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MicroRNA-195-5p inhibits malignant progression of gallbladder cancer by regulating Wnt3a

H.-D. SUN

Department of Hepatobiliary Surgery, Liaocheng People's Hospital, Liaocheng, China

Abstract. – OBJECTIVE: We aimed at investigating whether microRNA-195-5p inhibits the malignant proliferation of gallbladder cancer (GBC) *via* regulating Wnt3a; meanwhile, its relationship with the clinicopathological parameters and prognosis of patients with GBC was also explored.

PATIENTS AND METHODS: In this study, the tumor tissues and adjacent tissues of 47 GBC patients were tested for microRNA-195-5p expression level by real-time quantitative polymerase chain reaction (qPCR); the relationship between microRNA-195-5p expression and clinical indicators of GBC patients was further analyzed. Control group (NC mimic or NC inhibitor), microRNA-195-5p overexpression group (microRNA-195-5p mimic), and microRNA-195-5p knockdown group (microRNA-195-5p inhibitor) were set in GBC cell lines, respectively. In GBC cell lines GBC-SD and NOZ, cell counting kit-8 (CCK-8), plate cloning experiments and flow cytometry were carried out to assess microRNA-195-5p's effect on proliferation and apoptosis of GBC cells. Further, the interaction between microRNA-195-5p and its downstream gene Wnt3a was explored through Luciferase reporting assay.

RESULTS: Our data showed that microRNA-195-5p expression in tumor tissues of GBC patients was remarkably lower than that in adjacent ones. In comparison to patients in highly expressed microRNA-195-5p group, those in lowly expressed microRNA-195-5p had more advanced pathological stage and larger tumor size. Overexpression of microRNA-195-5p markedly attenuated the proliferation capacity of GBC cells as compared to the NC mimic group; in contrast, knockdown of microRNA-195-5p enhanced GBC cell proliferation function of GBC cells in comparison to NC inhibitor group. At the same time, the opposite tendency in cell apoptosis was observed in the above four groups. In GBC tissue specimens, Wnt3a showed an increased expression, which was negatively correlated with microRNA-195-5p. Meanwhile, Luciferase assay verified a binding relationship between microRNA-195-5p and Wnt3a. In addition, we found that over-expressing Wnt3a counteracted the influence of upregulation of microRNA-195-5p on proliferation and apoptosis of GBC cells and thus modulate the malignant progress of GBC cells.

CONCLUSIONS: In summary, the above studies suggest that low expression of microRNA-195-5p is remarkably relevant to pathological stage and tumor size of patients with GBC. In addition, microRNA-195-5p may suppress the malignant progression of GBC through down-regulating Wnt3a.

Key Words:
MicroRNA-195-5p, Wnt3a, Gallbladder cancer, Malignant progression.

Introduction

Gallbladder Cancer (GBC), a common biliary tract tumor with no specific clinical manifestations in the early stage, is often found in the middle or late stage with a poor prognosis[1,2]. By the time of clinical diagnosis of GBC, most of the adjacent organ infiltration and/or distant metastasis through the blood system had occurred, making it difficult to perform radical surgery, and thus leading to a poor surgical effect and a 5-year survival rate less than 5%[3,4]. Epidemiological surveys show that the incidence of GBC in most areas of China is on the rise[5,6]. Therefore, in order to improve the long-term survival rate of GBC patients, in addition to further standardization and exploration of surgery in terms of surgery combined with chemoradiation, the mechanism of the occurrence, development and metastasis of GBC should also be further studied at the molecular level[7,8]. Hence, it has become the focus and hotspot of current GBC biological research to ex-

Corresponding Author: Huidong Sun, MD; e-mail: yang4@mail.broward.edu

plore the pathological mechanism of GBC at the molecular level, look for specific biological markers to improve the early diagnosis rate, and thus provide clues for molecular targeted therapy and prognosis evaluation[9].

MicroRNAs (miRNAs) are a class of endogenous non-coding short-stranded RNAs (about 22 nucleotides in size) that inhibit mRNA translation and mediate post-transcriptional gene regulation mainly through incomplete complementary pairing with the 3 'UTR of target mRNA[10-12]. MiRNAs not only play critical part in biological functions such as the proliferation, differentiation and apoptosis of GBC cell lines, but also were closely relevant to the occurrence, metastasis and other biological effects of tumors[12]. A single miRNA can affect the expression of thousands of genes, while several miRNAs can also affect the expression of a single gene simultaneously. At present, we can be sure that miRNAs are engaged in the expression of about one third of human genes[13,14]. They can interfere with protein synthesis by affecting the initiation of translation, thus further influencing the biological effects of oncogenes and tumor suppressor genes, which are important initiating factors for tumor generation[15-17]. MicroRNA-195-5p, a member of the miRNA family, is abnormally expressed in a number of solid malignant tumors and plays a role similar to that of tumor suppressor genes[18-20].

