SNORA38B promotes proliferation, migration, invasion and epithelial-mesenchymal transition of gallbladder cancer cells *via* activating TGF-β/Smad2/3 signaling

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ABSTRACT

Evidence has shown that small nucleolar RNAs (snoRNAs) participate in the tumorigenesis in multiple cancers, including gallbladder cancer (GBC). Our results showed that SNORA38B level was increased in GBC tissues compared to adjacent normal tissues. Thus, this research aimed to explore the role and molecular mechanisms of SNORA38B in GBC. SNORA38B level between normal and GBC tissues was evaluated by RT-qPCR. Cell proliferation, apoptosis, migration, and invasion were tested by EdU assay, TUNEL staining and transwell assay, respectively on human intrahepatic biliary epithelial cells (HIBEpiCs) and the GBC cell lines, NOZ and GBC-SD. Expression of proteins in GBC cells was evaluated by immunofluorescence and Western blot assays. We found that, relative to normal tissues, SNORA38B level was notably elevated in GBC tissues. SNORA38B overexpression obviously enhanced GBC cell proliferation, migration, invasion and epithelial-mesenchymal transition (EMT), but weakened cell apoptosis. Conversely, SNORA38B downregulation strongly suppressed the proliferation and EMT of GBC cells and induced cell apoptosis and ferroptosis, whereas these phenomena were obviously reversed by TGF-β. Meanwhile, SNORA38B downregulation notably reduced the levels of phosphorylated-Smad2 and phosphorylated-Smad3 in GBC cells, whereas these levels were elevated by TGF-β. Collectively, downregulation of SNORA38B could inhibit GBC cell proliferation and EMT and induce ferroptosis *via* inactivating TGF-β1/Smad2/3 signaling. These findings showed that SNORA38B may be potential target for GBC treatment.

Key words: gallbladder cancer; small nucleolar RNAs; SNORA38B; epithelial-mesenchymal transition; TGF-β.

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Contributions: Yiyu Qin made major contributions to the design and manuscript drafting of this study; Jian Li, Hongchao Han, Yongliang Zheng, Haiming Lei, Yang Zhou, Hongyan Wu, Guozhe Zhang and Xiang Chen were responsible for data acquisition, data analysis and manuscript revision; Zhengping Chen made contributions to conception of the study and revised the manuscript. All the authors read and approved the final version of the manuscript and agreed to be accountable for all aspects of the work.

Conflict of interest: the authors declare that they have no competing interests, and all authors confirm accuracy.

Ethics approval: all procedures were approved by the Ethics Committee of the Yancheng Third People's Hospital (no. TYC2022024).

Funding: Project of Jiangsu Provincial Commission of Health and Family Planning, Grant/Award Number: M2022072; Jiangsu Province "333 High−level Talents Cultivating Project".

Introduction

Gallbladder cancer (GBC) is a common malignant type of the biliary tract tumor.[1] It has been shown that GBC exhibits a high propensity for metastasis with a poor prognosis.[2] In recent years, surgery is still the essential method for treating GBC.[3,4] However, if GBC patients appeared distant metastasis, they will miss the chance of surgery.[2,5] Thus, since only less than half of GBC patients can receive surgery therapy,[2,5] exploring novel therapeutic approaches for GBC are urgently needed. Furthermore, most GBC patients are often diagnosed at an advanced stage,[6] and a report has mentioned that their 5-year survival rate is less than 5%: thus, these patients often have an extremely worse prognosis.[7] Uncovering novel promising biomarkers might provide new strategies for the diagnosis and treatment of GBC.

Small nucleolar RNAs (snoRNAs) are a kind of noncoding RNA molecules ranging from 60-300 nucleotides.[8,9] snoRNAs could modify ribosomal RNA and spliceosome RNAs, thereby playing a role in ribosome biogenesis and RNA splicing.[10] The evidence showed a crucial role of snoRNAs in the production of protein synthesis.[11] Recently, it has been shown that snoRNAs participate in cellular processes including cell survival and inflammation.[12,13] Zhang *et al*. uncovered that snoRNAs could affect tumor cell proliferation and migration.[14] For example, SNORA21 overexpression could suppress the growth of GBC cells.[15] Deficiency of SNORA74B could suppress GBC cell proliferation through inactivating Akt/mTOR signaling.[16] Thus, targeting snoRNAs may be a promising therapeutic approach for GBC. Zhuo *et al*. found that SNORA38B was obviously upregulated in non-small cell lung cancer (NSCLC) tissues, and SNORA38B overexpression could enhance NSCLC cell proliferation, migration and invasion, suggesting that SNORA38B may play an oncogenic role in NSCLC.[17] Nevertheless, the level of SNORA38B in GBC is still elusive and its biological properties in GBC remains largely unstudied.

Thus, we aimed to explore whether SNORA38B could affect the proliferation, migration, invasion and epithelial-mesenchymal transition (EMT) in GBC. Additionally, it has been shown that TGF-β1/Smads signaling plays an important role in cancer development.[18,19] Activation of TGF-β1/Smads signaling can facilitate tumor growth and metastasis.[19] Thus, we also explored whether SNORA38B promoted GBC progression through modulating TGF-β1/Smads signaling. Our findings may provide a new insight in potential therapeutic targets for GBC.

Materials and Methods

Clinical samples

A total of 5 pairs of GBC tissues and adjacent normal tissues were collected from patients with GBC from the Yancheng Third People's Hospital. All participants have signed informed consent forms. This research was conducted according to the Declaration of Helsinki, and all the procedures were approved by the Ethics Committee of the Yancheng Third People's Hospital (approval no. TYC2022024). All samples were immediately frozen in liquid nitrogen and then stored under -80°C until use.

Real-time quantitative PCR (RT-qPCR)

Total RNA in tissues or cells was isolated using the Trizol reagent (No. 15596-026, Ambion®, Thermo Fisher Scientific, Waltham, MA, USA). Next, the extracted RNA was reverse-transcribed into cDNA using the HiScript Reverse Transcriptase (RNase H) kit (No. R101-01/02, Vazyme Biotech, Nanjing, China). Thereafter, qPCR was performed using a SYBR Green Master Mix kit (No. Q111-02, Vazyme Biotech) on an Applied Biosystems QuantStudio 6 Real-Time PCR Systems (ABI Scientific, Sterling, VA, USA). The qPCR procedure was as follows: 95°C for 10 min, 40 cycles of 95°C for 30 sec, 60°C for 30 sec. SNORA38B level was normalized to U6, and quantified with the $2^{-\Delta\Delta (ct)}$. All primers used in this study were listed in Table 1.

