




# LncRNA SNHG3 Facilitates the Malignant Phenotype of Cholangiocarcinoma Cells via the miR-3173–5p/ERG Axis

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## Abstract

**Background** Long noncoding RNA (lncRNA) small nucleolar RNA host gene 3 (SNHG3) is an oncogenic lncRNA that has been reported in many cancers, but the role of SNHG3 in cholangiocarcinoma (CCA) remains largely unknown. Bioinformatic analysis revealed a regulatory relationship among SNHG3, miR-3173–5p, and ERG. miR-3173–5p is a tumour suppressive miRNA, while ERG is an oncogene. In the present study, we focused on the regulatory effects and molecular mechanisms of SNHG3 in CCA.

**Method** The expression of SNHG3 and miR-3173–5p was evaluated using qRT–PCR analysis. Knockdown of SNHG3 was achieved by shRNA. Cell viability was assessed by MTT assay. Migration and invasion were determined by Transwell assay. Flow cytometry was used to assess cell apoptosis. Western blots were applied to quantify protein levels. Furthermore, using RNA pulldown and dual luciferase assays, the interactions between SNHG3 and miR-3173–5p and between miR-3173–5p and ERG in CCA cells were validated.

**Results** SNHG3 was significantly upregulated in CCA cells compared with normal human intrahepatic biliary epithelial cells. Knockdown of SNHG3 inhibited the proliferation and migration of CCA cells. Mechanistically, SNHG3-sponged miR-3173–5p, thus releasing the repression of ERG by miR-3173–5p. Rescue experiments showed that the miR-3173–5p/ERG axis mediated the oncogenic effect of SNHG3.

**Conclusion** Taken together, our data suggest that SNHG3 is a pleiotropic oncogenic lncRNA in CCA. Knockdown of SNHG3 expression suppressed malignant phenotypes in CCA cells via the miR-3173–5p/ERG axis.

**Keywords** SNHG3 · miR-3173–5p · ERG · Cholangiocarcinoma · Malignant phenotype

## Introduction

Cholangiocarcinoma (CCA) is a rare solid tumour among primary hepatobiliary malignancies.<sup>1</sup> CCAs can be classified into three subtypes: intrahepatic (iCCA), perihilar (pCCA), or distal (dCCA) according to their anatomical location. It has been reported that most cholangiocarcinomas arise de novo, and no risk factors have been identified to date, leading to a low 5-year survival rate.<sup>2</sup> Thus, improving the

understanding of molecular changes in the pathogenesis of CCA is of great importance.

Long noncoding RNAs (lncRNAs) are classified as a subset of noncoding RNA molecules > 200 nt in length with a regulatory effect on gene expression through a variety of mechanisms, including sponging miRNAs.<sup>3</sup> LncRNAs are widely involved in the regulation of cell proliferation, ageing, apoptosis, and other biological processes and are related to the malignant phenotypes of multiple tumours.<sup>4</sup> Small nucleolar RNA host gene 3 (SNHG3) is a recently reported lncRNA with oncogenic function in several cancers, including colorectal cancer, hepatocellular carcinoma, and gastric cancer.<sup>5–7</sup> In 2019, Li and colleagues revealed that lncRNA-SNHG3 was significantly increased in CCA tissues and could be an independent prognostic factor in intrahepatic CCA.<sup>8</sup> However, whether SNHG3 functions as a regulator in the progression of CCA remains utterly unknown.

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MicroRNAs (miRNAs) are a class of noncoding RNAs approximately 22 nt in length with abundant expression.<sup>9</sup> MiR-3173–5p, hosted by DICER, acts as a control point for DICER expression.<sup>9</sup> Previous studies have revealed that miR-3173–5p is downregulated and exhibits antitumour effects in B-cell acute lymphoblastic leukaemia.<sup>10</sup> However, there is no evidence concerning miR-3173–5p in CCA. Using online bioinformatics analysis, we predicted a potential binding site between SNHG3 and miR-3173–5p. Thus, we presumed that miR-3173–5p might be involved in SNHG3 regulation in CCA.

ETS-related genes (ERGs) are transcription factors from the E-26 transformation-specific (ETS) family. ERG plays multiple roles in development, including vasculogenesis, angiogenesis, haematopoiesis, and bone development. The oncogenic role of ERG has been validated in many cancers, such as prostate cancer,<sup>11</sup> cervical cancer,<sup>12</sup> and acute myeloid leukaemia.<sup>13</sup> However, no evidence has revealed the functional significance of ERG in CCA. Thus, it is of great importance to unveil the potential role of ERG in CCA. In the present study, we validated the functional roles of SNHG3 in regulating malignant phenotypes in CCA. The results revealed for the first time that knockdown of SNHG3 contributed to impairment of malignant phenotypes of CCA cells, which might be mediated through the miR-3173–5p/ERG axis.

## Materials and Methods

### Cell Culture

Normal human intrahepatic biliary epithelial cell HiBEC and CCA cell lines, HCCC9810 (Homo sapiens, intrahepatic cholangiocarcinoma, female, Japanese, CVCL\_4896), QBC939 (Homo sapiens, intrahepatic cholangiocarcinoma, female, Chinese, CVCL\_6942), RBE (Homo sapiens, intrahepatic cholangiocarcinoma, female, Chinese, CVCL\_6908), and HUCCT1 (Homo sapiens, intrahepatic cholangiocarcinoma, male, Japanese, CVCL\_0324), were obtained from the Cell Bank of Type Culture Collection (Chinese Academy of Sciences, Shanghai, China). HiBECs were grown in Dulbecco's modified essential medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% foetal bovine serum (FBS, Invitrogen, Carlsbad, CA, USA), 100 U/ml penicillin, and 100 µg/ml streptomycin. HCCC9810, QBC939, RBE, and HUCCT1 cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium (Invitrogen) supplemented with 10% foetal bovine serum, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cell culture system was a sterile humidified atmosphere in a 37 °C incubator containing 5% CO<sub>2</sub>.

