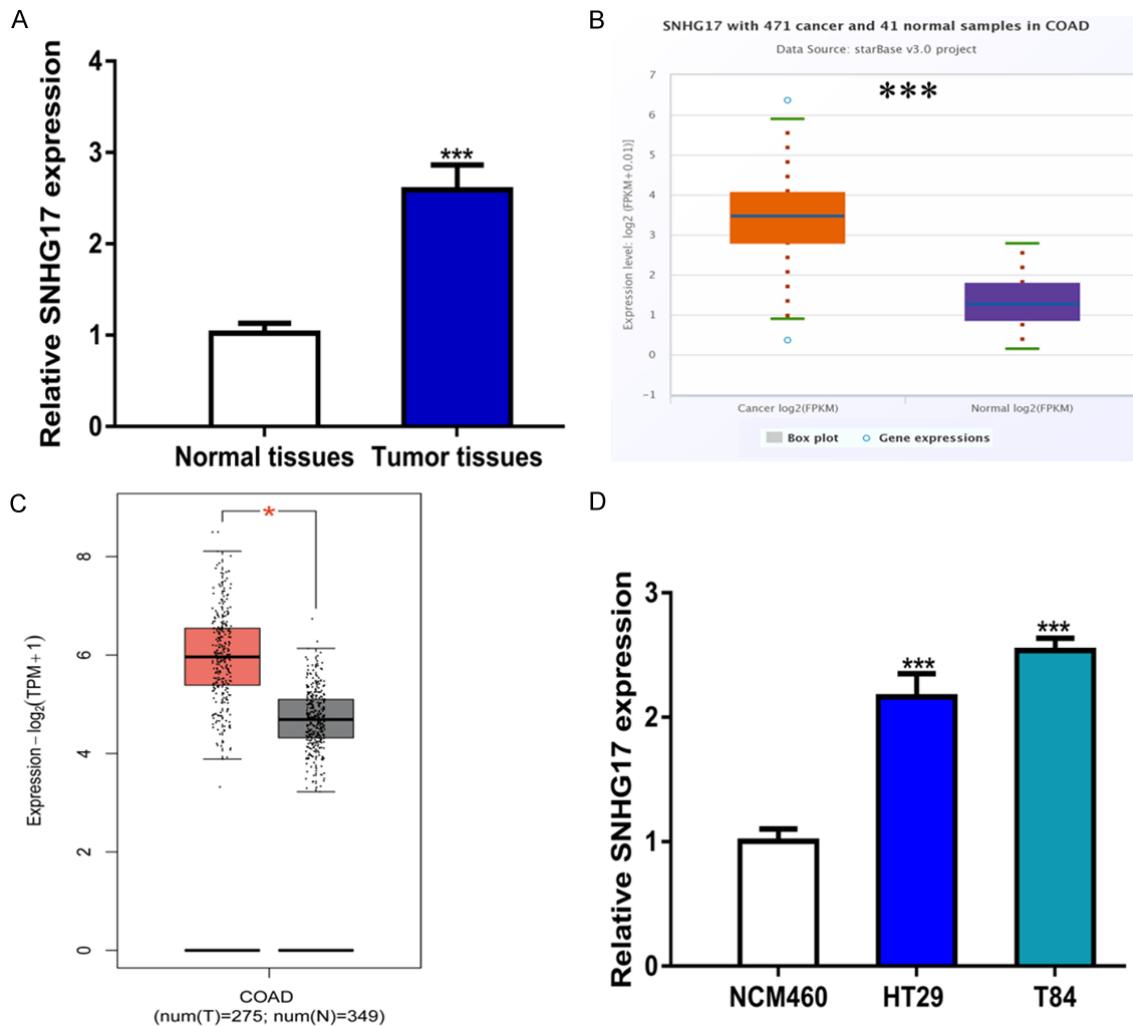


Figure 1. SNHG17 expression was elevated in COAD tissues and cell lines. A. SNHG17 expression was increased in COAD tissues compared with normal tissues as detected by RT-qPCR. B. SNHG17 expression was increased in COAD tissues compared with normal tissues as detected by ENCORI. C. SNHG17 expression was increased in COAD tissues compared with normal tissues as detected by GEPIA. D. SNHG17 expression was increased in COAD cells compared with normal cells as detected by RT-qPCR. ***P < 0.001, *P < 0.05. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: Real-time quantitative PCR.

BeyoECL (Beyotime) and analyzed with Bio-Rad software (Hercules, CA, USA).

Cell proliferation assay

Cells were seeded at the density of 2×10^4 cells/well in 96-well plate and incubated for 0, 1, 2, and 3 days. MTT solution (Beyotime, Haimen, Jiangsu, China) was added to each well and incubated for additional 4 h. Finally, DMSO was added to dissolve formazan crystal and then detect the optical density at 490 nm with microplate reader.

Colony formation assay

1,000 cells were seeded in 6-well plate and incubated for 2 weeks. Then, colonies were washed with PBS, fixed by methanol, and dyed by 0.05% crystal violet for 20 min. Colonies numbers were manually calculated under microscope.