Cell culture and transfection

Human intrahepatic biliary epithelial cells (HIBEpiCs) were cultured in complete medium [including DMEM, 10% fetal bovine serum (FBS) + 1% penicillin-streptomycin]. GBC cell line (NOZ) was cultured in complete medium (including Williams'E medium, 10% FBS + 1% penicillin-streptomycin). GBC cell line (GBC-SD) was cultured in complete medium (including RPMI-1640 medium, 10% FBS + 1% penicillin-streptomycin). All cells were maintained at 37°C in a 5% CO_2 atmosphere.

To obtain SNORA38B-overexpressing GBC cells, cells were transfected with pcDNA3.1 vector (OE-NC) or pcDNA3.1-SNORA38B (SNORA38B OE) plasmids using the Lipofectamine 2000 reagent. Meanwhile, siRNA NC and SNORA38B siRNAs (SNORA38B siRNA1, SNORA38B siRNA2 and SNORA38B siRNA3; RiboBio Co. Ltd., Guangzhou, China) were transfected into GBC cells using the Lipofectamine 2000 reagent to downregulate SNORA38B level in GBC cells.

Cell counting kit-8 (CCK-8) assay

The viability of GBC cells was evaluated using the CCK-8 kit (Dongren Chemical Technology Ltd., Shanghai, China). GBC cells were plated onto 96-well plates at 37°C. Following the indicated treatments, 10 μL of CCK-8 was then added into each well. Thereafter, all cells were then cultured at 37°C with 5% CO_2 for 4 h. Finally, a microplate reader (MULTISKAN MK3; Thermo Fisher Scientific) was used to read the absorbance value of each well at 450 nm. This assay was repeated in triplicate.

EdU staining assay

An EdU detection kit (Beyotime Institute of Biotechnology, Haimen, China) was used for assessing cell proliferation. Briefly, GBC-SD cells were incubated with EdU for 2 h, and then fixed in 4% paraformaldehyde for 15 min. Next, cells were incubated with Click solution and then stained with Hoechst 33342 solution (diluting with PBS at a volume ratio of 1:1000) in darkness for 30 min at room temperature. Finally, EdU-positive cells were captured using an Fi3 Nikon fluorescence microscope (objective: 20x). This assay was repeated in triplicate.

Table 1. Primer sequences.

Name		Primer sequences (5'-3')
U6	Forward	CGCTTCGGCAGCACATATAC
	Reverse	AAATATGGAACGCTTCACGA
SNORA38B	Forward	GGCATGTCTATAGTTCCTTGTCT
	Reverse	GGGATGGTTGATCTTGAGCC

Transwell migration and invasion assays

Cell migratory and invasive abilities were assessed using the transwell assays. 700 μL of complete medium containing 10% FBS were pre-loaded into 24-well plates. Next, transwell inserts were placed into the 24-well plate, and 200 μL of GBC-SD cells were then plated into transwell inserts. After 24 h incubation, cells on the lower surface of the insert were fixed in 70% pre-cooled alcohol for 1 h and then stained with 0.5% crystal violet (Servicebio Technology, Wuhan, China) for 20 min. Finally, the migrated and invaded cells were captured using a Ta2-FL Nikon light microscope (objective: 20x). For detecting cell invasive ability, the transwell inserts were pre-treated with 1 mg/mL Matrigel (Corning, Glendale, AZ, USA). This assay was repeated in triplicate.

Flow cytometry assay

The cell apoptosis detection kit (Keygen Biotech, Nanjing, China) was applied for evaluating cell apoptosis. GBC-SD cells were placed into 6-well plate overnight at 37°C. After treatment, cells were washed twice with PBS and then re-suspended in 500 μL of binding buffer and then stained with 5 μL of AnnexinV-FITC and 5 μL of propidium iodide (PI) for 15 min in darkness. Finally, a FACSCalibur cytometer (BD Biosciences, Franklin Lakes, NJ, USA) was used to analyze the apoptotic cells. This assay was repeated in triplicate.

Immunofluorescence (IF) assay

GBC-SD cells were fixed in 4% paraformaldehyde, and then probed with the primary antibodies including anti-caspase 3 (No. AF6311, 1:200, Affinity Biosciences, Cincinnati, OH, USA), anti-E-cadherin (No. 20874-1-AP, 1:300, Proteintech, Rosemont, IL, USA), anti-N-cadherin (no. ab18203, 1:200; Abcam, Cambridge, UK), ant-vimentin (no. BM0135, 1:200, BosterBio, Pleasanton, CA, USA) overnight at 4°C. Thereafter, cells were then probed with corresponding secondary antibodies (no. BA1031, BA1032, 1:100, BosterBio) for 1 h at 37°C. Subsequently, cells were photographed under a BX35 OLYMPUS fluorescence microscope (objective: 20x). 10 μg/mL of DAPI was used for staining the nuclei in the dark for 30 min at room temperature. This assay was repeated in triplicate. Sections lacking primary antibody were used as the negative control.

TUNEL staining assay

Cell apoptosis was detected using a TUNEL kit (Yeasen Biotechnology, Shanghai, China). Briefly, cells were fixed in 4% paraformaldehyde and then stained with the TUNEL reagent for 1 h in darkness. Subsequently, the TUNEL-positive cells were photographed under a BX35 Olympus fluorescence microscope (objective: 20x). 10 μg/mL of DAPI was used for staining the nuclei in the dark for 30 min at room temperature. This assay was repeated in triplicate.

Western blot assay

The BCA kit was used for determining the protein concentration. Next, proteins were then loaded on 10% SDS-PAGE and blotted onto a PVDF membrane. Next, the membrane was probed overnight at 4°C with the primary antibodies: anti-Smad2 (no. ab33875, 1:1000; Abcam), anti-p-Smad2 (no. ab188334, 1:1000; Abcam), anti-Smad3 (no. 66516-1-Ig, 1:1000; Proteintech), anti-p-Smad3 (no, ab52903, 1:1000; Abcam), and anti-β-actin (no. 66009-1-I,1:1000; Proteintech), followed by incubation with a secondary antibody (no. A0208, 1:2000; Beyotime Institute of Technology) for 50 min at room temperature. Thereafter, the blots were developed with an ECL kit. This assay was repeated in triplicate.

