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MiR-1-5p is down-regulated in gallbladder carcinoma and suppresses cell proliferation, migration and invasion by targeting Notch2

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ABSTRACT

Background: Numerous studies have demonstrated that aberrant microRNAs (miRNAs) are involved in tumorigenesis and tumor progression. Nevertheless, the precise role of miR-1-5p in gallbladder carcinoma cell growth and metastasis remains not fully revealed.

Material and methods: The levels of miR-1-5p were detected in gallbladder carcinoma tissues and cell lines using qRT-PCR method. A series of functional assays, including cell proliferation, colony formation, wound healing and Transwell invasion were conducted using miR-1-5p or miR-1-5p inhibitor transfected cells.

Results: MiR-1-5p was remarkably down-regulated in gallbladder carcinoma tissues and cell lines compared to normal. In addition, over-expression of miR-1-5p markedly suppressed the growth, migration and invasion of gallbladder carcinoma cell. Conversely, down-expression of miR-1-5p facilitated gallbladder carcinoma cell proliferation and aggressiveness. Mechanistic investigations demonstrated that neurogenic locus notch homolog protein 2 (Notch2) was the directly target of miR-1-5p and Notch2 mediated the inhibitory effect of miR-1-5p in gallbladder carcinoma cell growth and aggressiveness.

Conclusion: Our findings demonstrated that miR-1-5p acted as a suppressive miRNA and played vital roles in the growth, migration and invasion of gallbladder carcinoma cell through targeting Notch2.

1. Introduction

Gallbladder carcinoma still remains the most common and aggressive malignancy of bile duct, and the worldwide incidence of gallbladder carcinoma is increasing annually [1,2]. The prognosis of patients is poor, as the five-year survival rate of gallbladder carcinoma is lower than 5% [3,4]. Although the advances in the diagnosis and treatment of gallbladder carcinoma the clinical outcomes of patients have not significantly improved owing to the metastasis [5]. Currently, surgical resection is the only effective therapeutic strategy for gallbladder carcinoma, as therapeutic options capable of preventing gallbladder carcinoma metastasis are still lacking [6]. Hence, studies aiming to investigate the underlying molecular mechanisms in gallbladder carcinoma initiation and progression are urgently demanded. Identifying novel genes that are associated with gallbladder carcinoma progression might enable to develop novel therapies for preventing cancer metastasis.

MiRNAs, which are a category of endogenous non-coding 20–22 nucleotide RNAs, have recently been demonstrated as post-transcriptional regulated the expression of target genes [7]. MiRNAs regulate target genes through binding to the 3′-untranslated region (3′-UTR) of targets genes and future inhibit the translation or promote the degradation of genes [8]. Substantive evidences demonstrate that miRNAs participate in the progression of several cancer types [9–11]. Currently, increasing studies showed that alternation of miRNAs play crucial roles in tumorigenic processes, including cell proliferation, tumor angiogenesis, apoptosis, and cancer cell invasion [12,13]. Our microarray analysis revealed that the levels of miR-1-5p were significantly lower in gallbladder carcinoma than that in the normal tissues, which suggested that miR-1-5p was a potential cancer suppressor. Previous studies prove that miR-1 is down-expressed in esophageal squamous cell carcinoma (ESCC) through inhibiting MET proto-oncogene (MET), Cyclin

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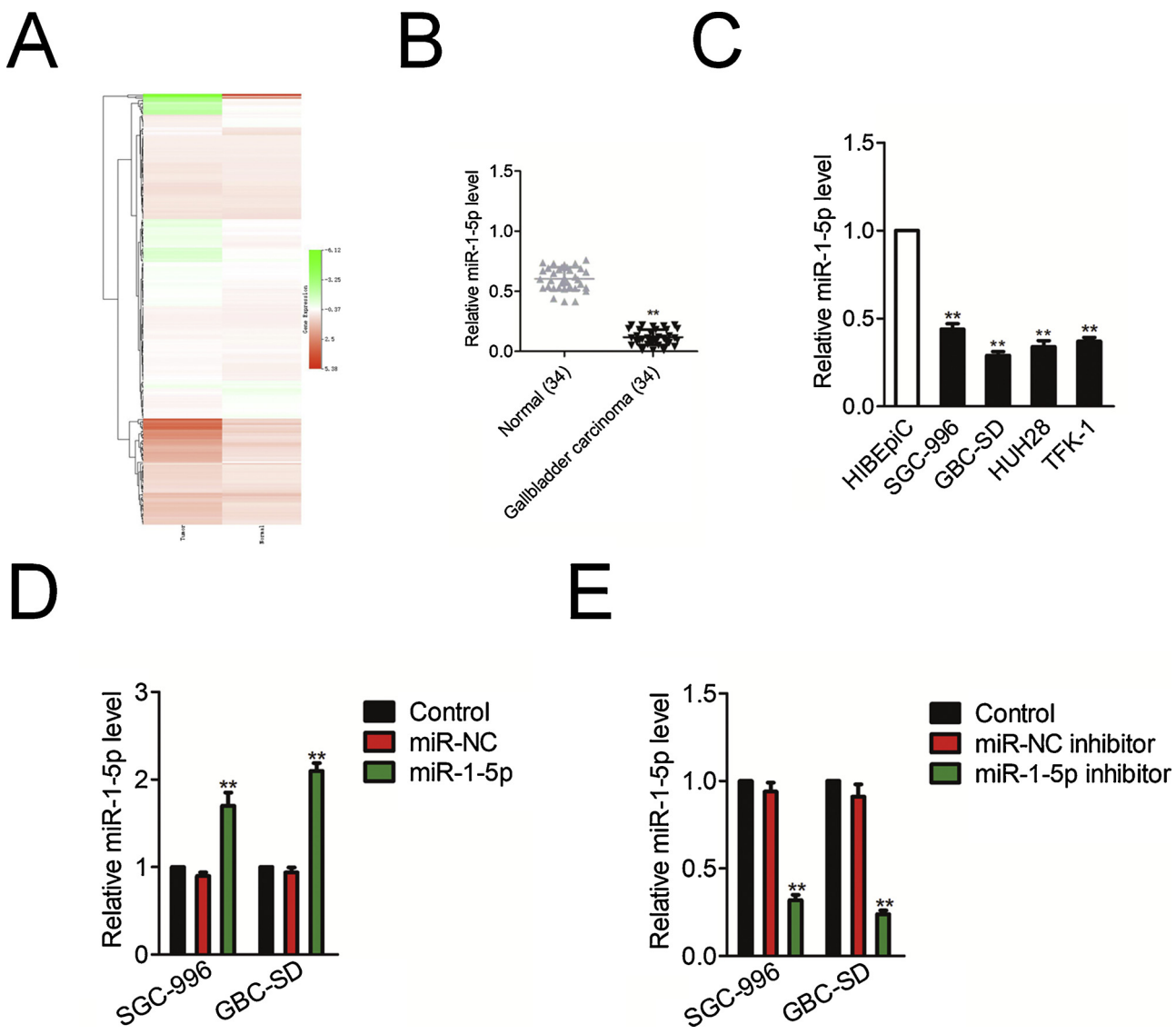