### Quantitative Real-Time PCR (qRT–PCR)

Total RNA was isolated from frozen tissue or cultured cells using TRIzol reagent (Invitrogen). cDNA was synthesized from total RNA using the Takara reverse transcription system (Dalian, Liaoning, China). qRT–PCR was performed on an ABI-7500 thermocycler using iQTM SYBR® Green Supermix (Bio Rad, Hercules, CA, USA). The primer sequences used for qRT–PCR analysis are shown in Table 1. U6 was used as an internal control for miRNA. GAPDH was used as an internal control for other genes. The 2<sup>–ΔΔCt</sup> method was used to calculate the relative expression of the target gene compared to the internal control gene.<sup>14</sup>

### Construction of Plasmids

SNHG3 shRNA (sh-SNHG3#1, sh-SNHG3#2), SNHG3-overexpressing plasmid, miR-3173–5p mimic, miR-3173–5p inhibitor, ERG-overexpressing plasmid, and all respective controls were synthesized and purchased from GenePharma (Shanghai, China). For transfection, the indicated plasmids were transfected into RBE cells with Lipofectamine 3000 (Invitrogen) according to manufacturer's instructions. At 48 h after transfection, the cells were subjected to subsequent treatment and determinations.

### Cell Viability Assay

The viability of RBE cells was determined by the 3-(4,5-dimethylthiazole-2)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, 2 × 10<sup>3</sup> cells at the logarithmic growth stage were inoculated in 96-well plates and cultured for 0, 24, 48, or 72 h. Then, 20 µl of 5 mg/ml MTT solution was added to each well at end points, and the cells were cultured at 37 °C for another 3 h. The

**Table 1** Oligonucleotide primer sets for real-time PCR

| Name          | Sequence (5'–3')  |
|---------------|---|
| SNHG3 F       | AGACAGATTCGCGAGTGGTCG                                       |
| SNHG3 R       | TTGCTCCAAGTCTGCCAAAGA                                       |
| miR-3173–5p F | GCTGCCCTGCCCTGTTTTTC  |
| miR-3173–5p R | GTCGTATCCAGTGCAGGGTCCGAG<br>GTATTCGCACTGGATACGAC AAA<br>GGA |
| ERG F         | ACTACGATAAGCTCAGCCGC  |
| ERG R         | GGGAGGTCTGAGGGGTA   |
| U6 F          | GCTTCGGCAGCACATATACTAAAAT                                   |
| U6 R          | CGCTTCACGAATTTGCGTGTTCAT                                    |
| GAPDH F       | GCCAAAAGGGTCATCATCTC  |
| GAPDH R       | GGCCATCCACAGTCTTCT  |

cells were then placed in the dark with 100 ml of dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO, USA) for 2 h. Finally, absorbance at 490 nm was measured in each well using a miniature tablet reader (Bio Rad 550).

### Flow Cytometry

RBE cells were inoculated in a 6-cm culture plate at a density of  $2 \times 10^6$ . An Annexin V/propidium iodide (PI) apoptosis kit (Beyotime, Jiangsu, China) was used to determine apoptosis rates after transfection according to manufacturer's instructions. RBE cells were collected and washed twice in cold PBS. The washed cells were resuspended in Annexin V binding buffer. FITC binding Annexin V and PI were added to the buffer and cultured at 25 °C for 10 min. After staining, the cell mixture was loaded into a flow cytometer. The apoptosis rate was calculated as the sum of the upper right quadrant and lower right quadrant.

### Transwell Migration/Invasion Assay

Transwell inserts (8- $\mu$ m pore size, Corning, Lowell, MA, USA) coated with Matrigel (BD Biosciences, Bedford, MA, USA) were used to determine the invasion capability of cells. The same inserts without Matrigel were used to determine the migration capability of cells. Briefly,  $1 \times 10^5$  RBE cells in serum-free RPMI 1640 were seeded in the upper chamber, and 500  $\mu$ l of DMEM containing 10% FBS was added to the lower chamber. After 24 h, the nonmigrating or noninvasive cells on the upper side of the membrane were cleared with a wet cotton swab. Cells in the lower chamber were fixed with 95% methanol for 10 min and stained with 1% crystal violet for 5 min. Under an inverted microscope (100 $\times$ ), the cells were visualized and captured in 5 fields. The number of cells was calculated by ImageJ software.

### RNA Pull Down Assay

RBE cells ( $1 \times 10^6$ ) were transfected with biotin-miR-3173-5p (biotin-miR-3173-5p) or its mutant (biotin-miR-3173-5p MUT). Forty-eight hours after transfection, cells were collected and centrifuged. Next, the pellets were resuspended in lysis buffer containing protease inhibitor. The cell lysates and bovine serum albumin (Sigma) were incubated with Dynabeads M-280 Streptavidin (Invitrogen, USA) overnight at 4 °C for 4 h. After three washes with cold lysis buffer and one wash with high salt buffer, RNA in the bead complex was extracted with TRIzol, and the enrichment was detected by qRT-PCR.