Wound-healing assay

Cells were seeded in 6-well plate and allow to grow until 100% confluence. Wound was gener-

SNHG17-miR-375-CBX3 axis in COAD

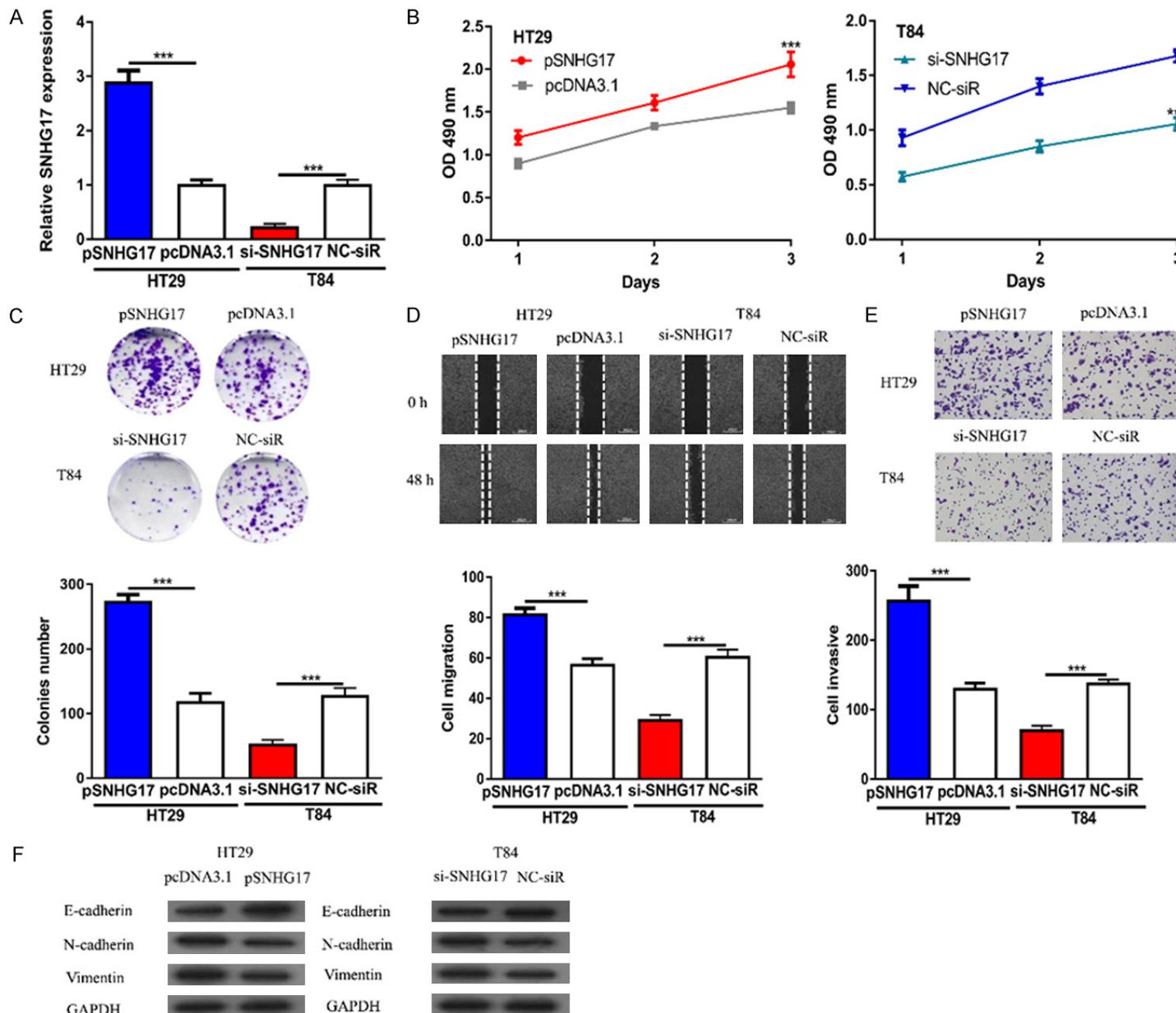


Figure 2. SNHG17 promotes COAD cell proliferation, colony formation, migration, and invasion. A. SNHG17 expression in COAD cells was increased by pSNHG17 and decreased by si-SNHG17 transfection. B. MTT assay showed cell proliferation in COAD cells was stimulated by pSNHG17 and suppressed by si-SNHG17 transfection. C. Colony formation assay showed colony formation ability in COAD cells was stimulated by pSNHG17 and suppressed by si-SNHG17 transfection. D. Wound-healing assay showed cell migration ability in COAD cells was stimulated by pSNHG17 and suppressed by si-SNHG17 transfection. E. Transwell invasion assay showed cell invasion ability in COAD cells was stimulated by pSNHG17 and suppressed by si-SNHG17 transfection. F. Western blot showed E-cadherin expression was increased and vimentin, N-cadherin expression was suppressed by pSNHG17, while the transfection of si-SNHG17 has opposite effects. ***P < 0.001. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: Real-time quantitative PCR; si-SNHG17: small interfering RNA targeting SNHG17; NC-siR: negative control siRNA.

ated at cell surface using a pipette tip. PBS was used to remove cell debris. Images were captured at 0 and 48 h after wounding creation and observed under microscope to measure wound area.

Transwell invasion assay

Cells in serum-free medium were placed into Matrigel (BD, Franklin Lakes, NJ, USA) coated upper chamber, while the 10% serum contained DMEM was added to the lower chamber. After incubation for 48 h, non-invasive cells were removed, while invasive cells were dyed by 1% crystal violet and manually calculated under microscope.

Tumor xenograft assay

Cells with sh-SNHG17 (5'-GAUUGUCAGCUGA-CCUCUGGUCCUGU-3') or sh-con (5'-UUCUCCG-UUCGUGUCACGUUUU-3') transfection were injected into mice. Tumor volume was measured every 4 days for a total of 7 times. After 4 weeks, these mice were sacrificed to remove tumor tissues and then weighted. Study protocol was approved by the ethic committee of Tianjin Medical University Cancer Institute and Hospital, National Clinical Research Center for Cancer, Key Laboratory of Cancer Prevention and Therapy, Tianjin, Tianjin's Clinical Research Center for Cancer. This work was performed followed for the welfare of the laboratory animals.