Fig. 1. MiR-1-5p is down-expressed in gallbladder carcinoma. A. Heatmap: The dys-expression pattern of miRNAs in gallbladder carcinoma tissues in comparison to corresponding normal tissues. B. The levels of miR-1-5p were detected in 34 pairs of gallbladder carcinoma samples and normal tissues by qRT-PCR analysis. $^{**}P < 0.01$ compared with Normal. C. The levels of miR-1-5p were assessed in biliary epithelial cell line, HIBEpiC and gallbladder carcinoma cell lines (SGC-996, GBC-SD, HUH28 and TFK-1) using qRT-PCR. The level of miR-1-5p was normalized to U6. $^{**}P < 0.01$ compared with HIBEpiC. D–E. The level of miR-1-5p in SGC-996 and GBC-SD cell transfected with miR-1-5p mimics or miR-1-5p inhibitor was assessed by qRT-PCR. $^{**}P < 0.01$ compared with control.

dependent kinase 4 (CDK4) and Cyclin D1 [14]. In addition, miR-1 increases the sensitivity of ESCC cell to the anti-cancer drug, gefitinib by inactivation of PIK3CA signaling axis [15]. Furthermore, miR-1-3p is markedly down-expressed in bladder cancer and miR-1-3p suppresses cell proliferation and invasion by regulating brain-derived neurotrophic factor-tyrosine kinase receptor B (BDNF-TrkB) signaling pathway in bladder cancer [16]. Nevertheless, the function of miR-1-5p in gallbladder carcinoma growth and metastasis is not well undefined and requires profound investigations.

The Notch signaling axis is a highly conserved and regulates multitudinous cellular processes, including cell growth, cellular apoptosis, tumor-associated angiogenesis and metastasis [17,18]. Notch signaling axis consists of Notch receptors, DSL protein ligands (Delta/Serrate/Lag-2) and intracellular effector molecules. Notch receptors are subdivided into four kinds: Notch1, Notch2, Notch3 and Notch4, in which Notch2 takes part into the tumorigenesis, facilitates tumor growth and reduces the chemo-sensitivity in various kinds of cancer [19,20]. In non-small-cell lung carcinoma (NSCLC), over-expression of Notch2 is associated with the clinical outcomes of patients with cancer [21]. In ESCC, the up-regulation of Notch2 is also closely connected with both overall and progression-free survival of patients [22]. However, current researches rarely focus on miRNAs and Notch2 interactions in gallbladder carcinoma.

In the current study, we identified the potential roles of miR-1-5p in gallbladder carcinoma cell growth, migration and invasion. Our detections demonstrated that miR-1-5p was significantly down-expressed in gallbladder carcinoma tissues in comparison with the non-tumor tissues. Furthermore, the experiments *in vitro* suggested that miR-1-5p suppressed the proliferation, colony formation, migration and invasion of gallbladder carcinoma cell. Additionally, Notch2 was a directly target gene of miR-1-5p and miR-1-5p inhibited the aggressiveness of gallbladder carcinoma cell through targeting Notch2. Altogether, our findings identified that miR-1-5p acted a tumor suppressor in gallbladder carcinoma proliferation, migration and invasion by inhibiting Nothc2 expression.