### Western Immunoblots

Total proteins were extracted from RBE cells using RIPA buffer (Thermo Fisher Scientific). After 10% SDS-PAGE and transfer to polyvinylidene difluoride (PVDF) membranes, the following primary antibodies (Cell Signalling Technologies, Denver, Massachusetts, USA) were used for detection: ERG (1:1000, #97,249) and GAPDH (1:5000, #5174). Membranes were incubated with primary antibodies overnight at 4 °C. On the second day, after incubation with horseradish peroxidase-bound secondary antibodies (Beyotime, Jiangsu, China), signals were detected by an enhanced chemiluminescence system. Bands were quantitatively analysed by using ImageJ software.

### Dual Luciferase Assay

The putative binding sites between SNHG3 and miR-3173-5p, miR-3173-5p, and ERG were predicted with starBase v3.0. 3'UTR fragments containing wild-type or the mutated binding site of ERG to miR-3173-5p were amplified and inserted into the psi-CHECK luciferase reporter (Promega, Fitchburg, Wisconsin, USA). Similarly, wild-type SNHG3 and the target site mutated SNHG3 were cloned into the psi-CHECK luciferase reporter. To generate mutated binding sites, a QuikChange Mutagenesis kit (Stratagene, La Jolla, CA, USA) was used. Then, RBE cells were cotransfected with the indicated luciferase reporters and miR-3173-5p mimic or mimic NC using Lipofectamine 3000 (Invitrogen). Luciferase activities were detected by the Dual Luciferase Reporter Assay System (Promega, WI, USA) 48 h after cotransfection.

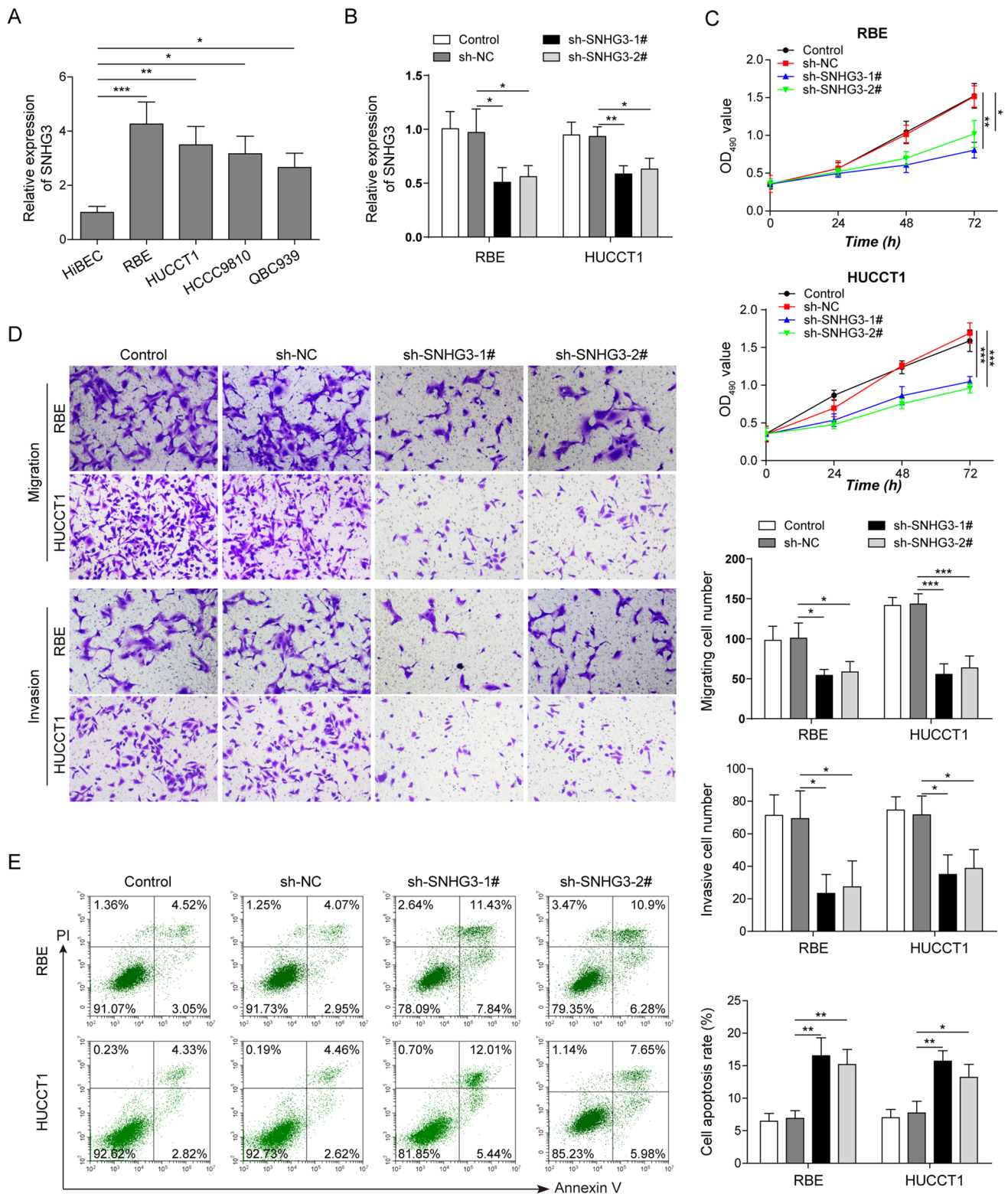
### Statistical Analysis

All data are presented as the mean  $\pm$  SD, and significance was analysed by GraphPad Prism 8.0 (San Diego, CA, USA) based on three independent experiments. Differences between two experimental groups were assessed by unpaired Student's *t* test. Differences between multiple experimental groups were assessed by one-way ANOVA followed by Tukey's post test. A *P* value < 0.05 was considered statistically significant.