ELISA assay

The levels of intracellular iron (Fe^{2+}, no. AB83366, Abcam), and reactive oxygen species (ROS), (no. S0033S, Beyotime Institute of Technology) in cells were detected using the ELISA kits, respectively. This assay was repeated in triplicate.

Statistical analysis

All data are shown as the mean±SD and all experiments were repeated in triplicate. Student's *t*-test or one-way analysis of variance (ANOVA) followed by Tukey's tests were used for comparisons between different groups. A p-value <0.05 was considered statistically significant.

Results

SNORA38B was elevated in GBC tissues

To determine the role of SNORA38B in GBC, SNORA38B level in GBC tissues and adjacent normal tissues obtained from GBC patients was evaluated using RT-qPCR. Relative to normal tissues, SNORA38B level was notably increased in GBC tissues (Figure 1A). Furthermore, to overexpress SNORA38B level in GBC cells, cells were transfected with SNORA38B-OE. The results showed that SNORA38B-OE strongly elevated SNORA38B level in GBC-SD and NOZ cells (Figure 1 B,C). To downregulate SNORA38B level in GBC cells, cells were transfected with si-SNORA38B-1, -2, or -3. The results showed that si-SNORA38B-2 greatly reduced SNORA38B level in GBC-SD and NOZ cells, which was utilized in the following studies (Figure 1 D,E).

SNORA38B deficiency suppressed GBC cell growth

To explore the function of SNORA38B in GBC, CCK-8, EdU staining and transwell assays were conducted. Overexpression of SNORA38B obviously enhanced GBC-SD cell viability and proliferation, whereas deficiency of SNORA38B greatly suppressed cell viability and proliferation (Figure 1F and Figure 2A). Moreover, SNORA38B overexpression strongly promoted GBC-SD cell migration and invasion, whereas SNORA38B deficiency displayed the opposite effects (Figure 2B). To sum up, SNORA38B could affect GBC cell proliferation, migration and invasion.

SNORA38B deficiency triggered GBC cell apoptosis

Next, we explored the effect of SNORA38B on apoptosis of GBC-SD cells. The results of flow cytometry indicated that relative to the siRNA-NC group, si-SNORA38B-2 obviously increased the early and late apoptotic rates of GBC-SD cells (Figure 3A). Moreover, SNORA38B overexpression obviously reduced the number of TUNEL-positive cells, whereas deficiency of SNORA38B notably elevated the number of TUNEL-positive cells (Figure 3B). Meanwhile, the results of IF staining assay indicated that SNORA38B downregulation greatly increased caspase 3 expression in GBC-SD cells (Figure 3C). Collectively, downregulation of SNORA38B could induce GBC cell apoptosis.

SNORA38B deficiency suppressed the EMT of GBC cells

To further explore whether SNORA38B could affect the EMT of GBC cells, the expressions of E-cadherin, N-cadherin and vimentin in GBC-SD cells were evaluated using IF staining assay. SNORA38B overexpression remarkably declined E-cadherin

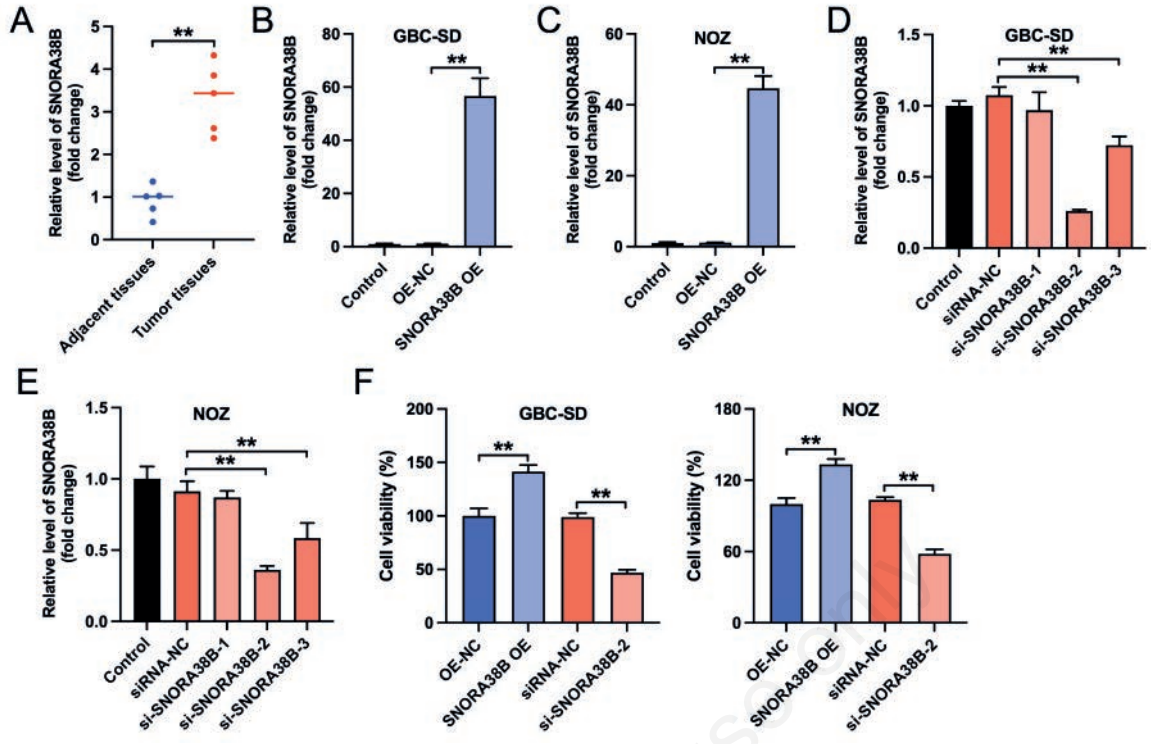

Figure 1. SNORA38B was remarkably elevated in GBC tissues. **A**) The level of SNORA38B in gallbladder cancer tissues and normal tissues was evaluated using RT-qPCR. B-E) RT-qPCR analysis of SNORA38B level in GBC-SD and NOZ cells transfected with (**B,C**) SNORA38B-OE or (**D, E**) si-SNORA38B-1, -2, or -3. **F**) GBC-SD and NOZ cells were transfected with SNORA38B-OE or si-SNORA38B-2. Cell viability was assessed using CCK-8 assay. **p<0.01.