In this study, miRNA microarray technology was applied to search for miRNAs differentially expressed in normal gallbladder epithelial cells and GBC cells, and microRNA-195-5p, remarkably down-regulated in GBC cells, was selected as our research object. Bioinformatics analysis suggests that Wnt3a may be one of the potential target genes of microRNA-195-5p. Therefore, we analyzed the mRNA expression of Wnt3a and microRNA-195-5p in 47 pairs of GBC tissues and explored their effects on the biological functions of GBC cells. The purpose of this study was to investigate whether microRNA-195-5p is implicated in malignant progression of GBC cells through modulating Wnt3a.

Patients and Methods

Patients and GBC Samples

All fresh tissue specimens were obtained from 47 cases of GBC patients in our hospital's general surgical resection specimens, placed in cryopreserved tubes 0.5 h after removal of tissue samples and stored in liquid nitrogen for future use. The normal tissue adjacent to the cancer (3 cm away from the tumor) was used as a control. Inclusion criteria: (1) pathologically confirmed as GBC and treated by cholecystectomy; (2) no radiotherapy or chemotherapy before operation was received; (3) clinical data and postoperative follow-up file were complete. In addition, the patients with distant metastasis; other malignancies; mental disease; myocardial infarction; heart failure or other chronic diseases, or those previously exposed to radioactive rays were excluded. This investigation was approved by the Ethics Committee of Liaocheng People's Hospital. Signed written informed consents were obtained from all participants before the study.

Cell Lines and Reagents

Human GBC cell lines GBC-SD and NOZ cells provided by the Cell Resource Center of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (Shanghai, China) were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, Rockville, MD, USA) and William's medium supplemented with 10% fetal bovine serum (FBS) (Gibco, Rockville, MD, USA) and 100 U/mL penicillin, 100 μg/mL streptomycin (Gibco, Rockville, MD, USA), in an incubator with 5% CO_2 at 37°C.

Transfection

Lipofectamine 3000 reagent (Invitrogen, Carlsbad, CA, USA) was mixed with microRNA-195-5p mimic or inhibitor (GenePharma, Shanghai, China) and then added into cells when cell density reached to 50-70%. 48 hours later, cells were collected for analysis.

Cell Counting Kit-8 (CCK-8) Assay

Cells were collected 48 h after transfection and seeded into 96-well plates (3000 cells/well). CCK-8 assay (Dojindo Molecular Technologies, Kumamoto, Japan) was conducted based on instructions.

Colony Formation Experiment

After transfection for 48 h, 200 cells were seeded in each well of a 6-well plate and cultured with complete medium for 2 weeks. After that, the cells were cloned and fixed in 2 ml of methanol for 20 minutes. After the methanol was aspirated, the cells were stained with 0.1% crystal violet, photographed and counted under a light-selective environment.

Flow Cytometry Analysis

The combination of Annexin V-FITC (fluorescein isothiocyanate) (Merck, Billerica, MA, USA) and Propidium Iodide (PI) was used for flow cytometry analysis. Collected cell density was adjusted to 1×10^6 / mL, and cell apoptosis was detected by flow cytometry (BD Biosciences, Franklin Lakes, NJ, USA) after 15 min of staining by Annexin V and PI.

Quantitative Polymerase Chain Reaction (qPCR)

1 mL of TRIzol (Invitrogen, Carlsbad, CA, USA) was used to lyse the cells to extract total RNA from the tissue. Real-time PCR was performed according to the instructions of SYBR® Premix Ex Taq™ kit (TaKaRa, Otsu, Shiga, Japan) on StepOne Plus Real-time PCR System, with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and U6 as internal reference. Primers used in the qPCR reaction: microRNA-195-5p: forward: 5'-GGGGTAGCAGCACAGAAAT-3'; reverse: 5'-TCCAGTGCGTGTCGTGGA-3'; U6: forward: 5'-GCTTCGGCAGCACATATACTAAAAT-3', reverse: 5'-CGCTTCACGAATTTGCGTGTCAT-3'; Wnt3a: forward: 5'-ATCTGGTGGTCCTTGGCTGTG-3', reverse: 5'-ACTCCTGGATGCCCGCTTT-3'; GAPDH: forward: 5'-GGAGCGAGATCCCTCCAAAAT-3', reverse: 5'-GGCTGTTGTCATACTTCTCATGG-3'.