## Results

### SNHG3 Was Upregulated in CCA Cell Lines, and Knockdown of SNHG3 Inhibited the Proliferation and Migration of CCA Cell Lines

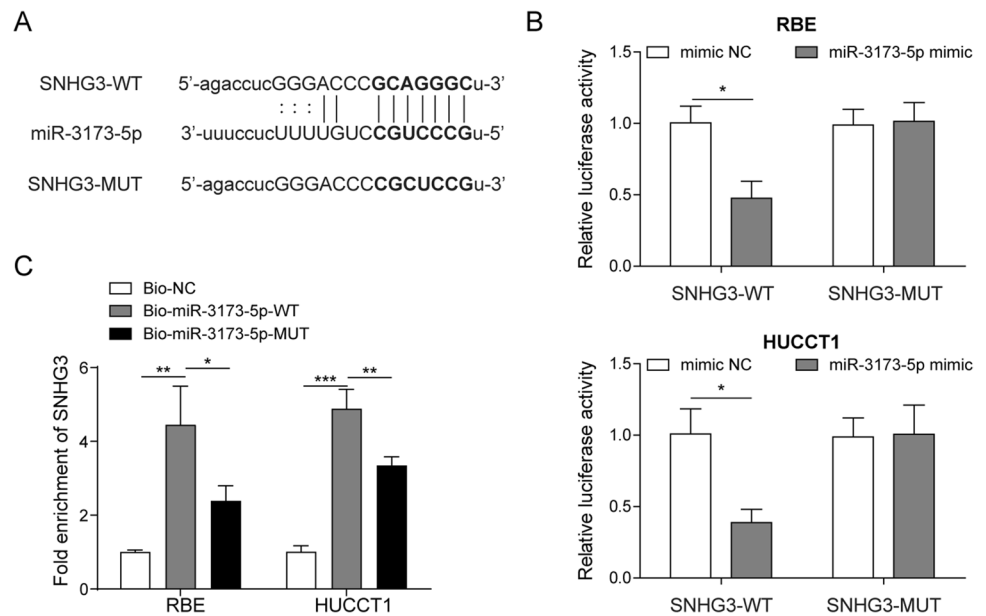
First, we evaluated the expression of SNHG3 in CCA cell lines. The human intrahepatic biliary epithelial cell



**Fig. 1** SNHG3 was upregulated in CCA cell lines, and knockdown of SNHG3 inhibited the proliferation and migration of CCA cell lines. **A** The expression of SNHG3 in different CCA cell lines and HiBECs was examined by qRT-PCR. **B** RBE cells and HUCCT1 cells were transfected with shRNA targeting SNHG3 (sh-SNHG3#1 and sh-SNHG3#2) or scramble shRNA (sh-NC). The expression of SNHG3

was examined by qRT-PCR. **C** RBE cell viability was evaluated by MTT assay. **D** Migration and invasion of RBE cells were determined by Transwell assay. **E** Apoptosis of RBE cells was examined by flow cytometry. *N* = 3, data were shown as the mean ± SD. A t test was used to determine the statistical significance. \**P* < 0.05, \*\**P* < 0.01, \*\*\**P* < 0.001

**Fig. 2** SNHG3-sponged miR-3173-5p. **A** Illustration of the predicted binding site between SNHG3 and miR-3173-5p by StarBase 3.0. **B** Direct binding was confirmed using a dual luciferase assay. **C** The binding was confirmed using RNA pull down.  $N=3$ , data were shown as the mean  $\pm$  SD. A *t* test was used to determine the statistical significance. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$



line HIBEC was used as a normal control. As shown in Fig. 1A, we found that SNHG3 was substantially upregulated in CCA cell lines compared with HIBECs. To investigate the regulatory work of SNHG3 in CCA, we applied shRNA to knock down SNHG3 in the RBE cell line and HUCCT1 cell line. The efficiency of shRNA was validated by qRT-PCR, as approximately 75% SNHG3 was diminished by shRNA#1 and shRNA#2 compared to sh-NC (Fig. 1B). The MTT assay showed that knock-down of SNHG3 significantly inhibited cell viability at 24, 48, and 72 h (Fig. 1C). Additionally, Transwell assays revealed that cell migration and invasion were impaired when SNHG3 was silenced by shRNA#1 and shRNA#2 (Fig. 1D). The results of flow cytometry by Annexin V/PI staining also suggested that SNHG3 knockdown shRNA#1 and shRNA#2 largely increased the proportion of apoptotic cells (Fig. 1E). Taken together, these results suggested that SNHG3 was essential for the maintenance of multiple malignant behaviours in the CCA cell line.