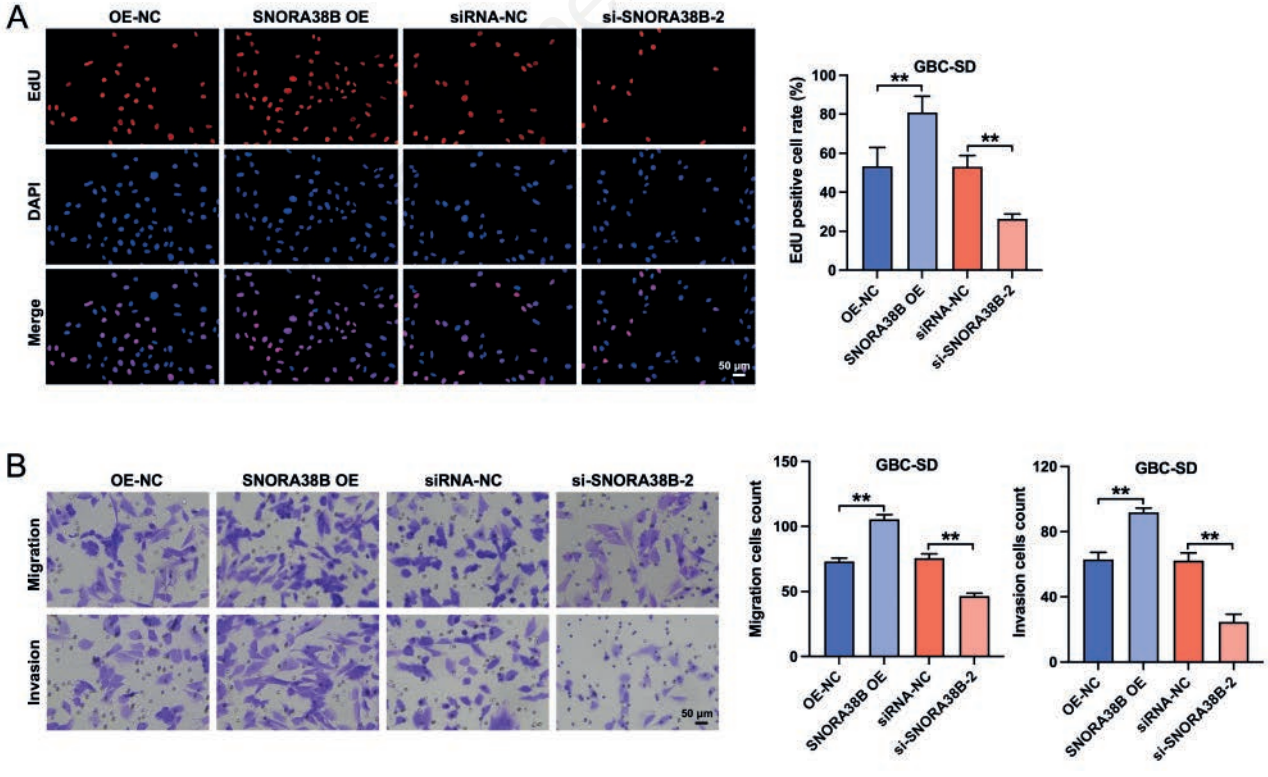

Figure 2. SNORA38B deficiency suppressed GBC cell proliferation, migration and invasion. GBC-SD cells were transfected with SNORA38B-OE or si-SNORA38B-2. **A**) Cell proliferation was assessed using EdU staining assay. **B**) Cell migration and invasion were detected by transwell assays. **p<0.01.

expression and elevated N-cadherin and vimentin expressions in GBC-SD cells, whereas SNORA38B downregulation displayed the opposite effects (Figure 4 A-C). To sum up, SNORA38B downregulation could suppress the EMT of GBC cells.

SNORA38B deficiency suppressed GBC cell growth *via* inactivating TGF-β/Smad2/3 signaling

It has been shown that TGF-β signaling plays a crucial role in human cancers.[20] Thus, we then explored whether SNORA38B could affect GBC cell growth *via* TGF-β signaling. TGF-β (10 ng/mL) strongly enhanced GBC-SD cell viability, proliferation, migration, and invasion and declined cell apoptosis (Figure 5 A-D). However, si-SNORA38B-2 obviously reduced GBC-SD cell viability, proliferation, migration and invasion and triggered cell apoptosis, whereas TGF-β treatment obviously abolished the anti-tumor activities of si-SNORA38B-2 on GBC-SD cells (Figure 5 A-D). Furthermore, compared to the si-SNORA38B-2 group, the upregulation of E-cadherin and downregulation of N-cadherin and vimentin by si-SNORA38B-2 in GBC-SD cells were obviously reversed by TGF-β (Figure 6 A-C). Furthermore, the downstream proteins (Smad2 and Smad3) of TGF-β signaling were detected using the Western blot assay. Compared to the siRNA-NC group, TGF-β notably upregulated p-Smad2 and p-Smad3 levels in GBC-SD cells (Figure 7 A,B). Meanwhile, compared to the siRNA-NC