Western Blot

Transfected cells were collected, and proteins were extracted for quantitative detection. Cells were lysed using PRO-PREP™ protein lysate, shaken on ice for 30 minutes, and centrifuged at 14,000 × g for 15 minutes at 4°C. Total protein concentration was calculated by GBCA protein quantification kit. Immunoblotting was carried out using primary antibodies against Wnt3a, β-catenin, GSK-3β, and MAPK rabbit anti-human monoclonal antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA), with GAPDH as internal reference. The intensity of protein expression was determined using alpha SP image analysis software.

Luciferase Assay

HEK293T cells were plated in 24-well plates and co-transfected with microRNA-195-5p mimic / NC and pMIR Luciferase reporter plasmids. The plasmid was then introduced into the cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA). 48 hours later, Luciferase activity of reporter was normalized to the control using luciferase reporter assay system (Promega, Madison, WI, USA).

Statistical Analysis

χ^2-test and the exact probability Fisher test was used for Univariate analysis; while COX regression analysis was for multivariate analysis. Besides, Kaplan-Meier method was used for survival analysis by using the log-rank test. Data are presented as X ± SD (standard deviation), and p less than 0.05 was statistically significant.

Results

MicroRNA-195-5p Is Underexpressed in GBC Patients

QPCR detected that microRNA-195-5p expression levels were remarkably reduced in tumor tissues of GBC patients as compared to their paracancerous ones (Figure 1A, 1B). We divided the 47 pairs of tumor tissue collected from GBC patients into high microRNA-195-5p expression and low expression group, and further explored the association of microRNA-195-5p with age, gender, pathological stage, incidence of distant metastasis and tumor size of GBC patients. Table I shows that lowly expressed microRNA-195-5p was positively correlated with GBC pathological stage and tumor size, but not with the other three indexes (Figure 1C). Therefore, the above results suggest that microRNA-195-5p may serve as a new biological indicator for predicting the malignant progress of GBC.

MicroRNA-195-5p Inhibits the Proliferation and Promotes the Apoptosis of GBC Cells

To specify the impact of microRNA-195-5p about the biological functions in GBC cell lines, we constructed microRNA-195-5p overexpression and knockdown models in GBC cell lines and verified the transfection efficiency by qPCR (Figure 2A). Subsequently, CCK-8 assay and plate cloning experiment demonstrated that microRNA-195-5p mimic significantly attenuated the proliferation ability of GBC cells while microRNA-195-5p inhibitor enhanced that (Figure 2B, 2C); meanwhile, the opposite tendency of cell apoptosis was observed in the above groups, measured by flow cytometry (Figure 2D).

MicroRNA-195-5p Specifically Binds to Wnt3a

To further clarify by which microRNA-195-5p inhibits the malignant progress of this cancer, we performed bioinformatics analysis to reveal that