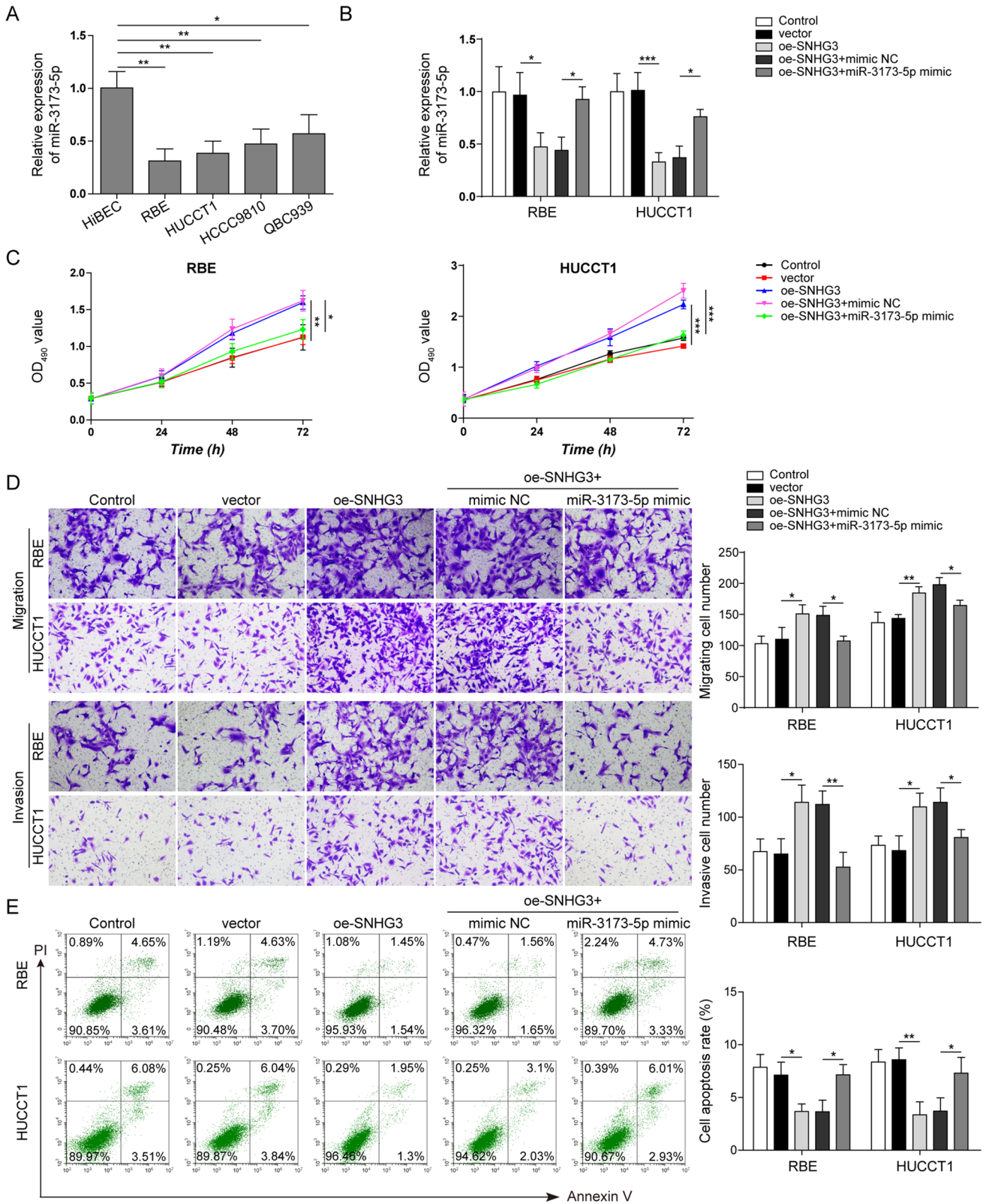
### SNHG3-Sponged miR-3173-5p

To understand the mechanical significance of SNHG3 in CCA, we used starBase 3.0 to predict candidate SNHG3-sponged miRNAs. The results revealed that SNHG3 had a binding site with miR-3173-5p (Fig. 2A). To ascertain the potential interaction, a luciferase assay was performed in RBE cells and HUCCT1 cells cotransfected with reporter containing wild-type or a mutated fragment of SNHG3 and miR-3173-5p mimic or mimic NC. The results showed that relative luciferase activity was substantially impaired by the

miR-3173-5p mimic in the wild-type group, whereas that in the mutated group remained unchanged (Fig. 2B). Further validation was conducted by RIP assay. As shown in Fig. 2C, biotin-labelled wild-type miR-3173-5p enriched SNHG3, whereas enrichment of SNHG3 in the mutated miR-3173-5p group was abolished (Fig. 3C). These above results indicated that SNHG3-sponged miR-3173-5p.

### SNHG3 Facilitated Malignant Phenotypes at least Partly by Sponging miR-3173-5p

Next, we further analysed the expression of miR-3173-5p in CCA cell lines. The results suggested that miR-3173-5p was dramatically downregulated in CCA cell lines (Fig. 3A). To address the significance of miR-3173-5p in SNHG3-regulated malignant phenotypes, we simultaneously overexpressed SNHG3 and miR-3173-5p in RBE cells and HUCCT1 cells. qRT-PCR validated that SNHG3 overexpression-inhibited miR-3173-5p was recovered by miR-3173-5p mimic transfection (Fig. 3B). MTT (Fig. 3C) and Transwell assays (Fig. 3D) indicated that overexpression of SNHG3 promoted the proliferation and migration/invasion of RBE cells and HUCCT1 cells, respectively. However, cotransfection of the miR-3173-5p mimic reversed this enhanced proliferative and migrative capability of RBE cells (Fig. 3C and Fig. 3D). Similarly, overexpression of SNHG3 slightly inhibited the basal apoptotic rate of RBE cells, while overexpression of miR-3173-5p compromised this effect (Fig. 3E). Collectively, these results suggested that miR-3173-5p was involved in the malignant phenotypes that SNHG3 facilitated in RBE cells.