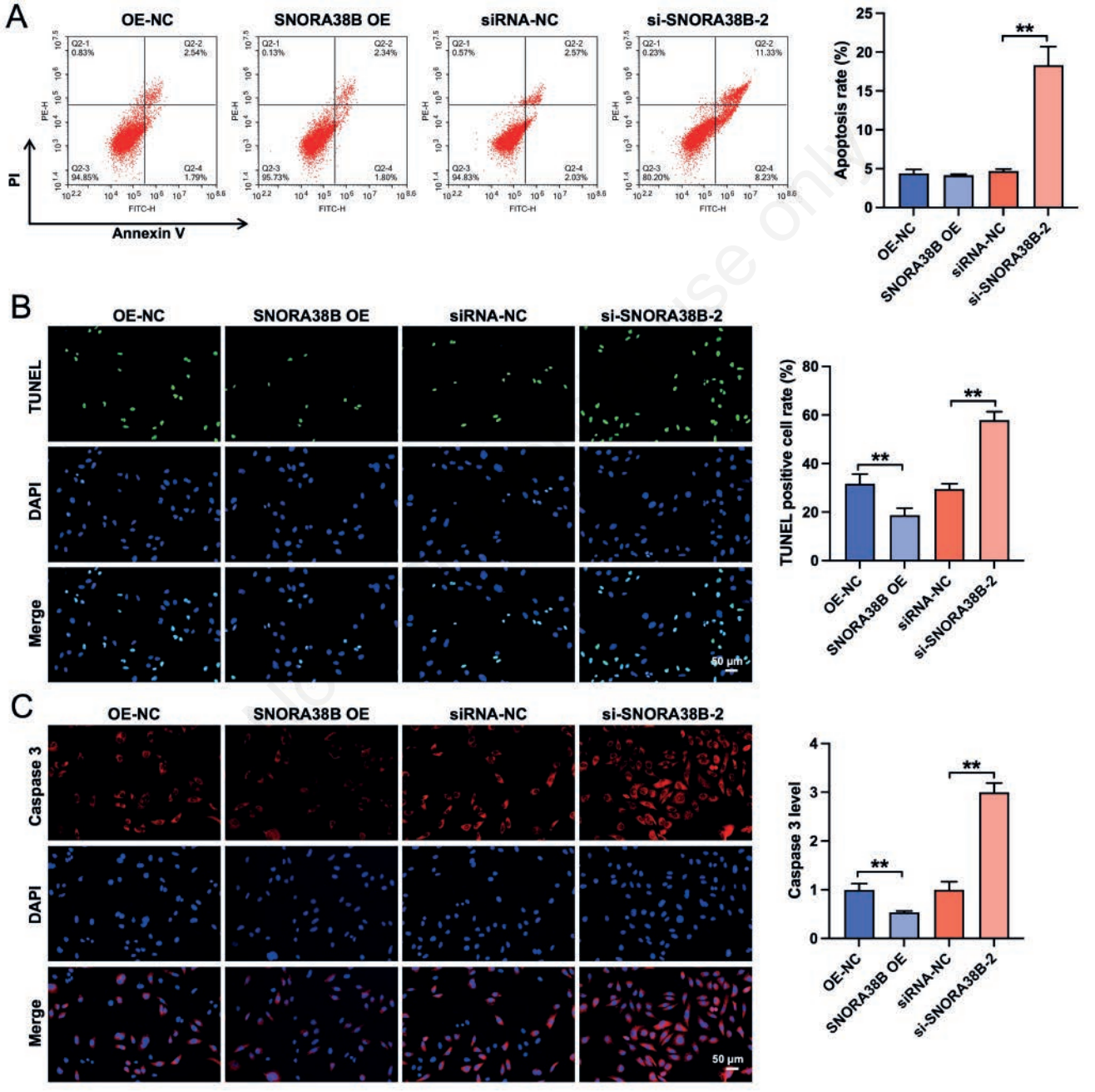

Figure 3. SNORA38B deficiency induced GBC cell apoptosis. GBC-SD cells were transfected with SNORA38B-OE or si-SNORA38B-2. **A,B**) Cell apoptosis was evaluated using (**A**) flow cytometry and (**B**) TUNEL staining assays. **C**) IF staining assay was performed to assess caspase 3 expression in cells. **p<0.01.

group, si-SNORA38B-2 remarkably reduced p-Smad2 and p-Smad3 levels in GBC-SD cells; however, TGF-β obviously reversed these changes (Figure 7 A,B). Collectively, SNORA38B deficiency could suppress the growth of GBC cells *via* inactivating TGF-β/Smad2/3 signaling.

SNORA38B deficiency triggered ferroptosis of GBC cells *via* TGF-β signaling

Next, the effect of SNORA38B on the ferroptosis in GBC was explored. As shown in Figure 8 A,B, compared to the siRNA-NC group, si-SNORA38B-2 greatly upregulated ROS level and Fe^{2+} levels in GBC-SD cells; however, TGF-β obviously abolished these changes. To sum up, SNORA38B deficiency could trigger ferroptosis of GBC cells *via* TGF-β signaling.

Discussion

snoRNAs have been found to be participated in tumorigenesis in multiple cancers *(e.g.* hepatocellular carcinoma, HCC and breast cancer).[21,22] For example, snoRNA U50A was related to the prognosis of breast cancer.[22] Deficiency of SNORA65, SNORA7A, or