Dual luciferase reporter assay

Bioinformatic analyses tools showed both SNHG17 and CBX3 contained binding site for miR-375 (<http://starbase.sysu.edu.cn/index.php>). The wild-type (WT) sequence of SNHG17 (SNHG17-WT) or CBX3 (CBX3-WT) was cloned into pmirGLO to build luciferase vectors. Mutant luciferase constructs (SNHG17-MT or CBX3-

MT) were built using site-direct mutagenesis kit (Takara). Cells were co-transfected with luciferase vectors and miRNAs using Lipo 2000. After 48 h of transfection, Dual-luciferase reporter system (Promega, Madison, WI, USA) was used to measure relative luciferase activity.

RNA pull-down assay

Pierce RNA Desthiobiotinylation Kit (Thermo Fisher Scientific, Waltham, MA, USA) was used to label biotin into lncRNA sequences (bio-SNHG17, bio-NC) and miRNAs (bio-miR-375-WT, bio-miR-375-MT, bio-NC). Then, cells were co-transfected using Lipo 2000 for 48 h and then lysed to obtain lysate. Magnetic beads were incubated with cell lysate. RNA was extracted with Trizol and then subjected to RT-qPCR assay.

Bioinformatic analyses of gene expression and correlation

Expression levels of SNHG17 and CBX3 in COAD tissues and normal tissues were analyzed at ENCORI (<http://starbase.sysu.edu.cn/index.php>) and GEPIA (<http://gepia2.cancer-pku.cn/#index>). Expression level of miR-375 in COAD tissues and normal tissues was analyzed at ENCORI. Correlation of SNHG17 or CBX3 with miR-375 was analyzed at ENCORI. Correlation of SNHG17 and CBX3 was analyzed at GEPIA. Moreover, the protein expression of CBX3 in tumor tissues and normal tissues detected by immunohistochemistry assay was analyzed at Human Protein Atlas (<https://www.proteinatlas.org>).

Statistical analysis

Data obtained from three independent experiments were analyzed at SPSS 22.0 (IBM, Armonk, NY, USA). Results were presented as

SNHG17-miR-375-CBX3 axis in COAD

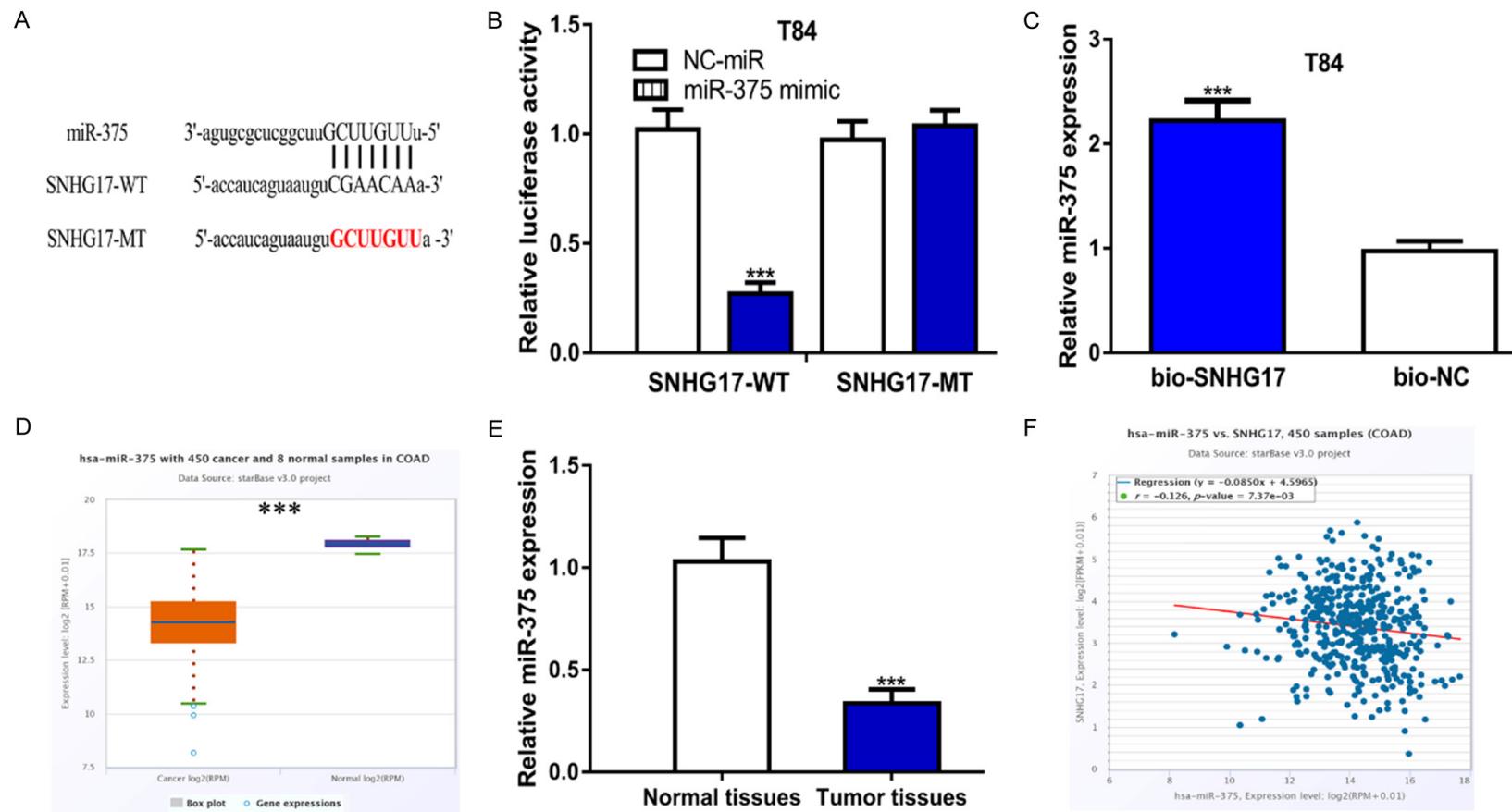
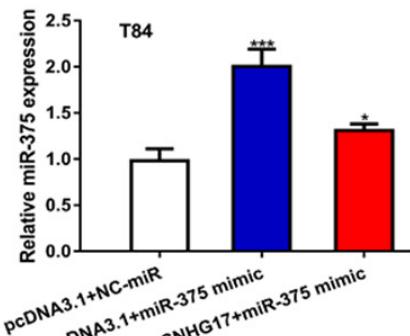