**Fig. 3** SNHG3 facilitated malignant phenotypes at least partly by sponging miR-3173-5p. **A** The expression of miR-3173-5p in different CCA cell lines and HiBECs was examined by qRT-PCR. **B** to **G** RBE cells and HUCCT1 cells were cotransfected with SNHG3-overexpressing plasmid and miR-3173-5p mimic. **B** The expression of miR-3173-5p was examined by qRT-PCR. **C** RBE cell viability was evaluated by MTT assay. **D** Migration and invasion of RBE cells were determined by Transwell assay. **E** Apoptosis of RBE cells was examined by flow cytometry.  $N=3$ , data were shown as the mean  $\pm$  SD. A t test was used to determine the statistical significance. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$

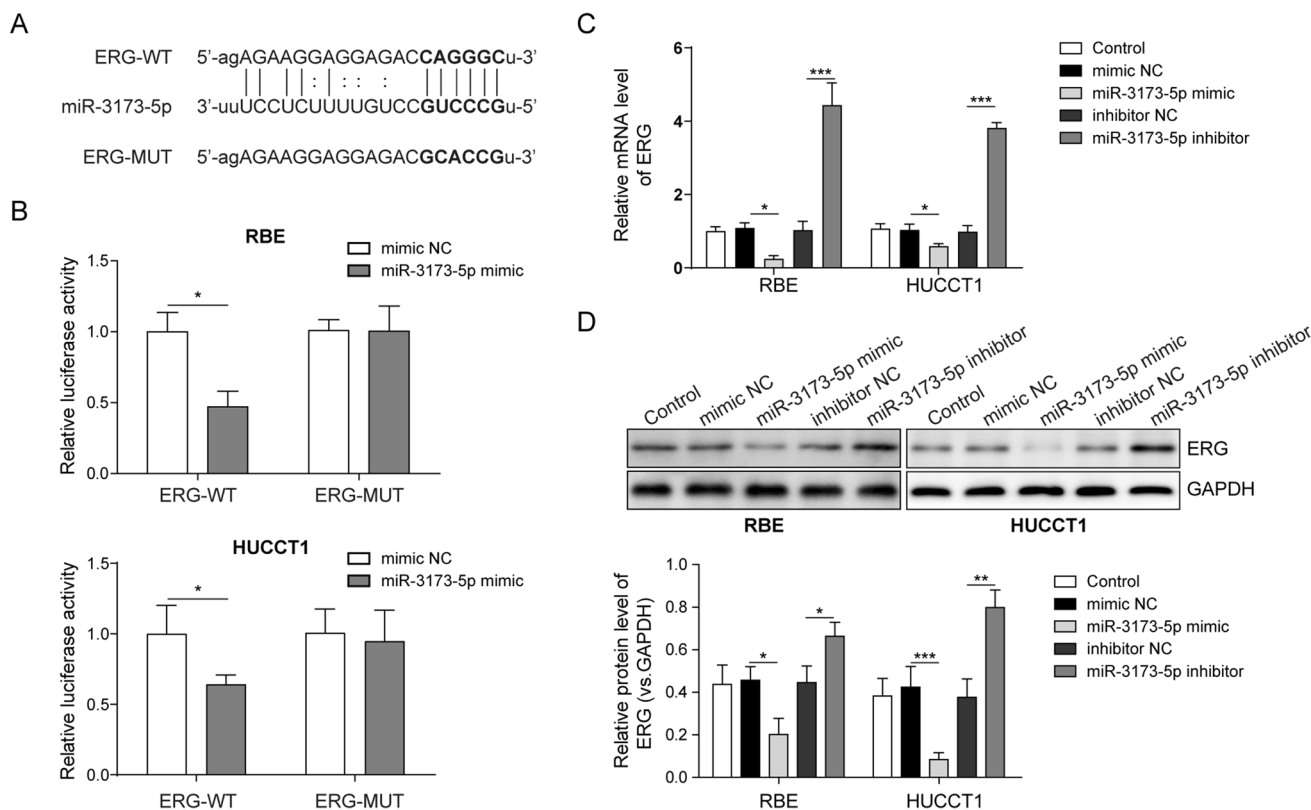
### miR-3173-5p Directly Bound and Decreased ERG

To further investigate the mechanism by which miR-3173-5p participates in the SNHG3-mediated malignant phenotype, starBase 3.0 was applied to predict the target of miR-3173-5p. We identified an oncogene named ETS-related gene (ERG) that has a binding site with miR-3173-5p in the 3' UTR (Fig. 4A). A luciferase assay validated that the relative activity was only repressed by miR-3173-5p in the reporter containing the wild-type binding site but remained unchanged in the reporter containing the mutated binding site in the 3' UTR of ERG (Fig. 4B). To determine whether miR-3173-5p decreased the expression of ERG

by direct binding, a miR-3173-5p mimic or miR-3173-5p inhibitor was transfected into RBE cells and HUCCT1 cells. qRT-PCR and western blots showed that the miR-3173-5p mimic suppressed ERG levels, whereas the miR-3173-5p inhibitor elevated ERG levels at both the mRNA and protein levels (Fig. 4C and Fig. 4D). Therefore, we confirmed that miR-3173-5p directly bound and negatively regulated the expression of ERG.