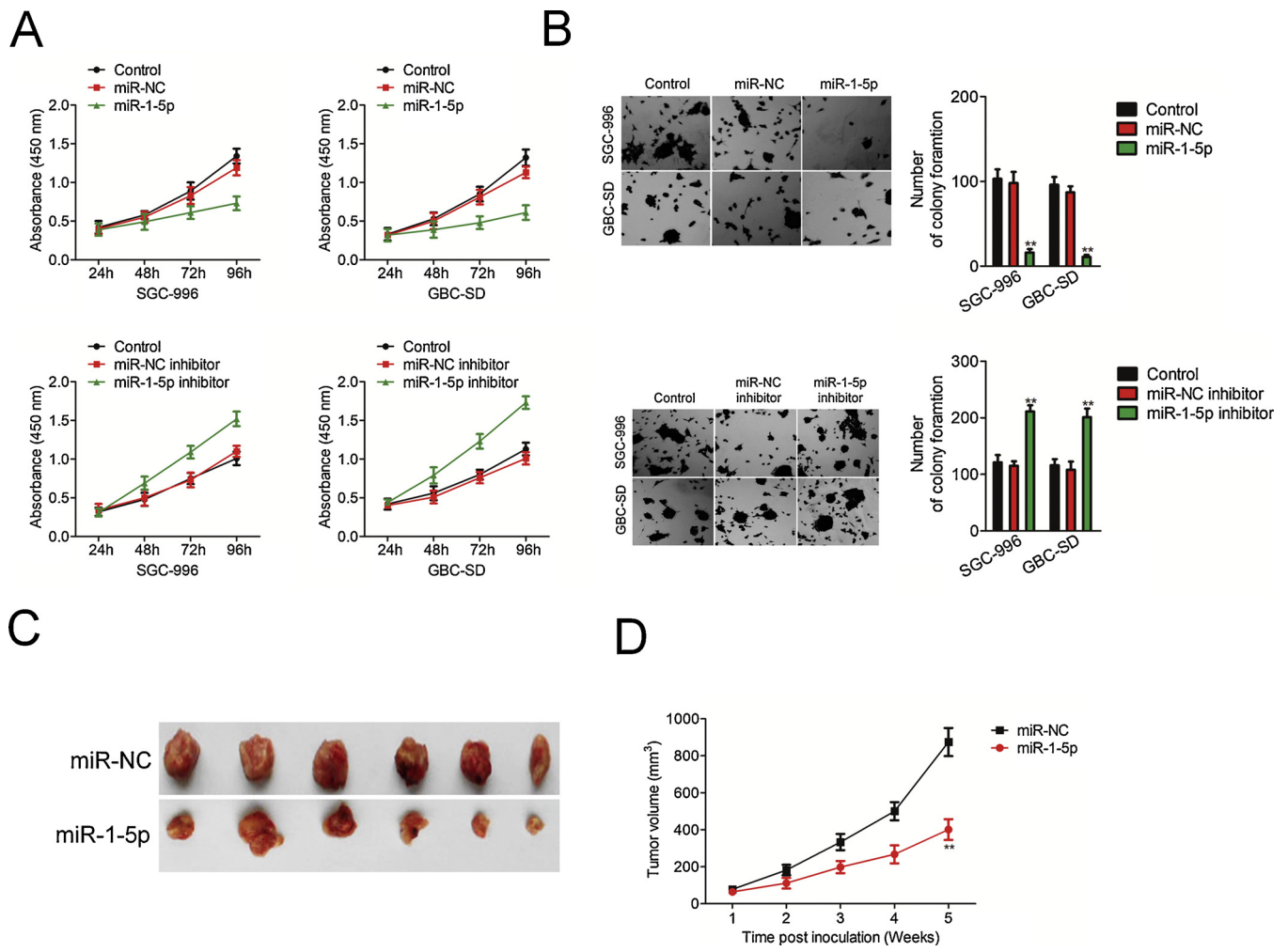

Fig. 2. MiR-1-5p suppresses the growth of gallbladder carcinoma cell. A. The cell viability of SGC-996 and GBC-SD cells that were transfected with miR-1-5p or miR-1-5p inhibitor was determined in MTT assays. B. Colony formation analysis was conducted in miR-1-5p or miR-1-5p inhibitor transfected cells. $^{**}P < 0.01$ compared with control. C–D. The effect of miR-1-5p on the gallbladder carcinoma SGC-996 cell growth in xenograft nude mice. $^{**}P < 0.01$ compared with miR-NC.

2. Materials and methods

2.1. Gallbladder carcinoma cells and tissues

Gallbladder carcinoma cells lines (GBC-SD, SGC-996, HUH28 and TFK-1) and normal biliary epithelial cells HIBEpiC were purchased from GuangZhou Jennio Biotech Co.,Ltd (Guangzhou. Guangdong, China) and maintained in DMEM or 1640 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS (Invitrogen), 100 μg/ml streptomycin and 100 μg/ml penicillin in a humidified incubator with 37 °C and 5% CO_2. 34 pairs of gallbladder carcinoma tissue and corresponding adjacent normal tissues were obtained from patients who received surgical treatment in Fifth Hospital of Harbin and Daqing oilfield iron man hospital. The tissues were frozen in liquid nitrogen until future use. The written consent was obtained from patients and this study was approved by the Ethics Committee of Fifth Hospital of Harbin and Daqing oilfield iron man hospital.

2.2. Cell transfections

Cells were transfected with miR-1-5p (20 nmol/l), miR-1-5p inhibitor (20 nmol/l) or miR-NC, miR-NC inhibitor which provided by GenePharma (Shanghai, China). SGC-996 cells were transfected with Notch2 shRNA (sh-Notch2, sequence: 5′-CGGTGTACCATTGACA TTG-3′) or control shRNA (sh-Con, 5′-GCAGGTAGCTCAGACCACT-3′) (Ribobo, Guangzhou, China) using Lipofectamine reagent in serum-free 1640 medium according to the manufacturer's instruction. Untreated cells were used as a negative control. For over-expression of Notch2, cells were transfected with Notch2 over-expressing recombinant vector (named as pcDNA3.1-Notch2) (GenePharma, Shanghai, China).

2.3. Quantitative real-time RT-PCR (qRT-PCR) analysis

RNA in either cells or tissues was extracted using TRIzol kit (Invitrogen). For miRNA determine, 2 μg small RNA was reverse transcribed to cDNA using miRNA first-strand cDNA synthesis kit (Invitrogen). The qRT-PCR detection of miR-1-5p was conducted using SYBR green PCR master mix (Takara, Dalian, Liaoning, China). U6 was used as control. To detect the level of Notch2, 2 μg RNA was reverse transcribed to cDNA using PrimeScript RT reagent kit (Takara, Dalian, Liaoning, China). GAPDH was the internal control. Data analysis was conducted using $2^{-\Delta\Delta Ct}$ method. The primers used for PCR were as follows (sense and antisense, respectively): GAPDH: 5′-TGTGGGCATC AATGGATTTGG-3′ and 5′-ACACCATGTATTCCGGGTCAAT-3′; Notch2: 5′-CAACCGCAATGGAGGCTATG-3′ and 5′-GCGAAGGCACAATCATCAA TGTT-3′; U6: 5′-AAAGCAAATCATCGGACGACC-3′ and 5′-GTACAACA CATTGTTTCCTCGGA-3′; miR-1-5p: 5′-TAAAGTGGGGACAGCAAAA TGC-3′ and 5′-AGCACAAGGTAGAGAAGGTAGAG-3′.