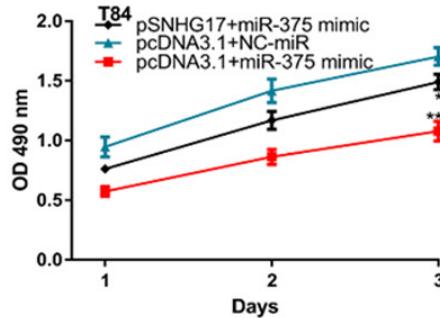
Figure 3. SNHG17 directly interacts with miR-375. A. Binding region between SNHG17 and miR-375. B. Luciferase activity indicated miR-375 mimic reduced luciferase activity of cells with SNHG17-WT, but not SNHG17-MT transfection. C. miR-375 expression was enriched in the complex pulled down by bio-SNHG17. D. miR-375 expression was increased in COAD tissues compared with normal tissues as detected by ENCORI. E. miR-375 expression was increased in COAD tissues compared with normal tissues as detected by RT-qPCR. F. Negatively correlation of SNHG17 and miR-375 as detected at ENCORI. ***P < 0.001. SNHG17: small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: real-time quantitative PCR; miR-375: microRNA-375; WT: wild type; MT: mutant.

SNHG17-miR-375-CBX3 axis in COAD

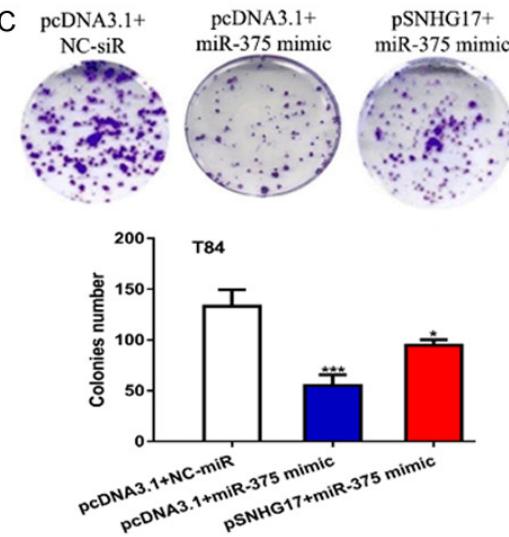
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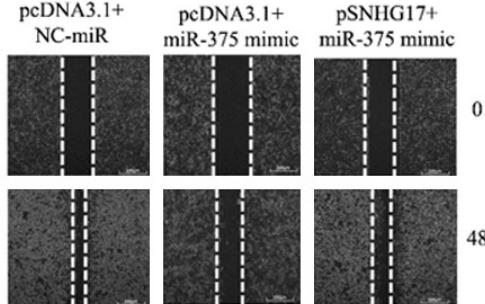
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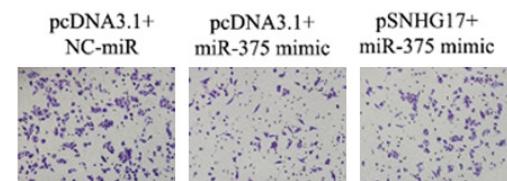
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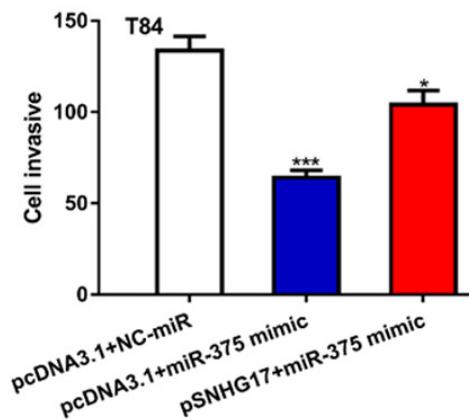
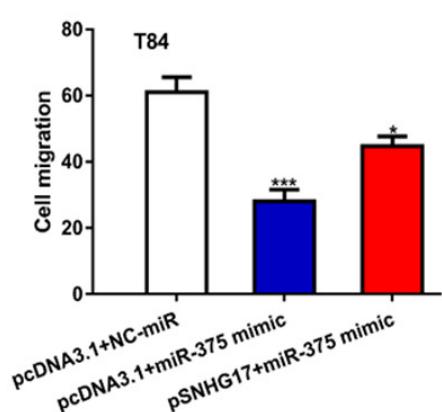
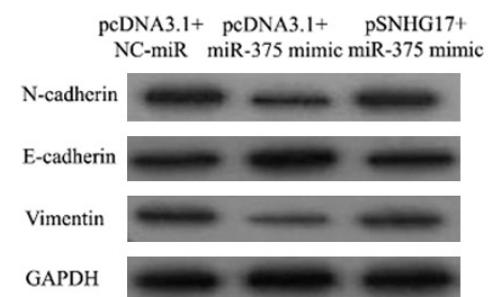


Figure 4. miR-375 mimics restore the effect of SNHG17 on COAD cells. A. miR-375 expression in COAD cells transfected with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic. B. MTT assay to detect cell proliferation in COAD cells with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic transfection. C. Colony formation assay to detect colony formation ability in COAD cells with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic transfection. D. Wound-healing assay to detect cell migration ability in COAD cells with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic transfection. E. Transwell invasion assay to detect cell invasion ability in COAD cells with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic transfection. F. N-cadherin, E-cadherin, and vimentin expression in COAD cells with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic transfection. ***P < 0.001, *P < 0.05. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: Real-time quantitative PCR; miR-375: microRNA-375; NC-miR: negative control miRNA.

mean \pm SD. Difference analyses were conducted using Student's t-test or one-way ANOVA and Tukey post-hoc test. P < 0.05 was considered to indicate significant difference.

