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BRMS1L mediated effect of miR-20a-5p expression on proliferation and apoptosis of gastric cancer cells

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Cancer causes most fatalities worldwide next only to cardiovascular disease. Early diagnosis helps to reduce such incidences. In gastric cancer, abnormal expressions of micro ribonucleic acids (miRNAs) could serve as a marker. However, studies involving both miR-20a-5p and BRMS1L expressions are limited. Here, we studied the expressions of micro ribonucleic acid (miR)-20a-5p and its target gene breast cancer metastasis suppressor 1-like (BRMS1L) in gastric cancer tissues and their effects on the proliferation and apoptosis of gastric cancer cells. The expression of miR-20a-5p was significantly higher and the expression of BRMS1L was lower in gastric cancer tissues than those in paracancerous tissues (P <0.05). The expression of miR-20a-5p was significantly correlated with tumor-node-metastasis stage, lymph node metastasis, infiltration depth and differentiation degree (P <0.05). The miR-20a-5p group had significantly raised cell proliferation ability and expressions of cyclin D1 and Bcl-2 as well as reduced apoptosis ability and expression of caspase-3 protein than those of control and miR-NC groups (P <0.05). MiR-20a-5p is highly expressed in gastric cancer tissues and cells, and overexpression of miR-20a-5p can facilitate the proliferation and suppress the apoptosis of gastric cancer cells.

Keywords: Micro ribonucleic acid, Tumor marker

Gastric cancer, as a common cancer of the digestive tract worldwide, ranks second in incidence and mortality rates among all malignancies[1,2]. The patients with gastric cancer in China account for about 50% of global cases. About 80% of patients have entered the advanced stage upon diagnosis, manifested as potential distal metastasis and invasion. Hence, early diagnosis and interventions are crucial to improvement of the prognosis of patients with gastric cancer[3,4]. As an invasive and high-cost diagnostic method for gastric cancer, gastroscopy combined with pathological biopsy is unsuitable for large-scale screening. In addition, the sensitivity and specificity of tumor markers carcinoembryonic antigen and carbohydrate antigen 199 are low. Therefore, it is necessary to find novel diagnostic markers for early gastric cancer[5].

At present, the pathogenesis of gastric cancer remains elusive, but the abnormal expressions of several micro ribonucleic acids (miRNAs) have been involved[6]. Micro RNAs are endogenous single-strand non-coding RNAs with the lengths of 20-25 nt. Through binding mRNA of target gene by complementary base pairs, miRNAs promote degradation of the corresponding mRNA and inhibit its translation, consequently down-regulating the expression of the target gene[7]. As oncogenes or tumor suppressors, miRNAs can modulate the biological processes of tumor cells, such as proliferation, differentiation, invasion and apoptosis[8]. For instance, miR-20a-5p has been reported to modulate the growth, cell differentiation and apoptosis of several cancers. Lu *et al.*[9] reported out that up-regulating miR-20a-5p expression facilitated the proliferation and suppressed the apoptosis of pancreatic cancer cells. Li *et al.*[10], noted low expression of miR-20a-5p in six types of gastric cancer cells and its capability of promoting the apoptosis of these cells and inhibiting their proliferation. Zhou *et al.*[11] found that HAGLROS inhibited hyperglycemia-induced cardiomyocyte proliferation and promoted cell apoptosis through suppressing the expression of miR-20a-5p. MiR-20a-5p is formed by shear of miR-20a,

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Abbreviations: BCA, Bicinchoninic acid; BRMS1L, Breast cancer metastasis suppressor 1-like; DMEM, Dulbecco's modified Eagle medium; GAPDH, Glyceraldehyde-3-phosphate dehydrogenase; miR, Micro ribonucleic acid; NC, Negative control; TNM, Tumor-node-metastasis; 3'-UTR, 3'-Untranslated region

which has been extensively studied using the serum of patients with gastric cancer. Nonetheless, the expression of miR-20a-5p in gastric cancer tissues or its effect on gastric cancer cells has rarely been reported. Besides, studies involving both miR-20a-5p and BRMS1L expressions are hardly available.