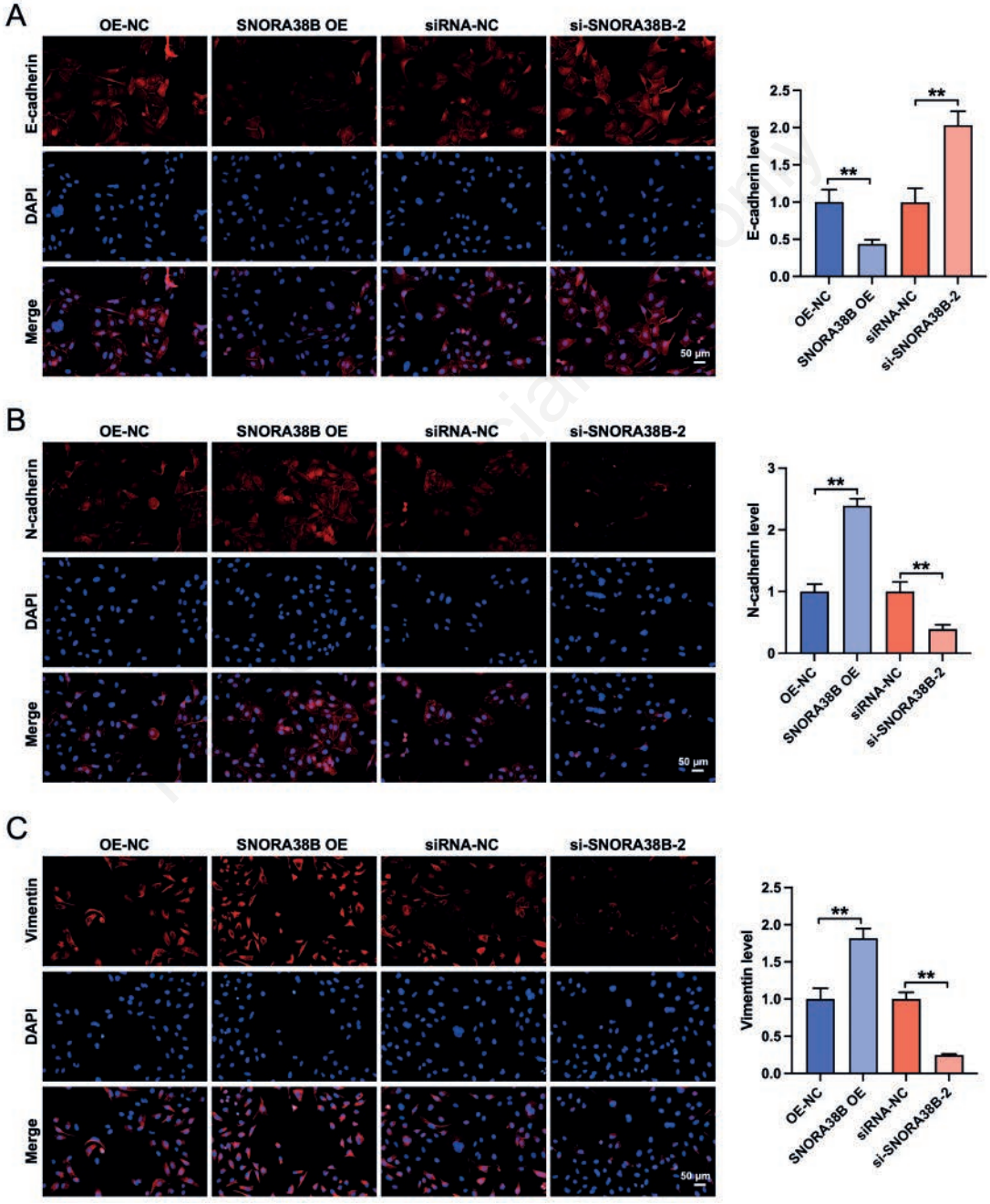

Figure 4. SNORA38B deficiency suppressed the EMT of GBC cells. GBC-SD cells were transfected with SNORA38B-OE or si-SNORA38B-2. **A-C**) IF staining assay was applied to evaluate E-cadherin, N-cadherin, and vimentin expressions in cells. **p<0.01.

SNORA7B could suppress lung cancer cell proliferation.[23] SNORA71A could enhance colorectal cancer cell growth.[13] SNORD17 could contribute to HCC progression *via* inhibiting p53 signaling.[24] snoRNA ACA11 could enhance HCC cell growth through modulating PI3K/AKT signaling.[25] Additionally, our previous research demonstrated that SNORA21 could suppress GBC cell growth.[15] These finding showed important roles of snoRNAs in cancer development. In the current research, SNORA38B level was notably elevated in GBC tissues, compared to normal tissues. Additionally, forced expression of SNORA38B greatly enhanced GBC cell proliferation, migration, and invasion in vitro; however, deficiency of SNORA38B exhibited the opposite effects. These results illustrated that SNORA38B may be an oncogene in GBC.

Ferroptosis is a type of iron-dependent form of cell death[26] that has been shown to play a crucial role in cancer development.[27] Promoting ferroptosis was able to suppress tumor progression.[28] Zhou *et al*. found that snoRNA host gene 1 (lncRNA SHNG1) could affect ferroptosis in HCC cells.[19] However, the effect of snoRNAs on ferroptosis in GBC has not been largely uncovered. In this study, our results showed that SNORA38B downregulation could elevate ROS level and Fe^{2+} levels in GBC-SD cells, suggesting that SNORA38B deficiency could induce GBC cell ferroptosis. Collectively, SNORA38B may affect GBC progression *via* regulating cell ferroptosis.