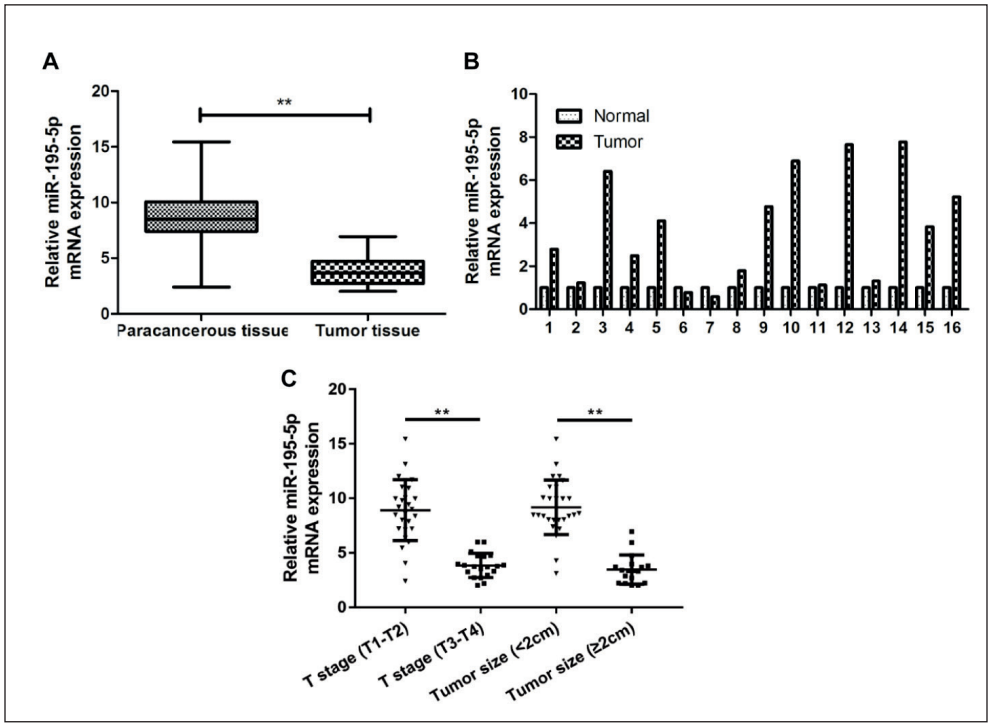

Figure 1. MiR-195-5p is underexpressed in gallbladder cancer tissues and cell lines. **A-B**, qRT-PCR detection of miR-195-5p expression in tumor tissues and adjacent tissues of gallbladder cancer; **C,** qRT-PCR detection of miR-195-5p expression in gallbladder cancer tissues with different pathological stages and tumor sizes; Data are average ± SD, ** p <0.01.

microRNA-195-5p may specifically bind to Wnt3a and further verified the interaction by luciferase assay (Figure 3A). Afterwards, the expression of Wnt/β-catenin pathway proteins Wnt3a, β-catenin, GSK-3β, and MAPK was examined by Western Blotting after microRNA-195-5p was overexpressed. It was found that microRNA-195-5p mimic remarkably reduced the above-mentioned proteins levels, which was conversely enhanced by microRNA-195-5p inhibitor (Figure 3B). Additionally, qPCR detected a significant increase in Wnt3a expression in GBC tissues specimens (Fig-

Table I. Association of miR-195-5p expression with clinicopathologic characteristics of gallbladder cancer.

Parameters	No. of cases	miR-195-5p expression		*p*-value
		High (n=24)	Low (n=23)	
Age (years)				0.871
<60	21	11	10	
≥60	26	13	13	
Gender				0.464
Male	24	11	13	
Female	23	13	10	
T stage				0.013
T1-T2	27	18	9	
T3-T4	20	6	14	
Tumor size (cm)				0.012
<2	29	19	10	
≥2	18	5	13	
Distance metastasis				0.192
No	27	16	11	
Yes	20	8	12	

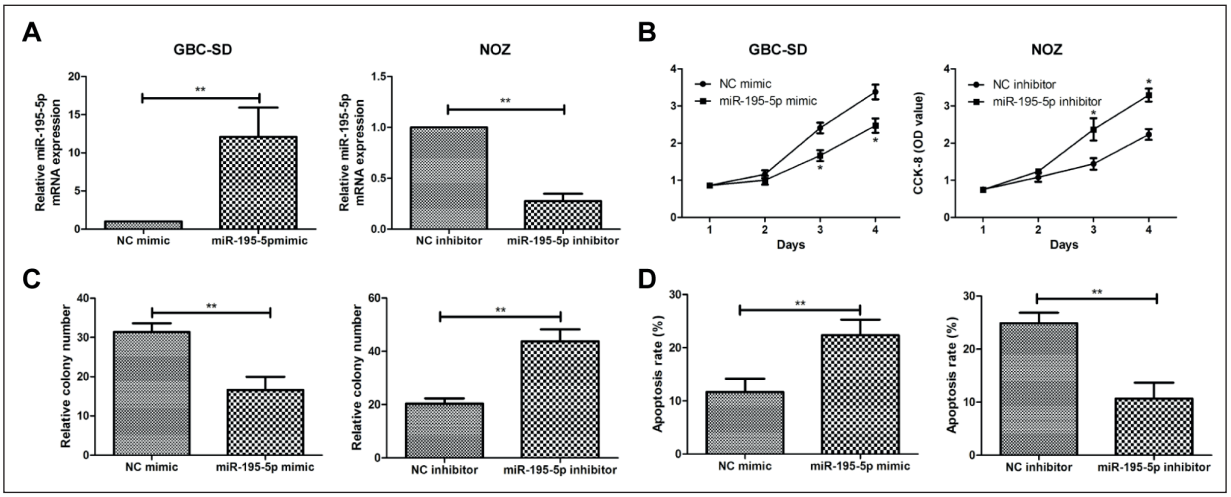

Figure 2. MiR-195-5p inhibits the proliferation of gallbladder cancer cells and increases its apoptotic capacity. **A**, qRT-PCR validates the transfection efficiency of miR-195-5p overexpression and knockdown of vectors in gallbladder cancer cell lines GBC-SD and NOZ, respectively; **B**, CCK-8 experimental detected proliferation ability of gallbladder cancer cells GBC-SD and NOZ transfected with miR-195-5p mimic or inhibitor; **C**, Plate cloning assay detected clone ability of gallbladder cancer cells transfected with miR-195-5p mimic or inhibitor; **D**, Flow cytometry assay detected apoptosis of gallbladder cancer cells transfected with miR-195-5p mimic or inhibitor. Data are average ± SD, * $p<0.05$, ** $p<0.01$.

ure 3C), suggesting that microRNA-195-5p and Wnt3a expression levels are negatively correlated in tumor tissues of GBC patients (Figure 3D).