Therefore, in this study, we explored the expression levels of miR-20a-5p and its target gene in gastric cancer tissues and cells, and tried to assess their effects on the proliferation and apoptosis of gastric cancer cells, thus providing theoretical basis for the early diagnosis and treatment of this cancer.

Material and Methods

Clinical data

Informed consent was obtained from all patients. This study was approved by the ethics committee of our hospital on 4th January 2020 (approval No. 202001005). The samples of surgically resected gastric cancer tissues and paracancerous tissues >5 cm away from the tumor margin were collected from 50 patients with gastric cancer treated in our hospital from January to October 2020, and then stored in liquid nitrogen. The general clinical data, including age, gender, smoking history, drinking history, tumor diameter, tumor location, tumor-node-metastasis (TNM) stage, lymph node metastasis, infiltration depth and differentiation degree, were recorded. Among the patients, there were 27 males and 23 females aged 32-81 years old.

Inclusion criteria

(a) Patients diagnosed as gastric cancer by gastroscopy and histopathological examination following Pasechnikov *et al*.[12]; and (b) those without diseases of vital organs.

Exclusion criteria:

(a) Patients with obvious inflammatory cell infiltration in paracancerous tissues; (b) those with gastric ulcer or erosion; or (c) those with other malignancies.

Materials and apparatus

Human gastric cancer cell lines (BGC-823, SGC-7901 and AGS cells) were provided by Cell Bank of Peking Union Cancer Institute (China), and gastric mucosal epithelial GES-1 cell line was provided by Obio Technology (Shanghai) Co., Ltd. (China). MiR-20a-5p mimic and mimic negative control (NC) were purchased from Shanghai GenePharma Co., Ltd. (China). MiR-20a-5p and BRMS1L primers were bought from Sangon Biotech (Shanghai) Co., Ltd. (China). Cyclin D1, Bcl-2, caspase-3 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) monoclonal antibodies were obtained from Cell Signal Technology (USA). TRIzol RNA Extraction Kit, Lipofectamine^{TM} 3000 and reverse transcription kit were provided by TaKaRa Biotech (Dalian) Co., Ltd. (China). Bicinchoninic acid (BCA) protein assay kit was purchased from Shanghai Beyotime Biotechnology Co., Ltd. (China). Fetal bovine serum, Dulbecco's modified Eagle medium (DMEM) and trypsin were bought from Gibco (USA).

DYCZ 425D double-plate vertical electrophoresis apparatus was obtained from Beijing Liuyi Instrument Plant (China). Carbon dioxide incubator and SpectraMax iD5 microplate reader were provided by Thermo Fisher Scientific (USA). IXplore Standard inverted microscope was purchased from Olympus (Japan).

Cell transfection and grouping

Gastric cancer cell lines (BGC-823, SGC-7901 and AGS cells) and gastric mucosal epithelial cell line (GES-1 cells) were resuscitated and inoculated in DMEM containing 10% fetal bovine serum, followed by incubation in an incubator with 5 percent CO_2 at 37°C. When the confluence reached 80%, the cells were digested with trypsin and then passaged.SGC-7901 cell solution (10 mL) was inoculated into a 6-well plate, and then miR-20a-5p (100 pmol) and mimic NC (100 pmol) were added into DMEM, respectively, followed by incubation with 4 μL of Lipofectamine^{TM} 3000 for 30 minutes. Then the cells were washed with mixture and incubated in DMEM for 24 h. Afterwards, the transfection efficiency was measured by flow cytometry.

Subsequently, SGC-7901 cells were divided into a control group (normal culture without transfection), a miR-NC group (transfected with mimic NC) and a miR-20a-5p group (transfected with miR-20a-5p).