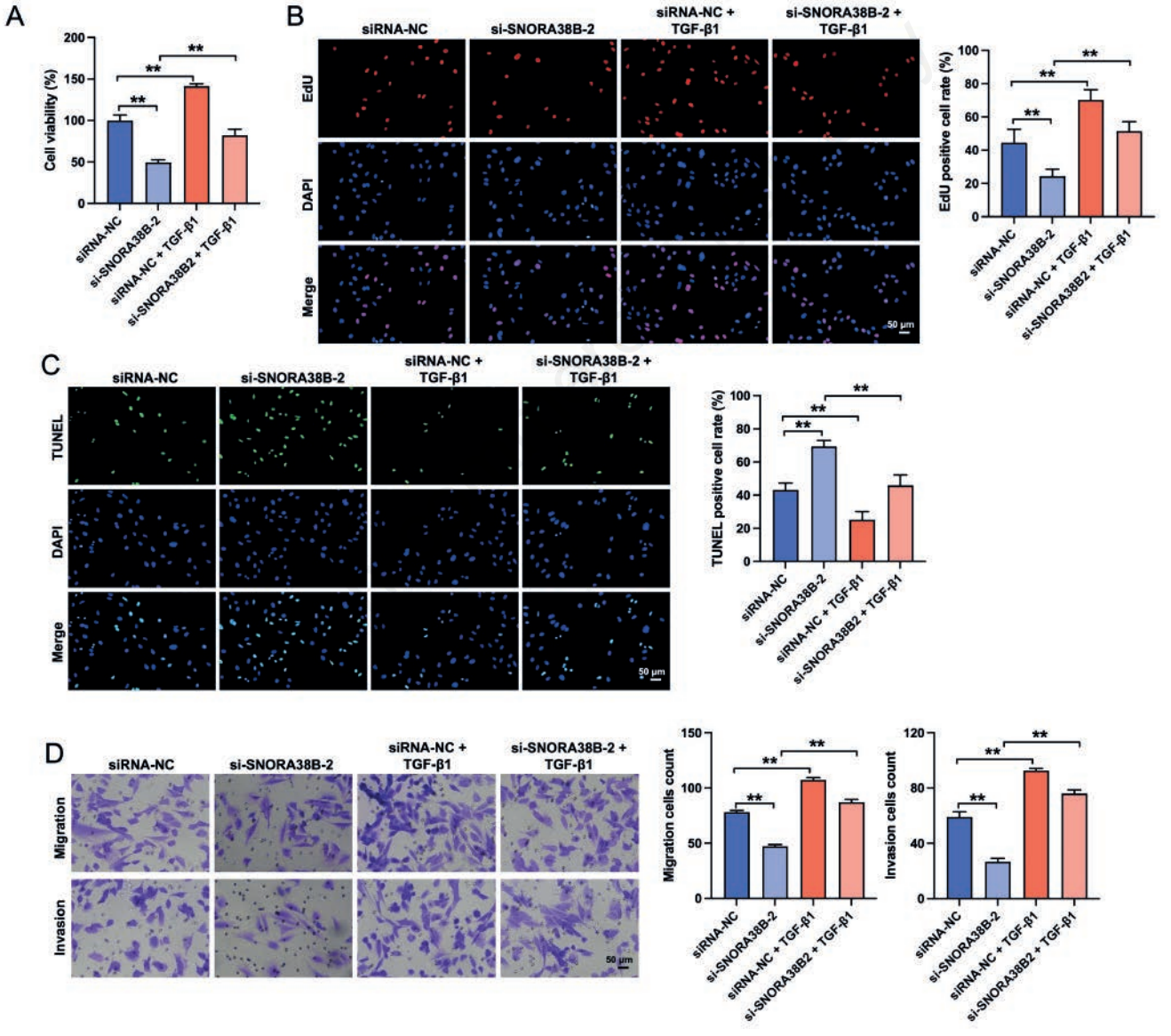

Figure 5. SNORA38B deficiency suppressed GBC cell growth *via* TGF-β signaling. GBC-SD cells were treated with si-SNORA38B-2, TGF-β1 or TGF-β1 + si-SNORA38B-2. **A**) Cell viability was assessed using CCK-8 assay. **B**) Cell proliferation was assessed using EdU staining assay. **C**) Cell apoptosis was evaluated using TUNEL staining assay. **D**) Cell migration and invasion were detected by transwell assays. **p<0.01.

EMT has been recognized to be related to tumor invasion and metastasis.[29] EMT is the process by which cells lose the epithelial phenotype with reduced adhesion property and acquire a mesenchymal phenotype with increased invasiveness.[30,31] E-cadherin and N-cadherin are two essential proteins in the EMT process.[32,33] It has been shown that E-cadherin, an epithelial cell marker, is downregulated during the EMT,[32] while the mesenchymal cell marker, N-cadherin is upregulated.[33] Zhang *et al*. indicated that snoRNA host gene 6 could promote the EMT in colorectal cancer cells *via* suppressing E-cadherin and elevating N-cadherin.[34] Our results showed that forced expression of SNORA38B could decline E-cadherin level and elevate N-cadherin and vimentin lev-

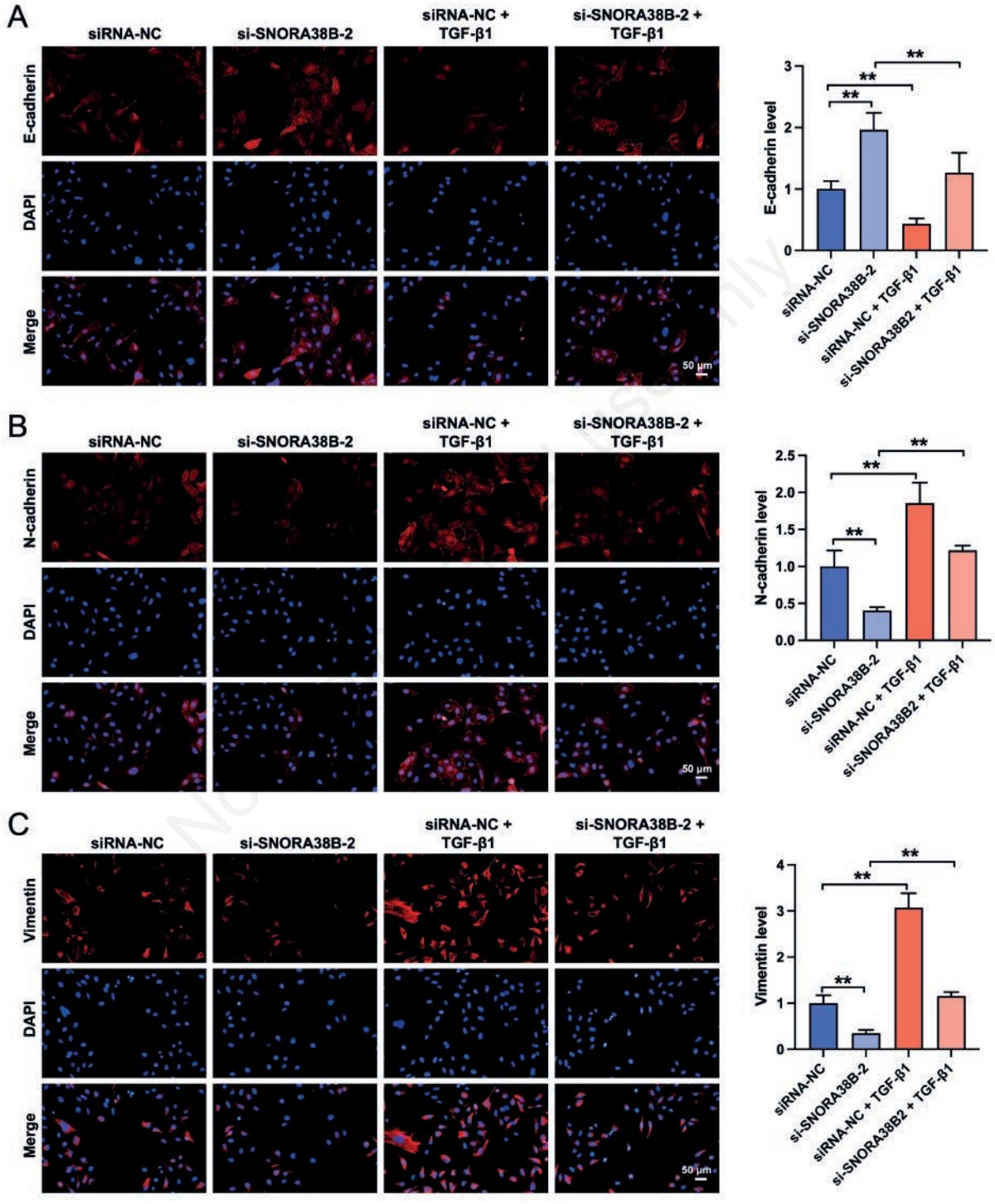

Figure 6. SNORA38B deficiency suppressed the EMT of GBC cells *via* TGF-β signaling. GBC-SD cells were treated with si-SNORA38B-2, TGF-β1 or TGF-β1 + si-SNORA38B-2. **A-C**) IF staining assay was applied for evaluating E-cadherin, N-cadherin, and vimentin expressions in cells. **p<0.01.

els in GBC cells; however, deficiency of SNORA38B exhibited the opposite effects. These results illustrated that SNORA38B overexpression could promote the EMT in GBC *via* increasing N-cadherin and vimentin levels and decreasing E-cadherin levels.