Results

SNHG17 was increased expression in COAD

We firstly explored SNHG17 expression in COAD, and we showed SNHG17 was significantly upregulated expression in COAD tissues compared with normal tissues (**Figure 1A**). Results from ENCORI and GEPIA confirmed SNHG17 was increased expression in COAD tissues compared with normal tissues (**Figure 1B** and **1C**). Moreover, we analyzed SNHG17 expression level in COAD cells and normal cell. As displayed in **Figure 1D**, we showed SNHG17 expression level in COAD cells was significantly higher than that in normal cell.

SNHG17 regulates COAD cell proliferation, colony formation, migration, and invasion

To explore the roles of SNHG17 in COAD, we upregulated its expression in HT29, while downregulated its expression in T84. RT-qPCR analysis results validated the successful transfection of pSNHG17 or si-SNHG17 (**Figure 2A**). MTT assay showed SNHG17 overexpression markedly promotes, whereas SNHG17 knockdown inhibits cell viability (**Figure 2B**). Colony formation assay confirmed the results of MTT assay, which was SNHG17 could promote cell growth (**Figure 2C**). Wound-healing assay indicated SNHG17 overexpression promotes, while SNHG17 knockdown inhibits cell migration (**Figure 2D**). Furthermore, the results of transwell invasion assay suggested SNHG17 overexpression stimulates, while its knockdown inhibits cell invasion (**Figure 2E**). Western blot showed vimentin and N-cadherin expression was decreased, while E-cadherin was increased by

SNHG17 overexpression (**Figure 2F**). In addition, the knockdown of SNHG17 has opposite effects on the expression of EMT markers (**Figure 2F**).

SNHG17 directly interact with miR-375

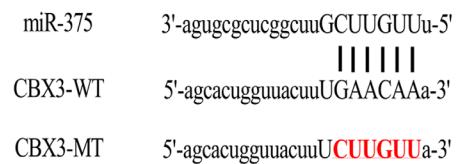
Bioinformatic analysis showed miR-375 could possibly interact with SNHG17 (**Figure 3A**). Luciferase activity assay showed miR-375 mimic can inhibit luciferase activity of SNHG17-WT but not SNHG17-MT (**Figure 3B**). Additionally, RNA pull-down assay showed miR-375 was significantly enriched in SNHG17 pulled down pellet (**Figure 3C**). Moreover, we explored miR-375 expression level in COAD tissues using RT-qPCR and ENCORI. We showed miR-375 expression level was significantly downregulated in COAD tissues compared with normal tissues (**Figure 3D** and **3E**). Furthermore, we showed SNHG17 and miR-375 was negatively correlated in COAD tissues (**Figure 3F**).

miR-375 mimic attenuated the effects of SNHG17 overexpression in COAD cells

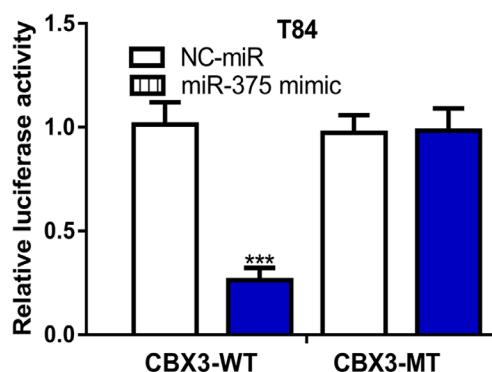
To explore whether the biological roles of SNHG17 were exerted via miR-375, miR-375 mimic was transfected into SNHG17 overexpressed HT29 cell. RT-qPCR showed miR-375 expression level was elevated by miR-375 mimic and decreased by pSNHG17 (**Figure 4A**). MTT assay and colony formation assay indicated co-introduction of miR-375 remarkably decrease cell proliferation compared with pSNHG17 group (**Figure 4B** and **4C**). Wound-healing assay and transwell invasion assay showed pSNHG17 partially reversed the effects of miR-375 mimic on cell migration and invasion (**Figure 4D** and **4E**). Western blot indicated miR-375 overexpression decreases E-cadherin expression and increases N-cadherin and vimentin expression (**Figure 4F**).