Wnt3a Reverses the Biological Function of MicroRNA-195-5p on GBC Cells

To further specify how microRNA-195-5p and Wnt3a mutually regulate and suppress the malignant progression of GBC, we simultaneously transfected microRNA-195-5p and Wnt3a overexpression or knockdown vectors into GBC cell lines. As a result, Western Blotting experiments revealed that co-transfection of microRNA-195-5p and Wnt3a overexpression vectors enhanced Wnt3a expression more conspicuously as compared to single transfection of microRNA-195-5p mimics; conversely, the co-transfection also reduced Wnt3a expression more significantly than single transfection of microRNA-195-5p inhibitor (Figure 4A). In addition, both over-expression and knockdown of Wnt3a reversed the impact of microRNA-195-5p mimics and microRNA-195-5p inhibitors on GBC cell proliferation and apoptosis, measured by CCK8, plate cloning formation experiment and flow cytometry analysis, respectively (Figure 4B, 4C, 4D).

Discussion

As a common malignant tumor in clinical practice, GBC has attracted extensive attention. At present, early radical surgical treatment is still the most effective means of clinical treatment of GBC and the main means to improve the prognosis[1-4]. However, most patients with evident symptoms of GBC have entered the middle and late clinical stage, often losing the chance of radical surgery and having a poor prognosis[5-8]. Therefore, it is of great significance to explore the mechanism of the occurrence and development of GBC to find out molecular targeted therapy for this cancer[8-11].

As negative regulators, miRNA can effectively inhibit protein translation, and some miRNA can even degrade mRNA. Currently, a great number of evidences show that abnormal miRNA expression may affect the occurrence of various tumors[17,18]. Moreover, it can regulate protein expression through binding to specific mRNAs[14-16]. In the present years, the antitumor effect of microRNA-195-5p was found in a variety of tumors. However, the function of microRNA-195-5p in GBC was not clear. Therefore, this study was aimed at investigating whether microRNA-195-5p inhibited the malignant proliferation of GBC, and its potential mechanism. In this study, by collecting the clinical samples of cancer tissues and non-cancer tissues from GBC patients, we found that microRNA-195-5p expression, remarkably lower in GBC tissues than in normal ones, was detected to have great relevance to pathological stage and tumor size, suggesting that microRNA-195-5p may serve as an indicator for the poor