TGF-β could affect cancer initiation and progression through regulating tumor cell growth and migration.[35] Moreover, TGF-β also plays a crucial role in inducing EMT during cancer development.[36] Mechanistically, TGF-β could facilitate cancer progression through the activation of Smads (e.g., Smad2/3).[37] Suppressing TGF-β/Smad2/3 signaling could prevent cancer progression.[38] Zhang *et al*. found that TGF-β, as a tumor promoter, could promote the progression of GBC.[39] Liu *et al*. reported that activation of TGF-β1/Smad3 signaling could facilitate GBC tumor progression.[40] Xu *et al*. found that snoRNA 113-1 (SNORD113-1) level was obviously reduced in HCC tissues, SNORD113-1 overexpression was able to suppress HCC cell growth through inhibiting TGF-β1/Smad2/3 signaling.[21] In the present study, we found that deficiency of SNORA38B could prevent the phosphorylation of Smad2 and Smad3 in GBC cells. Conversely, TGF-β treatment obviously upregulated the phosphorylation of Smad2 and Smad3 in si-SNORA38B-2-transfected GBC cells, indicating that SNORA38B deficiency could inactivate TGF-β1/Smad2/3 signaling. Additionally, evidence have shown that TGF-β1 could affect ferroptosis in cancer cells.[41,42] Inhibiting TGF-β/Smads signaling could promote ferroptosis in cancer cells.[43] In the current research, our results showed that deficiency of SNORA38B could elevate ROS and Fe^{2+} levels in GBC-SD cells; however, ROS and Fe^{2+} levels in cells were reversed by TGF-β treatment. Moreover, the inhibitory effects of SNORA38B deficiency on GBC cell proliferation, migration and invasion were reversed by treatment with TGF-β. These results showed that SNORA38B deficiency could inhibit GBC cell proliferation, migration, and invasion, and induce cell ferroptosis *via* inactivating TGF-β1/Smad2/3 signaling. For the first time, the current research demonstrated a relationship between SNORA38B and TGF-β1/Smad2/3 signaling in GBC. The limitation of this study is that the number of clinical samples is small, thus we need to collect more GBC samples to validate SNORA38B level in GBC tissues in the future.

Collectively, SNORA38B level was notably elevated in GBC tissues compared to the adjacent normal ones. Moreover, forced

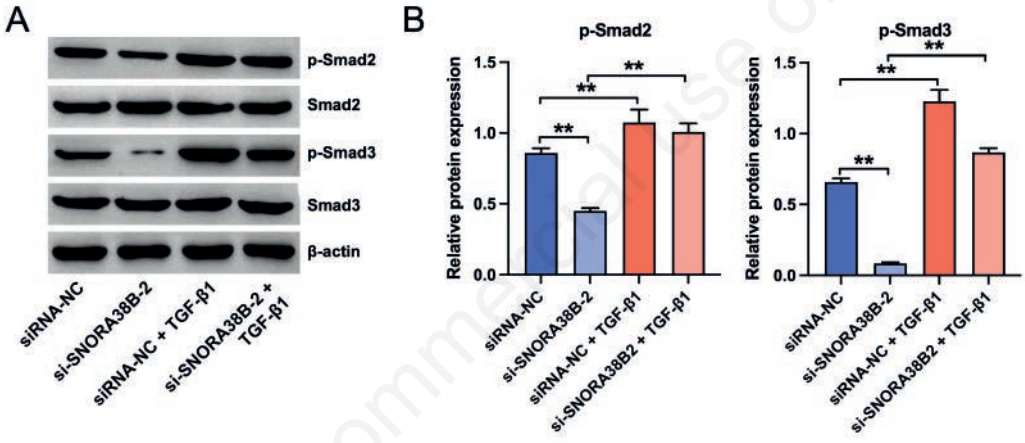

Figure 7. SNORA38B deficiency suppressed the growth of GBC cells *via* inactivating TGF-β/Smad2/3 signaling. GBC-SD cells were treated with si-SNORA38B-2, TGF-β1 or TGF-β1 + si-SNORA38B-2. **A,B**) Western blot assay was applied for detecting p-Smad2 and p-Smad3 levels in cells. **p<0.01.

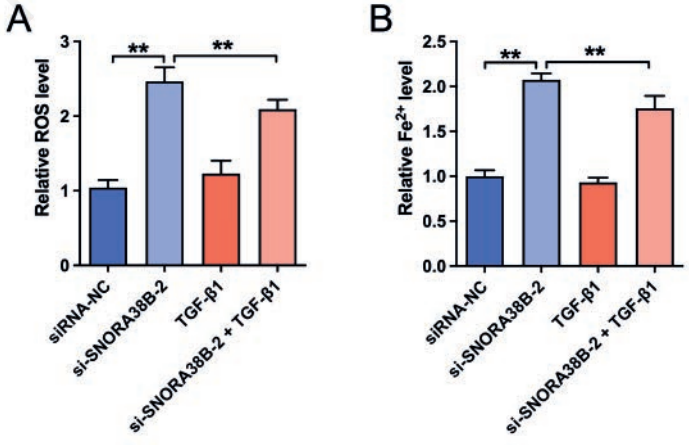

Figure 8. SNORA38B deficiency triggered ferroptosis of GBC cells *via* TGF-β signaling. GBC-SD cells were treated with si-SNORA38B-2, TGF-β1 or TGF-β1 + si-SNORA38B-2. **A,B**) The levels of ROS and Fe^{2+} levels in cells were detected using ELISA. **p<0.01.

expression of SNORA38B could enhance GBC cell growth and EMT *via* activating TGF-β1/Smad2/3 signaling. These findings showed that SNORA38B may be a potential target for GBC treatment.

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Received: 25 October 2023. Accepted: 4 December 2023.

European Journal of Histochemistry 2023; 67:3899
doi:10.4081/ejh.2023.3899