Bioinformatics prediction and dual-luciferase reporter assay

Using bioinformatics database TargetScanHuman (http://www.targetscan.org/vert_72/), BRMS1L was identified to be a potential target gene of miR-20a-5p. Then BRMS1L wild type 3'-untranslated region (3'-UTR) vector (pGL3-BRMS1L-3'-UTR-WT) and mutant 3'-UTR luciferase reporter vector (pGL3-BRMS1L-3'-UTR-MUT) were constructed. SGC-7901 cells in the logarithmic growth phase were inoculated into a 6-well plate and co-transfected with a mixture of the above two plasmids with mimic NC and miR-20a-5p mimic respectively using Lipo-fectamine^{TM} 3000.

After 48 hours of transfection, the cells were harvested to prepare lysates, and the luciferase activity was detected using the kit to determine whether miR-20a-5p bound 3'-UTR of BRMS1L.

Online bioinformatics database analysis

The expressions of BRMS1L in human tissues and organs, as well as normal gastric tissues and gastric cancer tissues, were analyzed by the online bioinformatics database Human Protein Atlas (https://www.proteinatlas.org/). In addition, the effect of BRMS1L expression on the survival time of patients was analyzed by the GEPIA database (http://gepia.cancer-pku.cn/).

Detection of miR-20a-5p and BRMS1L expressions in gastric cancer tissues and cells by qRT-PCR

Total RNA was extracted from tissues and cells using TRIzol reagent and reversely transcribed into cDNA using reverse transcription kit. With U6 as an internal reference, amplification was conducted by the SYBR Green method under the following conditions: pre-denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 s and annealing at 60°C for 30 s. The relative expression levels of miR-20a-5p and BRMS1Lwere calculated by the $2^{-\Delta\Delta CT}$ method.

The primer sequences were as follows: miR-20a-5p F: 5'-AGTCTATACAAGGGCAAGCTCTC-3', R: 5'-CCCAATACGACCAAATCCGTT-3'; U6 F: 5'-CTCGCTTCGGCAGCACA-3', R: 5'-AACGCTTCACGAATTTGCGT-3'; BRMS1L F: 5'-GGCACAGCATTGATATTACCTCA-3', R: 5'-TATGGACCTGAAACAACAACTGG-3'.

Detection of cell proliferationability and number of cancer cell colonies formed by methyl MTT assay

After 24 h of transfection, the cells were digested and inoculated into a 96-well plate at a density of 1×10^3/well, with 3 duplicated wells for each one. The cells were cultured for 24, 48, 72 and 96 h, respectively, and then added methyl thiazolyl tetrazolium (MTT) solution, followed by incubation at 37°C for 4 h. After centrifugation, the supernatant was discarded, and DMSO solution was supplemented. The optical density was measured at the wavelength of 490 nm using a microplate reader.

The cells were inoculated into a 6-well plate at a density of 1×10^3/well, with 3 duplicated wells for each one. After culture for 10 days, they were fixed with 4% paraformaldehyde solution and stained with crystal violet. Colony formation was observed under the microscope and photographed.

Detection of cell apoptosis by flow cytometry

The cells were inoculated into a 6-well plate at a density of 1×10^5/well and cultured for 48 h. After washing with PBS, they were resuspended with binding buffer and mixed well with Annexin V-FITC and PI, followed by incubation in dark for 10 min. Then cell apoptosis was detected by flow cytometry.

Detection of expression levels of cyclin D1, Bcl-2 and caspase-3 by Western blotting

After the cells were lyzed with protein lysate, total protein was routinely extracted from the samples and detected using BCA protein assay kit. The protein was denatured in boiling water, subjected to SDS-PAGE and transferred onto a PVDF membrane. Subsequently, the membrane was blocked with 5% skimmed milk for 2 h and incubated with primary antibodies against cyclin D1, Bcl-2, caspase-3 and GAPDH (1:2,000) at 4°C overnight. After washing, horseradish peroxidase-labeled secondary antibodies (1:10,000) were added, followed by incubation for 2 h and colour development with DAB. ImageJ software was applied for gray value analysis with GAPDH as internal reference.

Statistical analysis

SPSS 19.0 software was employed for statistical analysis. The normally distributed measurement data were expressed as ($x \pm s$) and compared by one-way analysis of variance among groups and independent-samples t test between two groups. $P < 0.05$ was considered statistically significant.