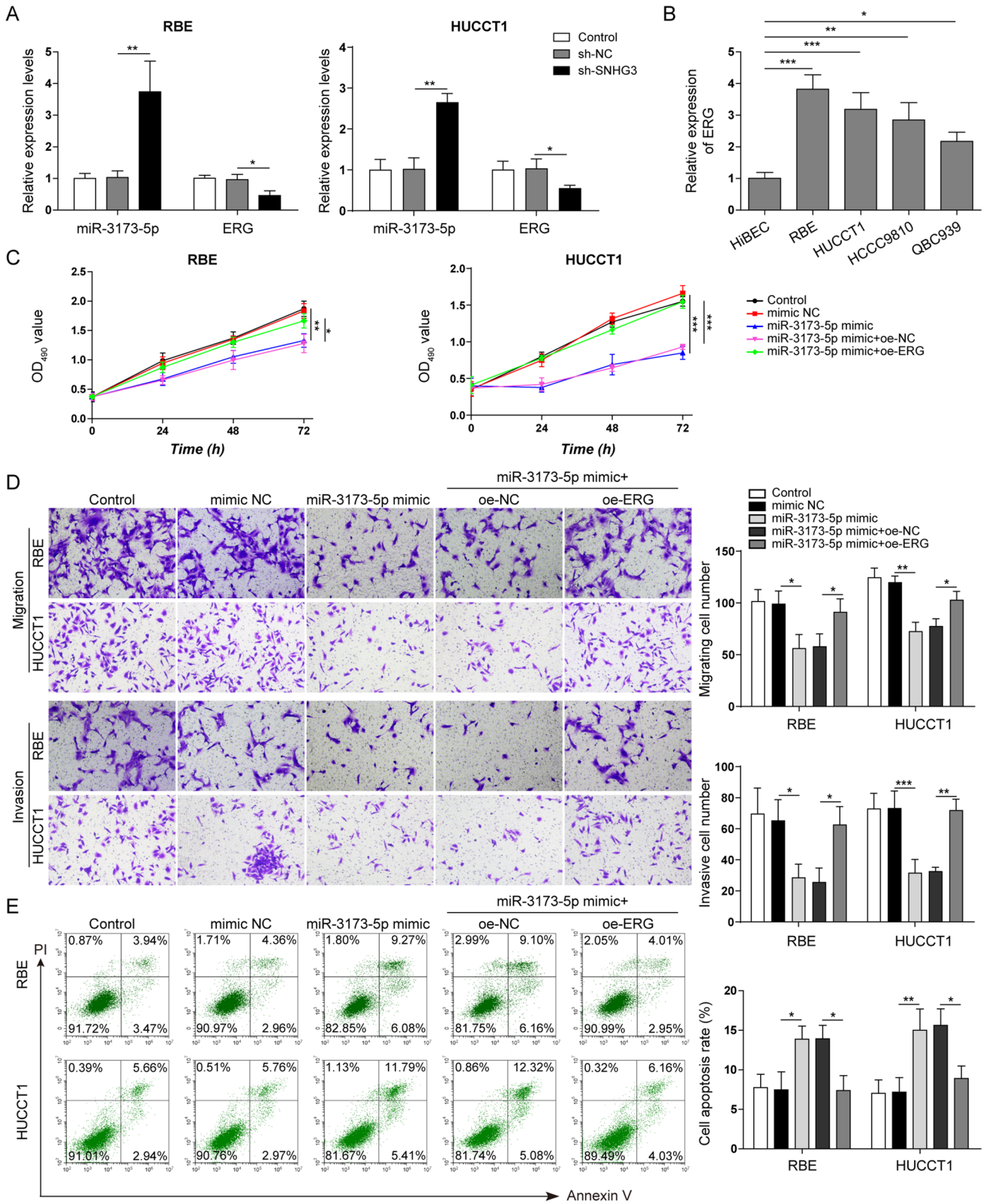
### miR-3173-5p Repressed the Malignant Phenotype at least Partly via ERG

Next, we further validated whether ERG participates in miR-3173-5p-repressed malignant phenotypes in the CCA cell line. qRT-PCR revealed that SNHG3 knockdown enhanced miR-3173-5p levels while suppressing ERG levels (Fig. 5A). Similar to SNHG3, ERG was substantially increased in CCA cell lines (Fig. 5B). To address the significance of ERG in miR-3173-5p-regulated malignant phenotypes, we simultaneously overexpressed miR-3173-5p and ERG in RBE cells and HUCCT1 cells. MTT (Fig. 5C) and Transwell assays (Fig. 5D) suggested that miR-3173-5p abrogated the viability and migrative capability of RBE cells



**Fig. 4** miR-3173-5p directly bound and decreased ERG. **A** Illustration of the predicted binding site between ERG and miR-3173-5p by StarBase 3.0. **B** Direct binding was confirmed using a dual luciferase assay. **C** The mRNA level of ERG was determined by qRT-PCR. **D**

The protein level of ERG was determined by western blots.  $N=3$ , data were shown as the mean  $\pm$  SD. A t test was used to determine the statistical significance. \* $P<0.05$ , \*\* $P<0.01$ , \*\*\* $P<0.001$





**Fig. 5** miR-3173–5p repressed malignant phenotypes at least partly via ERG. **A** The expression of ERG in sh-SNHG3-transfected cells was determined by qRT–PCR. **B** The expression of ERG in different CCA cell lines and HiBECs was examined by qRT–PCR. **C** to **G** RBE cells and HUCCT1 cells were cotransfected with ERG-overexpressing plasmid and miR-3173–5p mimic. **C** RBE cell viability was evaluated by MTT assay. **D** Migration and invasion of RBE cells were determined by Transwell assay. **E** Apoptosis of RBE cells was examined by flow cytometry.  $N=3$ , data were shown as the Mean  $\pm$  SD. A *t* test was used to determine the statistical significance. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$

and HUCCT1 cells, respectively. However, cotransfection of the ERG-overexpressing plasmid recovered this attenuated proliferative and migrative ability of RBE cells (Fig. 5C and Fig. 5D). Additionally, miR-3173–5p dramatically induced apoptosis in RBE cells and HUCCT1 cells, while this promotion was compromised by ERG overexpression (Fig. 5E). Collectively, these results suggested that miR-3173–5p was involved in SNHG3-facilitated malignant phenotypes in RBE cells and HUCCT1 cells.