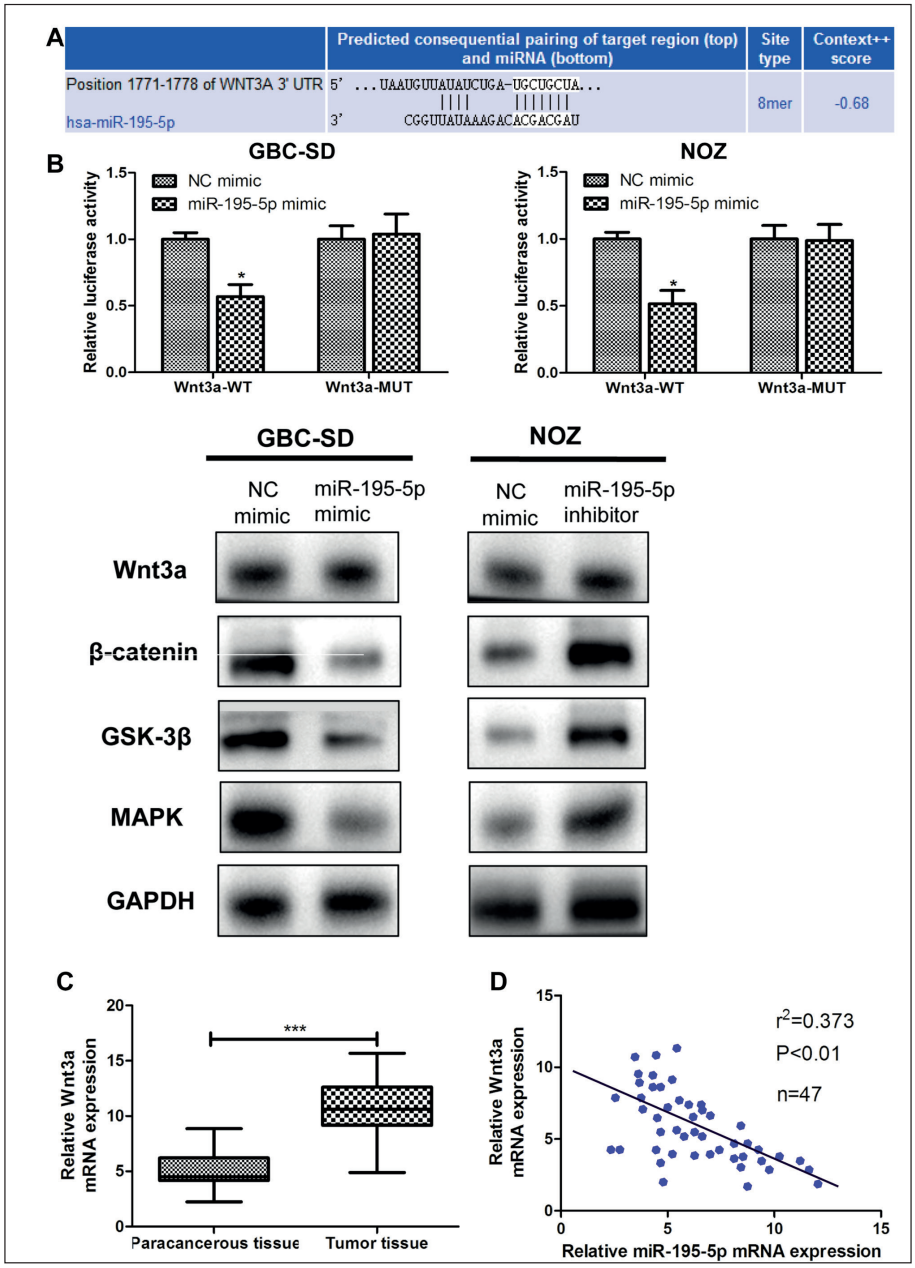

Figure 3. MiR-195-5p can bind to Wnt3a. **A**, Luciferase reporter gene experiments suggest that miR-195-5p can specifically bind to Wnt3a; **B**, Western blotting was used to verify the expression levels of Wnt / β-catenin pathway proteins Wnt3a, β-catenin, GSK-3β, and MAPK after transfection of miR-195-5p overexpression and knockdown of vectors in gallbladder cancer cell lines GBC-SD and NOZ; **C**, qRT-PCR was used to detect the expression of Wnt3a in gallbladder cancer tissues and adjacent tissues. **D**, There was a significant negative correlation between miR-195-5p and Wnt3a expression in gallbladder cancer. The data are average ± SD, * $p<0.05$, *** $p<0.001$.

prognosis of GBC. In addition, *in vitro* cell experiments demonstrated that microRNA-195-5p could inhibit the proliferation ability and promote the apoptosis of GBC cells, further confirming that microRNA-195-5p might play a role as a cancer-inhibiting gene in GBC.

To further clarify the biological functions of miRNAs in GBC, we need to search for its target genes. Bioinformatics analysis revealed that microRNA-195-5p could have inhibitory effect by acting on Wnt3a. We found that the protein expression level of Wnt3a was signifi-