Results

Bioinformatics prediction and dual-luciferase reporter assay

The prediction results based on the online database TargetScanHuman revealed that there were 4 binding sites between miR-20a-5p and BRMS1L, which were located at nucleotides 576-582, 948-954, 1128-1135 and 2852-2859 (Fig. 1). The dual-luciferase reporter assay showed that the luciferase activity of wild-type BRMS1L was significantly lower than that of the miR-NC group after transfection with miR-20a-5p ($P < 0.05$), while the luciferase activity of mutant BRMS1L showed no significant difference ($P > 0.05$), suggesting the targeted regulation of miR-20a-5p onBRMS1L (Fig. 2).

Bioinformatics database analysis

Human Protein Atlas database showed that BRMS1L widely exists in tissues and organs such as the cerebral cortex, thyroid, stomach, lung, skeleton, pancreas and lymph nodes (upper panel of Fig. 3). Compared with normal tissue, the expression of

	Predicted consequential pairing of target region (top) and miRNA (bottom)	Site type	Context++ score	Context++ score percentile	Weighted context++ score	Conserved branch length	P_{CT}
Position 576-582 of BRMS1L 3' UTR hsa-miR-20a-5p	5' ...UGAAAGCUAAAAGGAGCACUUUU... ||||||| 3' GAUGGACGUGAUAUUCGUGAAAU	7mer-m8	-0.24	96	-0.24	9.341	0.74
Position 948-954 of BRMS1L 3' UTR hsa-miR-20a-5p	5' ...UCUUUGGUAUUUCUACACUUUAA... |||||| 3' GAUGGACGUGAUAUUCGUGAAAU	7mer-A1	-0.04	79	-0.04	4.369	0.23
Position 1128-1135 of BRMS1L 3' UTR hsa-miR-20a-5p	5' ...UCCUGGUGGUCUUGUGCACUUUA... ||||||| 3' GAUGGACGUGAUAUUCGUGAAAU	8mer	-0.33	98	-0.32	10.272	0.96
Position 2852-2859 of BRMS1L 3' UTR hsa-miR-20a-5p	5' ...UUUUGAUGGUCUUGUGCACUUUA... ||||||| 3' GAUGGACGUGAUAUUCGUGAAAU	8mer	-0.21	95	0.00	5.195	0.93

Fig. 1 — Targets of miR-20a-5p on BRMS1L. [There were 4 binding sites between miR-20a-5p and BRMS1L, which were located at nucleotides 576-582, 948-954, 1128-1135 and 2852-2859. BRMS1L, Breast cancer metastasis suppressor 1-like]

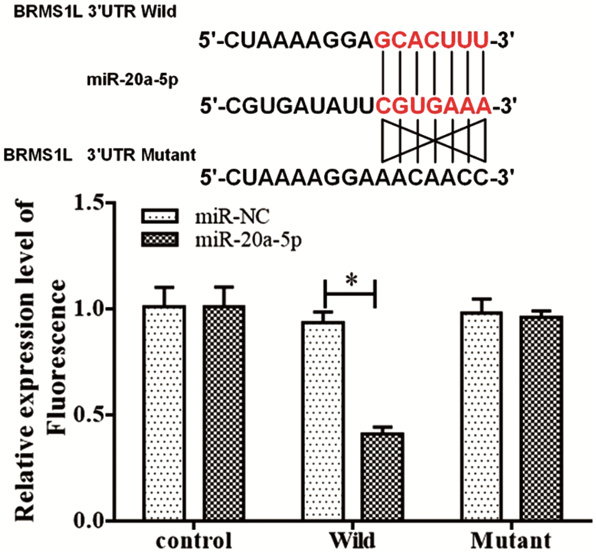

Fig. 2 — Result validation by dual-luciferase reporter assay. [**P* <0.05 *vs*. miR-NC group; NC, Negative control]