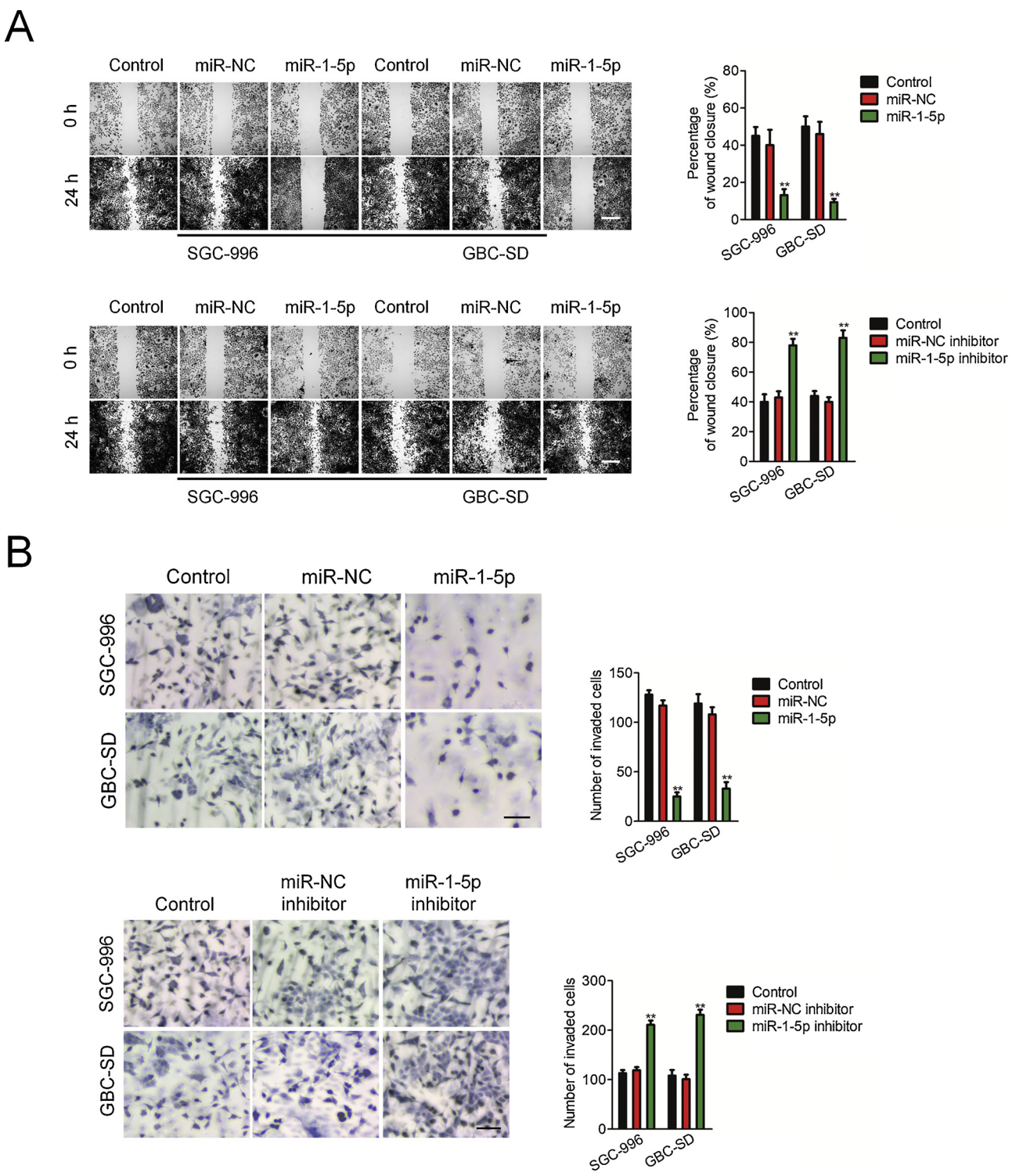

Fig. 3. MiR-1-5p inhibits the migration and invasion of gallbladder carcinoma cell. A. Wound healing analysis was conducted in both SGC-996 and GBC-SD cells. B. SGC-996 or GBC-SD cells were transfected with miR-1-5p or miR-1-5p inhibitor. Transwell invasion assay was conducted. Scale bar represents 100 μm. The data are represented by mean ± SD. ** $P < 0.01$ compared with control.

2.4. Cell proliferation and colonies formation analysis

Cell growth was performed using the MTT assay (Promega, Madison, WI, USA). Cell viability was detected at 1, 2, 3 and 4 days after miRNAs transfection. In colonies formation, cells (1×10^3/ml) were plated into 6-well plates and cultured in medium supplemented 10% FBS for total four weeks. Next, cell colonies more than 50 cells were stained using 0.1% crystal violet and were counted.

2.5. Tumorigenicity assay in vivo

Male BALB/c nude mice were purchased from Shanghai experimental animal center (Shanghai, China). The animal experiment was approved by the animal ethics committee of Fifth Hospital of Harbin, Daqing oilfield iron man hospital and BinZhou Medical University Hospital. 100 μl miR-1-5p transfected SGC-996 cell suspensions was injected s.c. into BALB/c nude mice. The tumor growth was measured