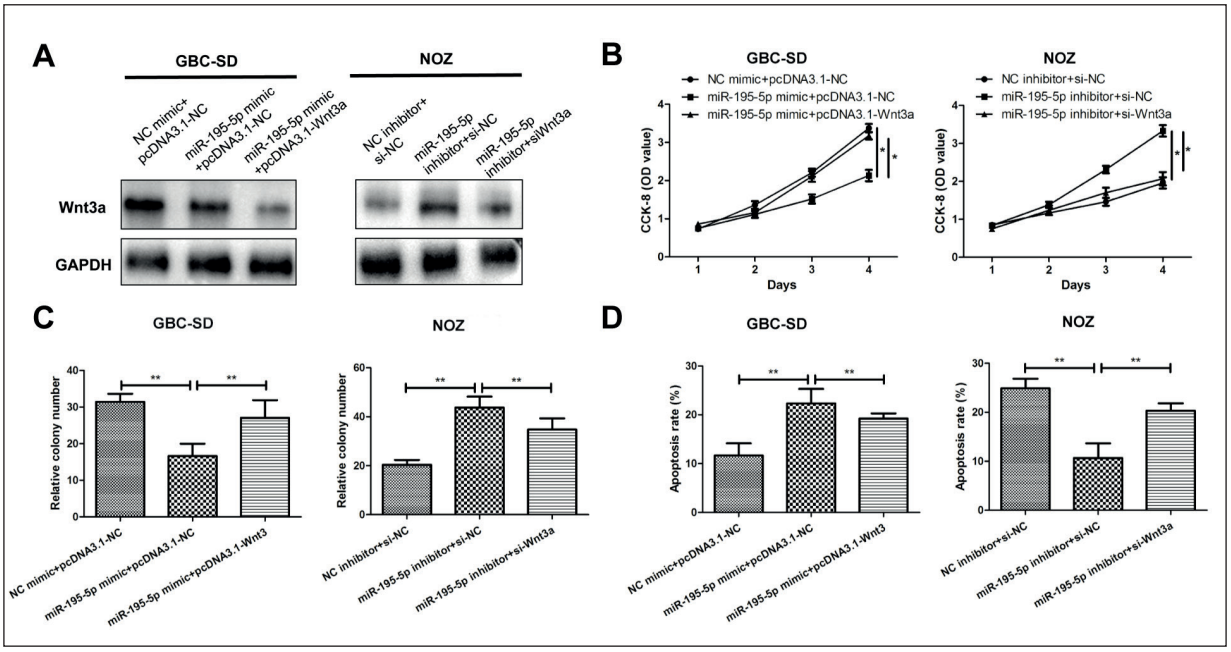

Figure 4. Wnt3a can reverse the effect of miR-195-5p on the biological function of gallbladder cancer cells. **A**, Western blotting was used to detect the expression level of Wnt3a after co-transfection of miR-195-5p and Wnt3a overexpression or knockdown vector in GBC-SD and NOZ cell lines. **B**, CCK-8 tested the gallbladder cancer cell proliferation ability after co-transfection of miR-195-5p and Wnt3a overexpression or knockdown vector in gallbladder cancer cell lines GBC-SD and NOZ. **C**, Plate cloning experiments were performed to test the gallbladder cancer cell clone formation ability in gallbladder cancer cell lines GBC-SD and NOZ after co-transfection of miR-195-5p and Wnt3a overexpression or knockdown vector. **D**, Flow cytometry was used to detect the apoptotic capacity of gallbladder cancer cells after co-transfection of miR-195-5p and Wnt3a overexpression or knockdown vector in GBC-SD and NOZ cells. Data are average ± SD, * $p<0.05$, ** $p<0.01$.

cantly decreased after overexpression of microRNA-195-5p. Meanwhile, qPCR detected an up-regulated Wnt3a expression in GBC tissue specimens as compared to that in adjacent normal ones. Wnt3a protein can activate the classic Wnt/β-catenin signaling pathway, and block phosphorylation of β-catenin by GSK-3β through phosphorylation of Dvl-1 in cells, thereby preventing β-catenin from being degraded. The increased β-catenin in the cytoplasm then transfer to the nucleus to bind to T cytokines / lymphocyte enhancers, specifically initiating and activating transcription of downstream target genes[21-23]. In our study, we verified *in vitro* that overexpression or knockdown of Wnt3a could offset the effects of overexpression or knockdown of microRNA-195-5p on GBC cell biological functions. To sum up, these findings suggested that microRNA-195-5p could inhibit the malignant progression of GBC through downregulating Wnt3a, which indicated it might be utilized in the clinical targeted therapy of GBC.

Conclusions

As reviewed, the above data suggested that the low expression of microRNA-195-5p was remarkably correlated with the pathological stage and tumor size of patients with GBC. In addition, microRNA-195-5p inhibited the malignant progression of GBC through the downregulation of Wnt3a.

Conflict of Interests

The authors declare that they have no conflict of interests.

References

1) Sharma A, Sharma KL, Gupta A, Yadav A, Kumar A. Gallbladder cancer epidemiology, pathogenesis and molecular genetics: recent update. World J Gastroenterol 2017; 23: 3978-3998.
2) Goetze TO. Gallbladder carcinoma: prognostic factors and therapeutic options. World J Gastroenterol 2015; 21: 12211-12217.