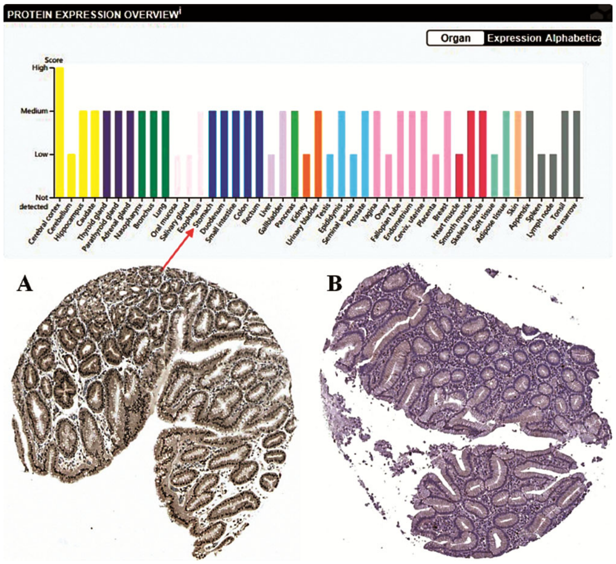

Fig. 3 — Expressions of BRMS1L in human (A) Normal gastric tissues; and (B) Gastric cancer tissues. [Compared with normal tissue, the expression of BRMS1L was significantly lower in gastric cancer tissue. BRMS1L, Breast cancer metastasis suppressor 1-like]

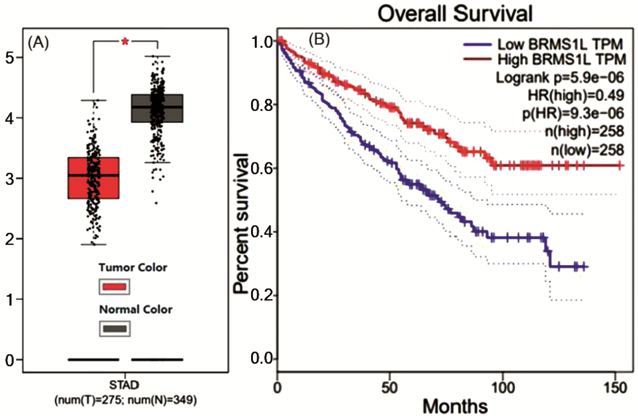

Fig. 4 — BRMS1L expression level in (A) Gastric tissues; and (B) Effects of different expression levels of BRMS1L on prognosis of patients in TCGA database and GTEx project. [Expression of BRMS1L in gastric cancer tissue was significantly lower than that in normal tissue, and the survival time was shorter in patients who had a lower expression of BRMS1L. **P* <0.01 *vs*. normal tissues. BRMS1L: Breast cancer metastasis suppressor 1-like]

BRMS1L was significantly lower in gastric cancer tissue (Fig. 3 A and B). According to the GEPIA database, the expression of BRMS1L in gastric cancer tissue was also significantly lower than that in normal tissue, and the survival time was shorter in patients who had a lower expression of BRMS1L (*P* <0.01), indicating that BRMS1L worked as a tumor suppressor (Fig. 4).

MiR-20a-5p and BRMS1L mRNA expressions in gastric cancer tissues and cell lines

The results of qRT-PCR exhibited that the expression of miR-20a-5p was significantly higher and the expression of BRMS1L was significantly lower in gastric cancer tissue than those in para-cancerous tissue (*P* <0.01) (Fig. 5). In comparison with normal gastric mucosal epithelial cell line (GES-1 cells), the expression of miR-20a-5p was significantly higher and the expression of BRMS1L was significantly lower in gastric cancer cell lines (BGC-823, SGC-7901 and AGS cells) (*P* <0.01).