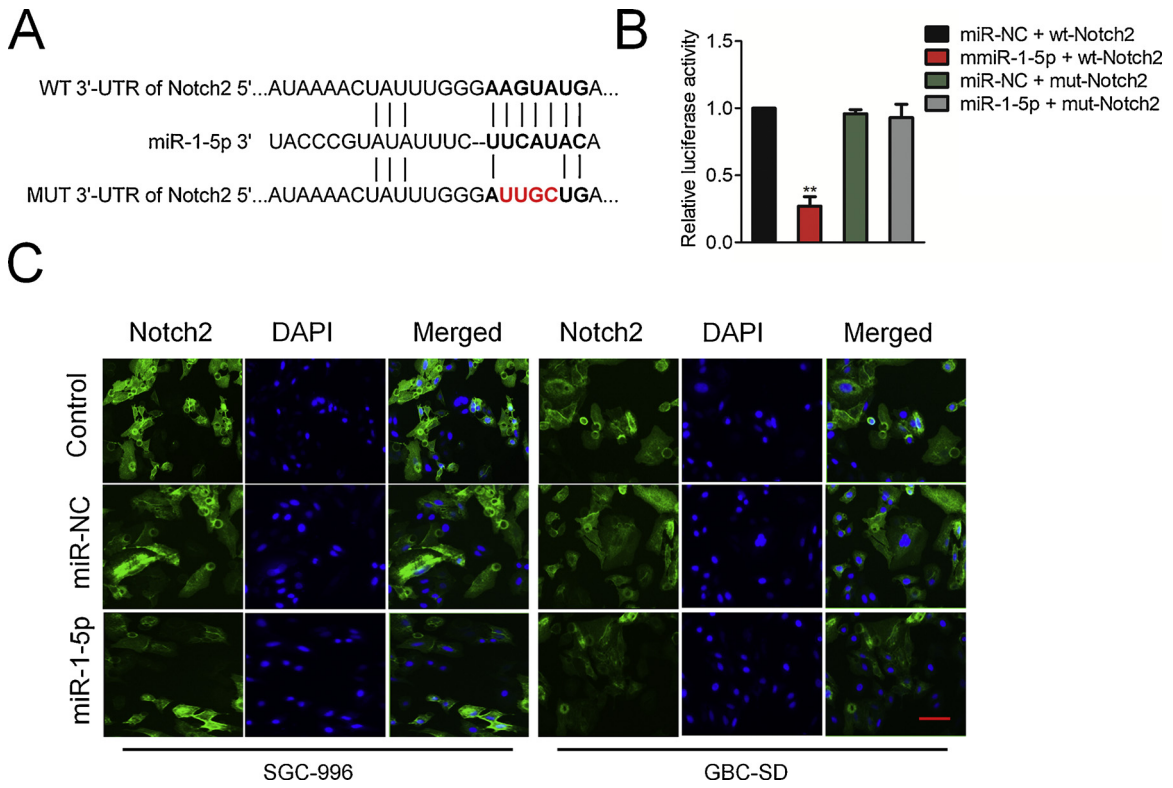

Fig. 4. Notch gene is the target of miR-1-5p. A. The predicted miR-1-5p binding sites within the 3′-UTR of Nothc2 and a mutated 3′-UTR of Nothc2 was shown. B. Luciferase reporter assay validated that Notch2 was a direct target gene of miR-1-5p. ** $P < 0.01$ compared with miR-NC + wt-Notch2. C. SGC-996 and GBC-SD cells were transfected with miR-1-5p or miR-NC and the expression of Notch2 was detected using immunofluorescence staining. Scale bar represents 100 μm.

once a week and the tumor volume was calculated using the formula: $V = 0.5 \times \text{length} \times \text{width}^2$. After five weeks, all mice were sacrificed and the tumors were dissected and photographed.

2.6. Cells migration analysis

Cells were cultured in 6-well plates to reach 90% confluence. Before assays, cells were treated with 25 μg/ml mitomycin C for 30 min to inhibit cell proliferation. Then, a gap was generated by scraping across cells monolayer. Cells wound closure was monitored under a phase-contrast microscope and the percentage of wound closure was counted [23].

2.7. Cells invasion assay

Cells invasion was measured using the Transwell chamber coated with polycarbonate membrane (8 μm pore size). The cells were re-suspended by serum-free media and cell number was adjusted to 1×10^5/ml. 200 μl cell suspensions was seeded into upper chambers. Medium containing 20% FBS were added into lower chamber and used as the attractant. Following 24-h incubation, the non-invasive cells on the upper side of chamber were removed, and the invaded cell on the lower side was fixed with 4% paraformaldehyde and stained with 0.1% crystal violet. Invaded cells in five fields for each well were counted by bright field microscopy [24].

2.8. Immunoblotting

Proteins were prepared using RIPA containing the proteinase inhibitor (Roche, Mannheim, Germany). 30 μg of protein was separated on 10% SDS-PAGE and transferred onto PVDF membranes (Millipore, Braunschweig, Germany). PVDF membrane was blocked using non-fat dried milk and then incubated with anti-Notch2 (Cell Signaling Technology, Danvers, MA, USA) and anti-GAPDH antibody (Cell Signaling Technology). After washed with TBST three times, the membrane was incubated with HRP-conjugated goat anti-rabbit antibody (Bioworld Technology, Nanjing, Jiangsu, China) for 2 h at RT. Protein band was detected using ECL reagents (Thermo, Rockford, USA).

2.9. Immunofluorescence staining

Cells were fixed in 4% paraformaldehyde and washed with ice cooled PBS for three times. Then, cells were incubated with 10% goat serum for 30 min at 37 °C and incubated the Notch 2 antibody overnight. After washed with ice cooled PBS, cells were incubated with FITC-conjugated goat anti-rabbit antibody (Bioworld Technology, Nanjing, Jiangsu, China). Cell nuclei were stained with DAPI (Bioworld Technology).

2.10. Luciferase reporter analysis

Wilde type of Notch2 3′-UTR sites (WT) or the mutant sites of Notch2 3′-UTR (MUT) was inserted into a firefly luciferase reporter vector, pGL3 promoter vector (Genscript, Nanjing, Jiangsu, China). Cells were plated into a 24 well plate and was cotransfected with luciferase pGL3 promoter vector and miR-1-5p or miR-NC using Lipofectamine® 2000 (Invitrogen). Luciferase activities were measured using luciferase reporter assay kit (Promega, Madison, WI, USA).

2.11. Statistical analysis

Data were presented as Means ± SD of three experiments. Statistical analysis in this study was completed using Graphpad Prism 5. Multiple comparisons were performed using one-way ANOVA followed by Tukey's multiple-comparison test. Two-tailed Student's *t*-test was