SNHG17-miR-375-CBX3 axis in COAD

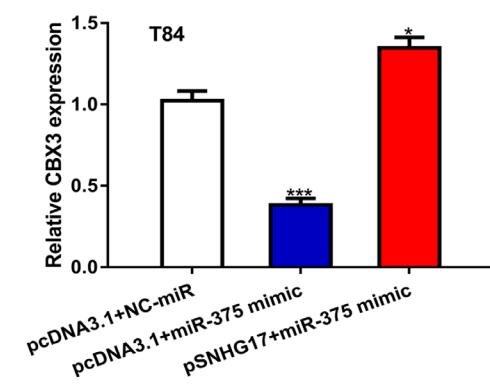
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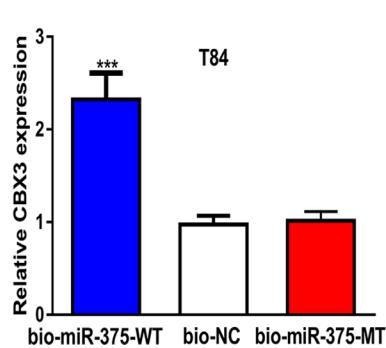
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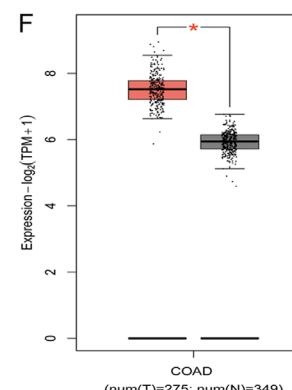
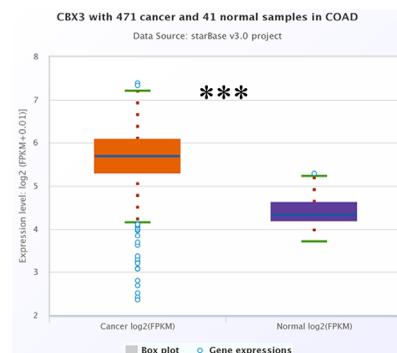
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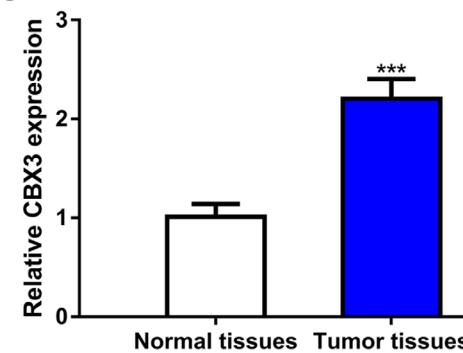
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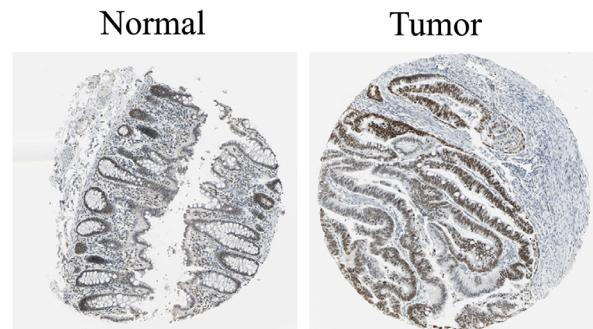
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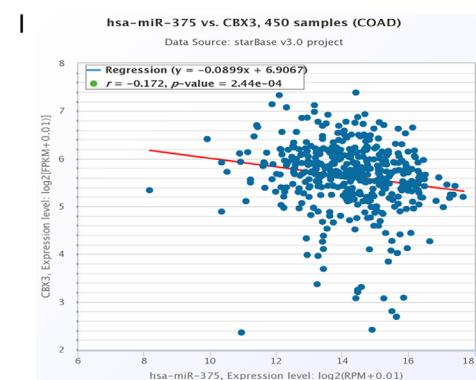
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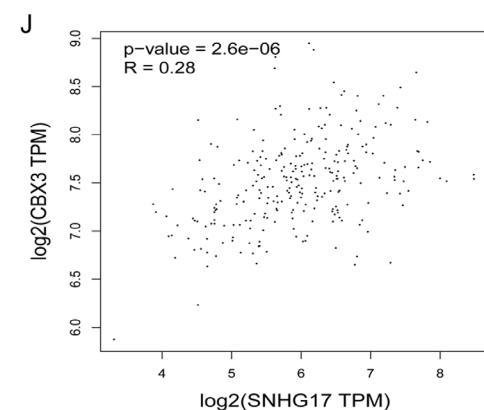


Figure 5. CBX3 was a target of miR-375. A. Binding region between CBX3 and miR-375. B. Luciferase activity indicated miR-375 mimic reduced luciferase activity of cells with SNHG17-WT, but not SNHG17-MT transfection. C. CBX3 expression in COAD cells transfected with pcDNA3.1+NC-miR, pcDNA3.1+miR-375 mimic, or pSNHG17+miR-375 mimic. D. CBX3 expression was enriched in the complex pulled down by bio-miR-375-WT, bio-miR-375-MT, or bio-NC. E. CBX3 expression was increased in COAD tissues compared with normal tissues as detected by ENCORI. F. CBX3 expression was increased in COAD tissues compared with normal tissues as detected by GEPPIA. G. CBX3 expression was increased in COAD tissues compared with normal tissues as detected by RT-qPCR. H. CBX3 protein expression was increased in COAD tissues compared with normal tissues as detected by IHC assay. I. Negatively correlation of CBX3 and miR-375 as detected at ENCORI. J. Positively correlation of CBX3 and SNHG17 as detected at GEPPIA. ***P < 0.001, *P < 0.05. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: Real-time quantitative PCR; miR-375: microRNA-375; WT: wild type; MT: mutant; CBX3: chromobox 3.

miR-375 directly interact with CBX3

Next, we explored the target of miR-375 and found CBX3 contains binding site for miR-375 (**Figure 5A**). Then, we found luciferase activity of cells transfected with CBX3-WT was significantly inhibited by miR-375 mimic (**Figure 5B**). Besides that, we showed CBX3 expression was decreased by miR-375 mimic and increased by pSNHG17 (**Figure 5C**). RNA pull-down assay further validated the interaction between miR-375 and CBX3 (**Figure 5D**). Moreover, we found CBX3 was significantly upregulated in COAD tissues compared with normal tissues (**Figure 5E-H**). At last, we found miR-375 and CBX3 was negatively correlated (**Figure 5I**), whereas SNHG17 and CBX3 was positively correlated in COAD tissues (**Figure 5J**).