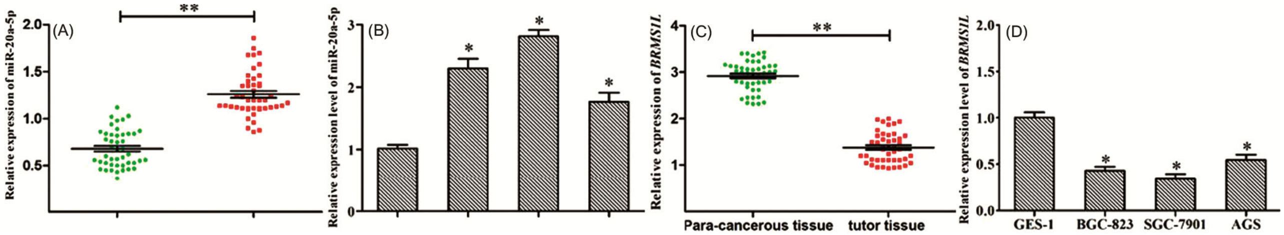

Fig. 5 — Expressions of miR-20a-5p and BRMS1L in (A and C) Gastric cancer tissues; and (B and D) Gastric cancer cells. [Expression of miR-20a-5p was significantly higher and the expression of BRMS1L was significantly lower in gastric cancer tissue than those in paracancerous tissue. *P <0.01 *vs*. normal tissues. BRMS1L: Breast cancer metastasis suppressor 1-like]

Table 1 — Correlations of miR-20a-5p expression level in gastric cancer tissues with clinicopathological parameters of patients [n (%)]

Parameters	n	miR-20a-5p expression		χ^2	P
		Low	High		
Age (Y)				1.299	0.254
<65	22	13 (52.00)	9 (36.00)		
≥65	28	12 (48.00)	16 (64.00)		
Gender				0.725	0.395
Male	27	15 (60.00)	12 (48.00)		
Female	23	10 (40.00)	13 (52.00)		
Smoking history				0.368	0.544
Yes	16	7 (28.00)	9 (36.00)		
No	34	18 (72.00)	16 (64.00)		
Drinking history				0.439	0.508
Yes	12	5 (20.00)	7 (28.00)		
No	38	20 (80.00)	18 (72.00)		
Tumor diameter (cm)				1.389	0.239
≤5	18	11 (44.00)	7 (28.00)		
>5	32	14 (56.00)	18 (72.00)		
Tumor location				3.949	0.139
Gastric antrum	23	15 (60.00)	8 (32.00)		
Gastric body	11	4 (16.00)	7 (28.00)		
Gastric fundus and cardia	16	6 (24.00)	10 (40.00)		
TNM stage				6.650	0.010
Stage I-II	29	19 (76.00)	10 (40.00)		
Stage III-IV	21	6 (24.00)	15 (60.00)		
Lymph node metastasis				5.195	0.023
Yes	28	10 (40.00)	18 (72.00)		
No	22	15 (60.00)	7 (28.00)		
Infiltration depth				6.876	0.009
Mucosa	31	11 (44.00)	20 (80.00)		
Submucosa	19	14 (56.00)	5 (20.00)		
Differentiation degree				5.882	0.015
Low	34	13 (52.00)	21 (84.00)		
Moderate/high	16	12 (48.00)	4 (16.00)		

Correlations of miR-20a-5p expression with clinicopathological features of patients

To validate the role of miR-20a-5p in the onset and progression of gastric cancer, 50 patients were assigned into high and low expression groups. The results displayed that miR-20a-5p expression was not significantly correlated with age, gender, smoking history, drinking history, tumor diameter or tumor location (P >0.05), whereas it was significantly associated with TNM stage, lymph node metastasis, infiltration depth and differentiation degree (P <0.05) (Table 1). A higher miR-20a-5p expression corresponded to a later TNM stage, a larger infiltration depth, and a higher risk of lymph node metastasis.

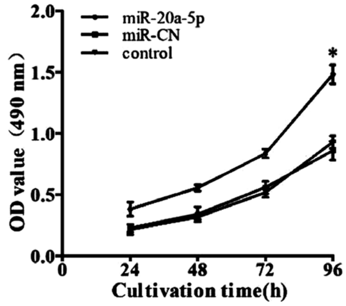

Fig. 6 — Detection of cell proliferation ability by MTT assay. [The miR-20a-5p group had a significantly higher cell viability than those of control and miR-NC groups, while the viability showed no significant difference between control and miR-NC groups.*P <0.05 *vs*. miR-NC group. NC, Negative control]