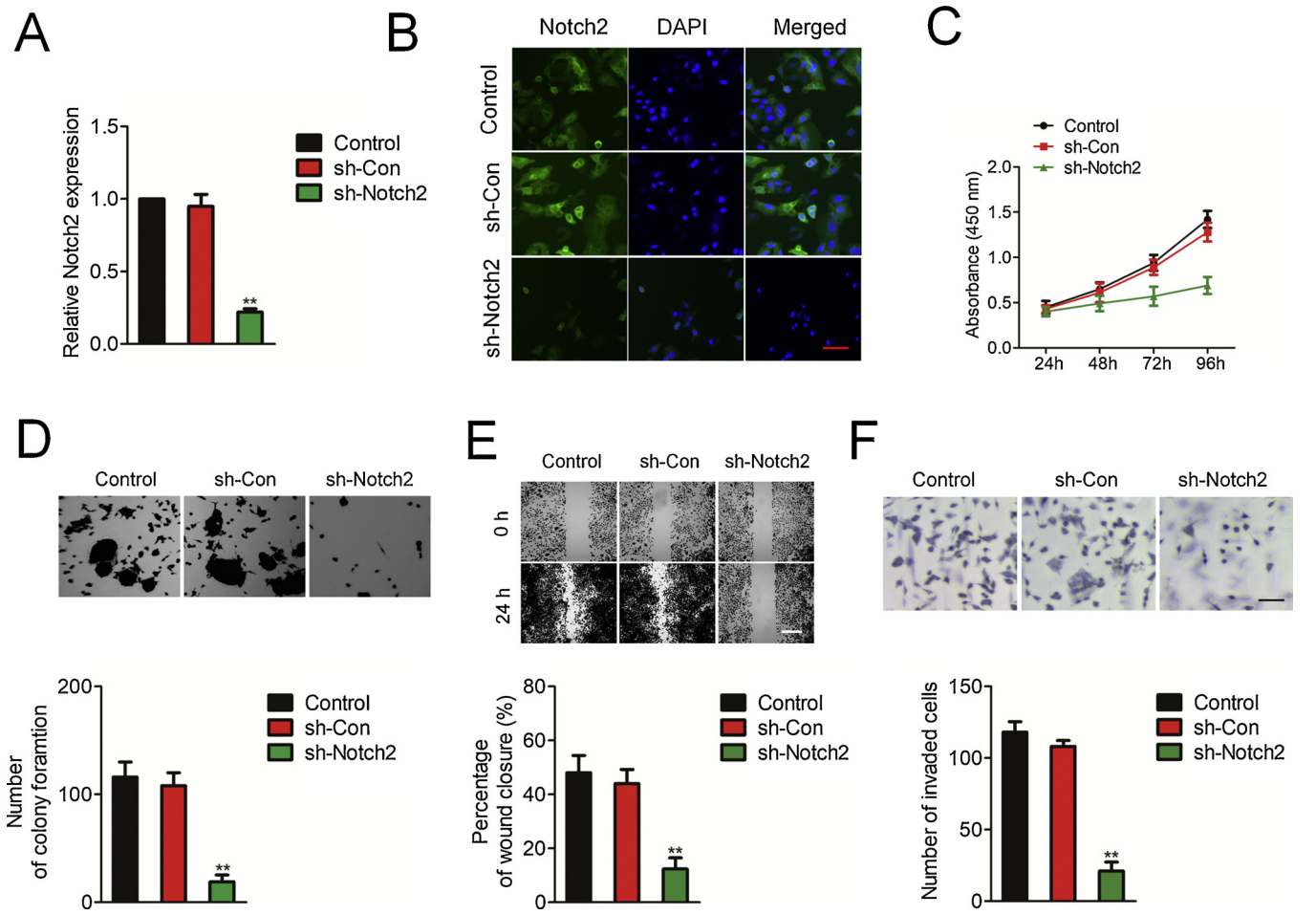

Fig. 5. Down-regulation of Notch2 inhibits the growth, migration and invasiveness of SGC-996 cell. A–B. The level of Notch2 in SGC-996 cells that were transfected with sh-Notch2 was inhibited compared to cells that were transfected with sh-Con. C–D. Down-regulation of Notch2 decreased the proliferation and colony formation of SGC-996 cell. E. Notch2 knocked-down suppressed the migration of SGC-996 cell. F. SGC-996 cells were transfected with sh-Notch2 and Transwell invasion assay was conducted. Scale bar represents 100 μm. ** $P < 0.01$ compared with control.

used for other comparisons. $P < 0.05$ was considered statistically significant.

3. Results

3.1. MiR-1-5p is down-expressed in gallbladder carcinoma

To identify the expression pattern of miRNAs in gallbladder carcinoma, microarray GEO (GSE104165) analysis was applied to explore the differential expressed miRNAs in gallbladder carcinoma tissues (Tumor) and adjacent tissues (Normal). As shown in Fig.1A, the levels of miR-1-5p, whose function is not yet well investigated, were markedly down-expressed in gallbladder carcinoma tissues compared with the paired normal tissues. To explore the potential functions of miR-1-5p in gallbladder carcinoma, we assessed the levels of miR-1-5p in 34 pairs of gallbladder carcinoma tissues and adjacent normal tissues by qRT-PCR assay. The levels of miR-1-5p were remarkably down-expressed in gallbladder carcinoma samples in comparison with the corresponding normal tissues (Fig. 1B). Additional, we determined the levels of miR-1-5p in a panel of gallbladder carcinoma cells lines (SGC-996, GBC-SD, HUH28 and TFK-1) and normal biliary epithelial cell line, HIBEpiC using qRT-PCR assay. In Fig. 1C, the levels of miR-1-5p were significantly lower in four gallbladder carcinoma cells than that in HIBEpiC. SGC-996 cells exhibited the relatively highest level of miR-1-5p, and GBC-SD cells had the lowest level of miR-1-5p. Hence, these two cell lines were selected for future functional investigation. Both SGC-996 and GBC-SD cells were transfected with miR-1-5p mimic or miR-1-5p inhibitor. As shown in Fig. 1D, the level of miR-1-5p in the miR-1-5p transfected cell was markedly increased than that in the miR-NC transfected cell. Moreover, the level of miR-1-5p in cell transfected with miR-1-5p inhibitor was remarkably decreased than that in the miR-NC inhibitor group (Fig. 1E).

3.2. MiR-1-5p suppresses gallbladder carcinoma cell growth

MTT assays were conducted to determine the growth of gallbladder carcinoma cell after transfected with miR-1-5p or miR-5p inhibitor. The proliferation of SGC-996 and GBC-SD cell was inhibited by miR-1-5p transfection when compared with the parental cell (Fig. 2A). Conversely, miR-1-5p inhibitor transfection obviously increased the growth of the SGC-996 and GBC-SD cell (Fig. 2A). These findings suggested that miR-1-5p remarkably inhibited the growth of gallbladder carcinoma cell *in vitro*. To future verify the suppressive effect of miR-1-5p on gallbladder carcinoma cell growth, the colony formation assay was conducted using SGC-996 and GBC-SD cells that were transfected with miR-1-5p or miR-1-5p inhibitor. As showed in Fig. 2B, miR-1-5p the colony formation of SGC-996 and GBC-SD cell that was transfected with miR-1-5p was obviously inhibitedwhen compared with the non-treated cell, whereas down-regulation of miR-1-5p increased the colony formation of gallbladder carcinoma cell. To future prove the results *in vitro*, an *in vivo* mouse xenograft model was constructed. MiR-1-5p transfected SGC-996 cells were subcutaneously inoculated into nude