Knockdown of CBX3 reversed the effects of SNHG17 overexpression in COAD cells

Furthermore, we speculated SNHG17/miR-375 may influence COAD progression via CBX3. As shown in **Figure 6A**, si-CBX3 decreased, while pSNHG17 increased CBX3 expression in HT29 cell. MTT and colony formation assay indicated CBX3 knockdown decreases cell growth and partially attenuated the effects of SNHG17 overexpression on cell growth (**Figure 6B** and **6C**). Wound-healing assay and transwell invasion assay showed a large reduction in cell migration and invasion ability when transfected with si-CBX3 (**Figure 6D** and **6E**). Moreover, we observed silenced CBX3 expression restores the stimulation effects of SNHG17 overexpression on cell migration and invasion (**Figure 6D** and **6E**). Western blot indicated CBX3 knockdown stimulates N-cadherin and vimentin expression and suppresses E-cadherin expression (**Figure 6F**).

SNHG17 regulates tumor growth in vivo

Finally, we detected the roles of SNHG17 on COAD tumor growth using animal model. As

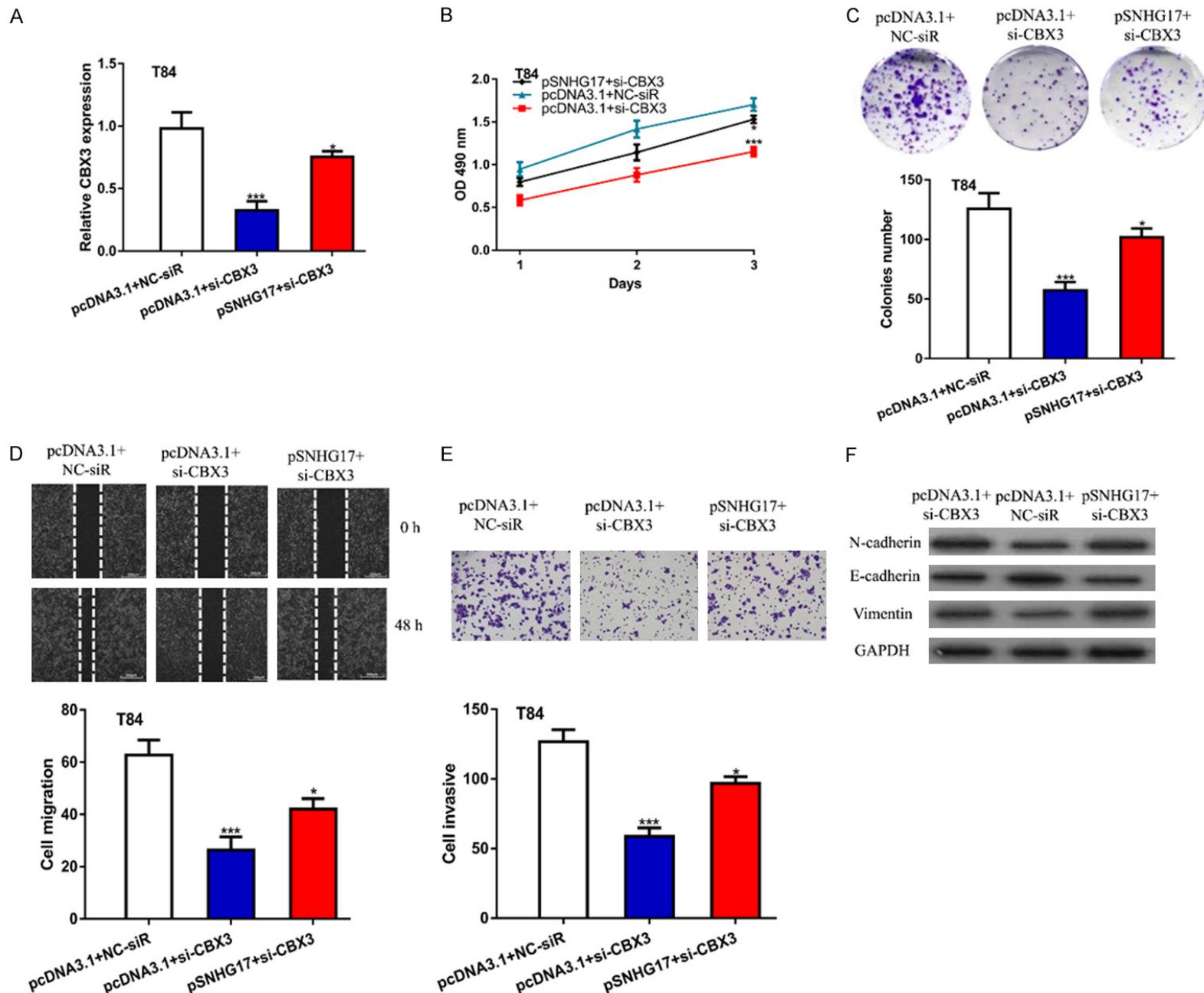
shown in **Figure 7A**, the sizes of tumor tissues resected from SNHG17 knockdown groups were lower than those from control groups. Consistently, tumor volume and tumor weight were also remarkably reduced by SNHG17 knockdown (**Figure 7B** and **7C**). Importantly, we observed decreased SNHG17 expression in the sh-SNHG17 groups compared with the control groups (**Figure 7D**).