3) Shyr BU, Chen SC, Shyr YM, Lee RC, Wang SE. Metastatic polyp of the gallbladder from renal cell carcinoma. BMC Cancer 2017; 17: 244.
4) Wang H, Ling W, Luo Y. Contrast-enhanced ultrasound findings of gallbladder adenocarcinoma with sarcomatoid carcinoma accompanied by intrahepatic metastasis: a case report and literature review. Medicine (Baltimore) 2018; 97: e10773.
5) Bai Y, Liu ZS, Xiong JP, Xu WY, Lin JZ, Long JY, Miao F, Huang HC, Wan XS, Zhao HT. Nomogram to predict overall survival after gallbladder cancer resection in China. World J Gastroenterol 2018; 24: 5167-5178.
6) Shen HX, Song HW, Xu XJ, Jiao ZY, Ti ZY, Li ZY, Ren B, Chen C, Ma L, Zhao YL, Zhang GJ, Ma JC, Geng XL, Zhang XD, Shi JS, Wang L, Geng ZM. Clinical epidemiological survey of gallbladder carcinoma in northwestern China, 2009-2013: 2379 cases in 17 centers. Chronic Dis Transl Med 2017; 3: 60-66.
7) Shapera E, Bitting C. Survival: a rare outcome in large cell neuroendocrine carcinoma of the gallbladder. Acta Gastroenterol Belg 2019; 82: 433-436.
8) Kuroki T, Tajima Y, Matsuo K, Kanematsu T. Genetic alterations in gallbladder carcinoma. Surg Today 2005; 35: 101-105.
9) Sicklick JK, Fanta PT, Shimabukuro K, Kurzrock R. Genomics of gallbladder cancer: the case for biomarker-driven clinical trial design. Cancer Metastasis Rev 2016; 35: 263-275.
10) Khandelwal A, Malhotra A, Jain M, Vasquez KM, Jain A. The emerging role of long non-coding RNA in gallbladder cancer pathogenesis. Biochimie 2017; 132: 152-160.
11) Tutar Y. miRNA and cancer; computational and experimental approaches. Curr Pharm Biotechnol 2014; 15: 429.
12) Lu TX, Rothenberg ME. MicroRNA. J Allergy Clin Immunol 2018; 141: 1202-1207.
13) Rafiei H, Ashrafizadeh M, Ahmadi Z. MicroRNAs as novel targets of sulforaphane in cancer therapy: The beginning of a new tale? Phytother Res 2020; 34: 721-728.
14) Van Meter EN, Onyango JA, Teske KA. A review of currently identified small molecule modulators of microRNA function. Eur J Med Chem 2020; 188: 112008.
15) Pontecorvi G, Bellenghi M, Puglisi R, Care A, Mattia G. Tumor-derived extracellular vesicles and microRNAs: functional roles, diagnostic, prognostic and therapeutic options. Cytokine Growth Factor Rev 2020; 51: 75-83.
16) Pedroza-Torres A, Romero-Cordoba SL, Justo-Garrido M, Salido-Guadarrama I, Rodriguez-Bautista R, Montano S, Muniz-Mendoza R, Arriaga-Canon C, Fragoso-Ontiveros V, Alvarez-Gomez RM, Hernandez G, Herrera LA. MicroRNAs in tumor cell metabolism: roles and therapeutic opportunities. Front Oncol 2019; 9: 1404.
17) Matsuyama H, Suzuki HI. Systems and synthetic microRNA biology: from biogenesis to disease pathogenesis. Int J Mol Sci 2019; 21: 132.
18) Jiao X, Qian X, Wu L, Li B, Wang Y, Kong X, Xiong L. MicroRNA: the impact on cancer stemness and therapeutic resistance. Cells-Basel 2019; 9: 8.
19) Chai L, Kang XJ, Sun ZZ, Zeng MF, Yu SR, Ding Y, Liang JQ, Li TT, Zhao J. MiR-497-5p, miR-195-5p and miR-455-3p function as tumor suppressors by targeting hTERT in melanoma A375 cells. Cancer Manag Res 2018; 10: 989-1003.
20) Lin X, Wang S, Sun M, Zhang C, Wei C, Yang C, Dou R, Liu Q, Xiong B. miR-195-5p/NOTCH2-mediated EMT modulates IL-4 secretion in colorectal cancer to affect M2-like TAM polarization. J Hematol Oncol 2019; 12: 20.
21) Jiang L, Yin M, Wei X, Liu J, Wang X, Niu C, Kang X, Xu J, Zhou Z, Sun S, Wang X, Zheng X, Duan S, Yao K, Qian R, Sun N, Chen A, Wang R, Zhang J, Chen S, Meng D. Bach1 represses Wnt/beta-catenin signaling and angiogenesis. Circ Res 2015; 117: 364-375.
22) Wang J, Qiu J, Bo L, Wu Z, Zhou A, Xu W, Mao C. WT1 influences apoptosis and proliferation of immature mice granular cells through regulation of the wnt/beta-catenin signal pathway. Cell Mol Biol (Noisy-le-grand) 2019; 65: 138-145.
23) Zheng W, Yao M, Fang M, Pan L, Wang L, Yang J, Dong Z, Yao D. Oncogenic Wnt3a: a candidate specific marker and novel molecular target for hepatocellular carcinoma. J Cancer 2019; 10: 5862-5873.