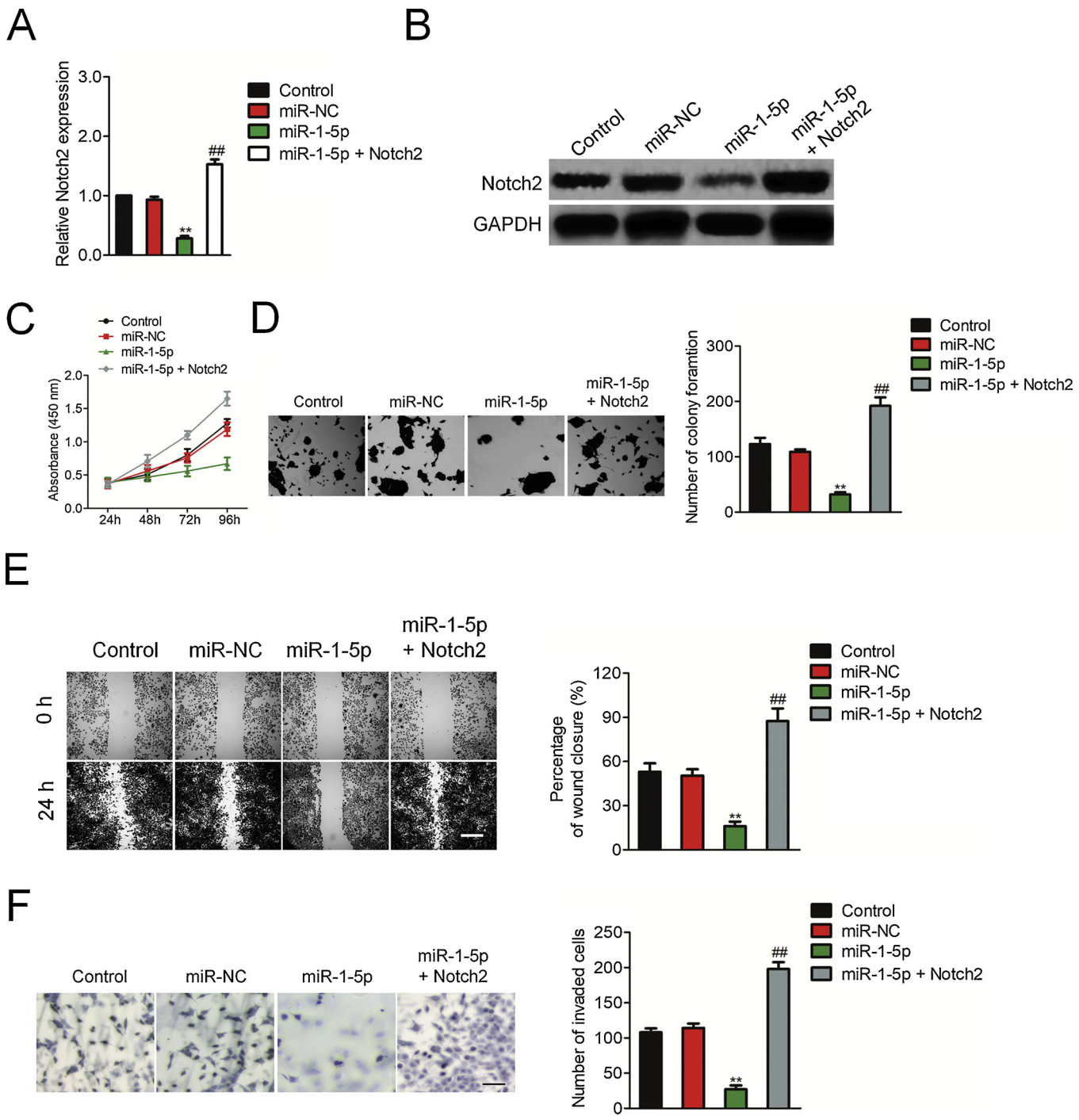

Fig. 6. Over-expression of Notch2 reverses the inhibitory effects of miR-1-5p. A–B. The level of Notch2 in SGC-996 cell that was transfected miR-1-5p alone or were cotransfected miR-1-5p and Notch2 was determined by qRT-PCR and western blotting. C–D. Over-expression of Notch2 counteracted the suppressive effects of miR-1-5p on SGC-996 cell proliferation and colony formation. E–F. Over-expression of Notch2 attenuated the inhibitory effects of miR-1-5p on SGC-996 cell migration and invasion. Scale bar represents 100 μm. ** $P < 0.01$ compared with control, $^{\#\#}$ $P < 0.01$ compared with miR-1-5p.

mice. After five weeks, the tumor volume curve suggested that the tumor growth of miR-1-5p over-expressing cell was markedly inhibited when compared to the control group (Fig. 2C,D). Altogether, these findings indicate that miR-1-5p strikingly suppresses gallbladder carcinoma SGC-996 cell growth *in vitro* and in xenograft model.

3.3. MiR-1-5p suppresses gallbladder carcinoma cell migration and invasion

In order to investigate the roles of miR-1-5p on both SGC-996 and GBC-SD cell migration and invasion *in vitro*, cells were transfected with miR-1-5p or miR-1-5p inhibitor and we evaluated the aggressiveness of gallbladder carcinoma cell *in vitro*. As shown in Fig. 3A, the migration of the cell that was transfected with miR-1-5p was significantly decreased compared to the parental cell. However, miR-1-5p inhibitor increased the migration of both two gallbladder carcinoma cell lines. In addition, Transwell invasion assay revealed that the invasion ability of miR-1-5p down-expressing cell was remarkably increased in comparison to the parental cell. Conversely, over-expression of miR-1-5p dramatically decreased the invasiveness of the gallbladder carcinoma cell *in vitro* (Fig. 3B). Altogether, these results suggest that miR-1-5p plays

vital roles in the migration and invasiveness of gallbladder carcinoma cell.