Discussion

Multiplies lncRNAs have been revealed to play crucial roles in regulating COAD progression [17]. lncRNA FOXD3-AS1 was found upregulated in COAD and implied worse overall survival of cancer patients [14]. It was also found FOXD3-AS1 knockdown could inhibit cancer cell growth, migration, and invasion [18]. Moreover, MNX-AS1 was found functioned as a ceRNA for miR-218-5p to upregulate Sec61 translocon alpha 1 subunit expression and thus to influence COAD progression [19]. SNHG17 was located at chromosome 20 with the length of 1186 bp and has been reported to function as an oncogenic lncRNA in several cancers [5-7]. In this study, we showed SNHG17 was elevated expression in COAD tissues and cells. Gain and loss-of-function experiments indicated SNHG17 promotes COAD cell proliferation, colony formation, cell migration, and invasion *in vitro* as well as regulates tumor growth *in vivo*, indicating SNHG17 also functions as oncogenic role in COAD. As we analyzed the migration and invasion abilities of COAD cells after gene expression alteration, hence, we also detected the markers for Epithelial-Mesenchymal Transition (EMT). We revealed SNHG17 overexpression could stimulate EMT processes by upregulating the key markers E-Cadherin.

Although the numbers of newly identified lncRNAs were steadily increasing, the underlying

SNHG17-miR-375-CBX3 axis in COAD



SNHG17-miR-375-CBX3 axis in COAD

Figure 6. SNHG17 affects COAD behaviors through regulating CBX3. A. CBX3 expression in COAD cells transfected with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3. B. MTT assay to detect cell proliferation in COAD cells with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3 transfection. C. Colony formation assay to detect colony formation ability in COAD cells with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3 transfection. D. Wound-healing assay to detect cell migration ability in COAD cells with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3 transfection. E. Transwell invasion assay to detect cell invasion ability in COAD cells with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3 transfection. F. N-cadherin, E-cadherin, and vimentin expression in COAD cells with pcDNA3.1+NC-siR, pcDNA3.1+si-CBX3, or pSNHG17+si-CBX3 transfection. ***P < 0.001, *P < 0.05. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; RT-qPCR: Real-time quantitative PCR; CBX3: chromobox 3; si-CBX3: small interfering RNA targeting CBX3; NC-siR: negative control siRNA.

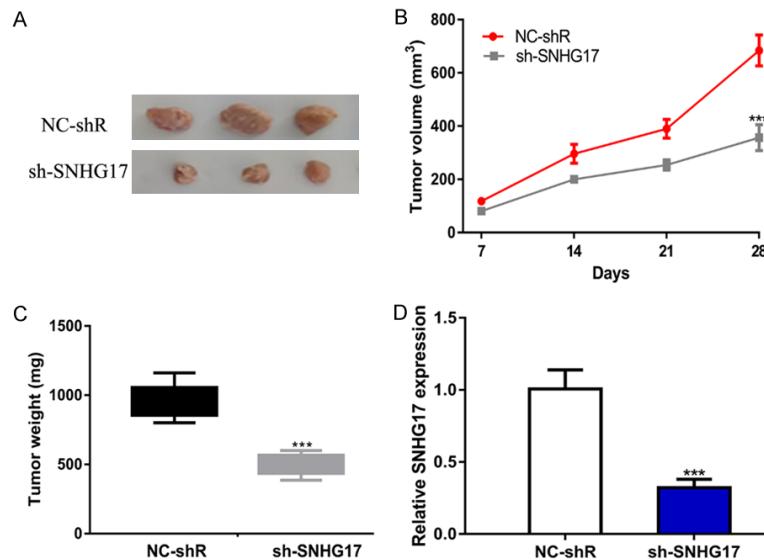


Figure 7. SNHG17 knockdown restrains tumor growth *in vivo*. A. Tumor images from COAD cells transfected with sh-SNHG17 or sh-NC. B. Tumor volume was decreased by sh-SNHG17. C. Tumor weight was decreased by sh-SNHG17. D. SNHG17 expression was decreased in tumors transfected with sh-SNHG17 compared with those with sh-NC. ***P < 0.001. SNHG17: Small nucleolar RNA host gene 17; COAD: colon adenocarcinoma; sh-SNHG17: small hairpin RNA targeting SNHG17; sh-NC: negative control shRNA.

mechanisms of lncRNAs in regulating cancer progression remain to be further explored. Previous studies have identified several miRNA targets for SNHG17 but no study employed ceRNA theory to understand the mechanisms of SNHG17. Here, we identified miR-375 was a potential target for SNHG17. This connection was further confirmed by luciferase activity assay and RNA pull-down assay. miR-375 was previously reported to function as a tumor suppressive miRNA in cancers [10-12]. In this study, we showed miR-375 overexpression could inhibit COAD cell proliferation, colony formation, migration and invasion. Furthermore, our study demonstrated miR-375 was involved in SNHG17 regulated COAD cell behaviors.

CBX3 belongs to heterochromatin protein families and found to be upregulated expression in

cancers. For example, CBX3 was increased expression in pancreatic cancer and regulated aerobic glycolysis via suppressing FBP1 [20]. In addition, a recent work demonstrated that high CBX3 expression was associated with malignant clinicopathological parameters and worse overall survival of hepatocellular carcinoma patients [21]. In our work, CBX3 was validated as a target for miR-375 by bioinformatic analysis, luciferase activity assay and RNA pull-down assay. Overexpression of CBX3 could promote the malignant behaviors of COAD cells. Hence, we made a bold hypothesis that SNHG17 may regulate COAD progression via miR-375/CBX3 axis. Rescue experiments were further performed to validate the SNHG17/miR-375/CBX3 triplets

in regulating COAD progression. There is still limitation in this work which is we did not further exploration the upstream mechanisms behind the upregulation status of SNHG17 in COAD.

Conclusions

To sum up, we demonstrated that SNHG17 promotes COAD progression via miR-375/CBX3 axis. The results in this work provided novel mechanisms regarding the roles of SNHG17 in regulating COAD progression, which might provide novel treatment markers for COAD.

Disclosure of conflict of interest

None.

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