## Discussion

Due to its aggressive biology, cholangiocarcinoma has a low 5-year survival rate.<sup>2</sup> Few lncRNAs have been identified as potential biomarkers and critical regulatory molecules in CCA. It was reported that oxidative stress induced lncRNA H19 and lncRNA HULC to promote cell migration and invasion in cholangiocarcinoma.<sup>15</sup> lncRNA ASAP1-IT1 was also validated to promote the progression of cholangiocarcinoma.<sup>16</sup> In the present study, we revealed for the first time an oncogenic role of SNHG3 in a CCA cell line. SNHG3 facilitated proliferation, migration, and invasion in CCA cells. Mechanistically, it was suggested that SNHG3 sponged miR-3173–5p; thus, derepressed miR-3173–5p inhibited ERG to achieve these oncogenic effects.

SNHG3 was defined as an oncogene in several cancer types.<sup>6</sup> In colorectal cancer, SNHG3 acts as an endogenous competitive RNA (ceRNA) to sponge miR-539, leading to enhanced tumour growth and metastasis.<sup>17</sup> In hepatocellular carcinoma, SNHG3 was suggested to sponge miR-128 to induce sorafenib resistance.<sup>18</sup> In terms of migration and invasion, lncRNA SNHG3wa regulates invasion and migration in osteosarcoma.<sup>19</sup> It was also reported that SNHG3 facilitated the migration of non-small-cell lung cancer cells by activating IL-6/JAK2/STAT3 signalling.<sup>20</sup> In the present work, we confirmed for the first time that SNHG3 exhibited an oncogenic effect by serving as a sponge of miR-3173–5p. However, we should also note that SNHG3 might exert regulatory activities via pathways other than ceRNA.

For instance, in gastric cancer, SNHG3 was demonstrated to regulate MED18 expression through EZH2-mediated methylation modulation.<sup>6</sup>

miR-3173–5p is a rarely studied miRNA with antitumour effects reported in B-cell acute lymphoblastic leukaemia and with protumour effects reported in ovarian cancer.<sup>9,10</sup> In the present study, we revealed that SNHG3 promoted malignant phenotypes by sponging miR-3173–5p and that miR-3173–5p was downregulated in CCA cell lines, suggesting an anti-oncogenic role of miR-3173–5p in CCA. In addition, our results revealed for the first time that miR-3173–5p overexpression was able to inhibit the proliferation and migration of CCA cells.

ERG is widely considered an oncogene in many cancers and is especially well studied in prostate cancer.<sup>21</sup> ERG was also reported to be an oncogene in other cancers, such as gastric cancer<sup>22</sup> and colorectal cancer.<sup>23</sup> Functionally, ERG was proven to facilitate the proliferation and migration of multiple cancers. A study revealed that ERG promoted prostate cancer cell proliferation and migration.<sup>24</sup> Another study validated that ERG enhanced osteosarcoma growth and metastasis.<sup>25</sup> However, no previous study investigated ERG in CCA. In the present study, our data suggested that ERG was upregulated in CCA cell lines and that overexpression of ERG reversed the antitumour effect of miR-3173–5p, which might be involved in SNHG3-mediated regulation. However, this research still has certain shortcomings. More proof at the clinical and in vivo levels should be presented, and the correlation between SNHG3 and glycolysis-related genes should be validated by gain and loss of function assays. In terms of miR-3173–5p, more functional studies should focus on the role of miR-3173–5p before further conclusions are drawn. Similarly, more investigation should be performed to validate the role of ERG in CCA and the mechanism by which ERG regulates the malignant phenotype of CCA.

## Conclusion

In summary, we identified lncRNA SNHG3 as a regulator of proliferation and migration in a CCA cell line. More importantly, our results suggested that the oncogenic activities of SNHG3 were achieved by targeting the miR-3173–5p/ERG axis. Therefore, understanding the mechanisms controlling SNHG3 expression during CCA development may provide novel insight into the progression of CCA.

**Abbreviations** CCA: Cholangiocarcinoma; lncRNA: Long non-coding RNAs; SNHG3: Small nucleolar RNA host gene 3; miRNAs: MicroRNAs; ERG: ETS-related gene; qRT-PCR: Quantitative real-time polymerase chain reaction; RIP assay: RNA immunoprecipitation; 3' UTR: 3' Untranslated region

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Data acquisition: Zeng-Peng Sun and Zhi-Guo Tan.  
Data analysis: Chuang Peng and Wei-Min Yi.  
Manuscript drafting: Wei-Min Yi.  
Manuscript revising: Chuang Peng.

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**Availability of Data and Material** All data generated or analysed during this study are included in this article. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

## Declarations

**Ethical Approval** Not applicable. This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for Publication** Not applicable. This article does not contain any studies with human participants performed by any of the authors.

**Conflict of Interest** The authors declare no competing interests.

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