3.4. Notch2 is a direct target of miR-1-5p

MiRNAs exert their critical functions *via* inhibiting the expression of target genes. To investigate the underlying mechanism of miR-1-5p in gallbladder carcinoma, the miRNA target prediction online tools (TargetScan and MiRanda) were selected to bioinformatics analyses the target of miR-1-5p. As shown in Fig. 4A, the result from bioinformatics analysis indicated the binding sites between miR-1-5p and Notch2 gene. Additional, luciferase reporter assays were utilized to further verify miR-1-5p binding to the 3′-UTR of Notch2. Wild-type 3′-UTR of Notch2 that containing the miR-1-5p-binding sites or a mutated 3′-UTR sequence of Notch2 was cloned into pGL-3 luciferase reporter vector, and then were co-transfected into 293 T cell together with miR-NC or miR-1-5p. As shown in Fig. 4B, miR-1-5p transfected remarkably suppressed the luciferase activity in cell that was transfected with wild-type Notch2 3′-UTR whereas luciferase activity in cell that was transfected with mutated Notch2 3′-UTR was not significantly inhibited by miR-1-5p. In addition, the expressions of Notch2 were significantly inhibited in gallbladder carcinoma cells that were transfected with miR-1-5p (Fig. 4C). Altogether, these observations indicate that Notch2 is a target gene of miR-1-5p.

3.5. Knock-down of Notch2 inhibits gallbladder carcinoma cell growth

On account of the above results, we deduced that miR-1-5p inhibited gallbladder carcinoma cell growth, migration and invasion by suppressing Notch2. Firstly, SGC-996 cell was transfected with Notch2 shRNA (sh-Notch2) and the transfection efficiency was verified using qRT-PCR and immunofluorescence assays (Fig. 5A,B). Then, the proliferation, migration and invasion of SGC-996 cell that was transfected with sh-Notch2 or sh-Con was detected using MTT, colony formation, wound healing and Transwell invasion assay. As shown in Fig. 5C-F, down-expression of Notch2 markedly inhibited the proliferation, colony formation, migration and invasion of SGC-996 cell *in vitro*.

3.6. Notch2 is involved in miR-1-5p mediated gallbladder carcinoma cell growth and invasion

To investigate the roles of Notch2 on the growth, mobility and invasion of SGC-996 cell, SGC-996 cell was cotransfected with miR-1-5p mimics and pcDNA3.1-Notch2. The expression of Notch2 in SGC-996 cell was measured using qRT-PCR and western blotting assay (Fig. 6A,B). Next, the proliferation of colony formation assay using SGC-996 cell was conducted. As shown in Fig. 6C,D, over-expression of Notch2 counteracted the inhibitory effects of miR-1-5p in SGC-996 cell growth and colony formation. Consistently, up-regulation of Notch2 reversed the inhibitory effect of miR-1-5p on the migration and invasion of SGC-996 cell (Fig. 6E,F). Thus, these results indicate that miR-1-5p regulates the migration and invasion of gallbladder carcinoma cell through regulating Notch2.

4. Discussion

Accumulating evidences demonstrate that miRNAs play crucial roles in cancer progression and directly facilitate or suppress cell proliferation, apoptosis, invasion and metastasis [25–27]. Identifying the miRNAs and their target genes that are essential for malignant tumor progression may provide potential therapeutic options [28]. MiRNAs exert their biological functions through binding to the 3′-UTR of genes. Recently, investigations demonstrated that plenty of miRNAs are abnormally expressed in cancer, and are closely associated with the clinical features, including prognosis, pathological types, lymph node metastasis and TNM stages [29–31]. Previous studies confirm that miR-1 functions as an anti-oncogene in diverse malignant cancers [14,32–34]. In bladder cancer, miR-1 inhibits growth, migration and invasion of bladder carcinoma cell *via* up-regulating the expression of secreted frizzled-related protein 1 (SFRP1) [35]. Additional, miR-1 suppresses the growth, metastasis and induces apoptosis of breast cancer and gastric carcinoma cell [36,37]. In esophageal squamous cell carcinoma (ESCC), miR-1 acts as a tumor suppressor and inhibits the growth and invasiveness of ESCC cell [14]. Altogether, these findings suggest that miR-1 has crucial roles in cancer progression. Nevertheless, the precise functions of miR-1-5p in gallbladder carcinoma are still unclear, and it needs further investigated.

In our study, we found that miR-1-5p was down-expressed in gallbladder carcinoma cell. Furthermore, we assessed the level of miR-1-5p in gallbladder carcinoma clinical tissues and observed that miR-1-5p was remarkably down-regulated in gallbladder carcinoma tissues when compared to the paired normal tissues. All these results indicated that miR-1-5p participated into the progression of gallbladder carcinoma. To investigate the function of miR-1-5p in gallbladder carcinoma, gallbladder carcinoma cell lines: SGC-996 and GBC-SD were transfected miR-1-5p or miR-1-5p inhibitor and then, we performed a series of functional experiments. Over-expression of miR-1-5p markedly suppressed the proliferation, colony formation, migration and invasion of gallbladder carcinoma cell *in vitro*. In contrast, down-expression of miR-1-5p increased gallbladder carcinoma cell proliferation and promoted cellular migration and invasion. These results indicated that miR-1-5p acted as anti-oncogene whose over-regulation decreased the aggressiveness of gallbladder carcinoma.

Recently, the involvements of miR-1 in the regulation of numerous target genes in cancers have been revealed. To explore the precise mechanism of miR-1-5p in gallbladder carcinoma, bioinformatics online analysis and luciferase reporter assay were applied and we found Notch2 was the target gene of miR-1-5p. As a major receptor in the Notch signaling pathway, Notch2 plays a critical role in the progression of various malignant tumors, including acute myeloid leukaemia, gastric cancer, bladder cancer, esophageal squamous cell carcinoma and hepatocellular carcinoma progression. We identified that miR-1 down-regulated the expression of Notch2 in gallbladder carcinoma cell. Over-expression of Notch2 was able to attenuate the suppressive impact of miR-1 on the migration and invasion in gallbladder carcinoma cell. All these results proved that miR-1-5p suppressed the growth, migration and invasion of gallbladder carcinoma cell by targeting Notch2.

In conclusion, our study demonstrates that miR-1-5p serves as a tumor suppressor in gallbladder carcinoma *via* targeting Notch2 and provides a molecular basis for the precise ole of miR-1-5p/Notch2 signaling in the growth, migration and invasion of human gallbladder carcinoma cell.

Conflict of interest

None.

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None.

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