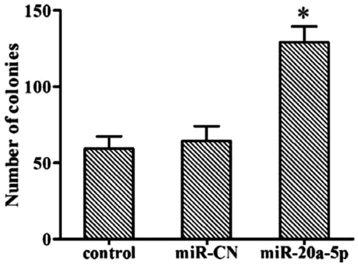

Fig. 7 — Number of formed cancer cell colonies. [Number of formed colonies in the miR-20a-5p group significantly exceeded those of control and miR-NC groups, while there was no significant difference in the number between control and miR-NC groups. *P <0.05 *vs*. miR-NC group. NC, Negative control]

Cell proliferationability and number of cancer cellcolonies

The MTT assay revealed that the miR-20a-5p group had a significantly higher cell viability than those of control and miR-NC groups (P <0.05), while the viability showed no significant difference between control and miR-NC groups (P >0.05) (Fig. 6).

The monoclonal formation assay exhibited that the number of formed colonies in the miR-20a-5p group significantly exceeded those of control and miR-NC groups (P <0.05), while there was no significant difference in the number between control and miR-NC groups (P >0.05) (Fig. 7).

Cell apoptosis

The results of flow cytometry demonstrated that the miR-20a-5p group had a significantly lower apoptosis rate than those of control and miR-NC groups ($P < 0.05$), while no significant difference was found in the rate between control and miR-NC groups ($P > 0.05$) (Fig. 8).

Expression levels of cyclinD1, Bcl-2 and caspase-3

Western blotting showed that the miR-20a-5p group had significantly higher expression levels of cyclin D1 and Bcl-2 and lower expression of caspase-3 than those of control and miR-NC groups ($P < 0.05$), while the expressions of these proteins had no significant differences between control and miR-NC groups ($P > 0.05$) (Fig. 9).

Discussion

Micro RNAs are able to modulate the expressions of target genes and participate in the proliferation, migration, invasion and apoptosis of tumor cells as oncogenes or tumor suppressors[13,14]. It is well-documented that miR-20a-5p works as an oncogene. Guo *et al.*[15] has shown that miR-20a-5p relieved the endothelial cell injury induced by oxidized low-density lipoprotein through targeted regulation on myocardin-related transcription factor-A. Wang *et al.*[16] reported that acupuncture down-regulated the expression of miR-20a-5p, thereby promoting cell proliferation and inhibiting apoptosis in the rats with cerebral ischemia-reperfusion injury. Additionally, Bai *et al.*[17] found that miR-20a-5p was highly expressed in triple-negative breast cancer and facilitated the growth of tumor cells by targeting human runt-related transcription factor 3. However, Yu *et al.*[18] reported that miR-20a-5p was lowly expressed in neuroblastoma and suppressed the proliferation of tumor cells through targeted regulation on autophagy-related gene 7. Overall, miR-20a-5p plays various regulatory roles in the onset and progression of tumors. In this study, gastric cancer tissues had a significantly higher expression of miR-20a-5p than that in paracancerous tissues, and miR-20a-5p expression was closely associated with TNM stage, lymph node metastasis, infiltration depth and differentiation degree ($P < 0.05$). Furthermore, a higher miR-20a-5p expression corresponded to a later TNM stage, a larger infiltration depth and a higher risk of lymph node metastasis ($P < 0.05$). The proliferative ability of SGC-7901 cells was markedly enhanced and their apoptosis ability was attenuated after transfection with miR-20a-5p mimic, suggesting that miR-20a-5p functioned as an oncogene.

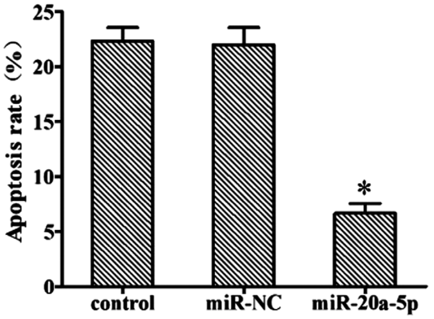

Fig. 8 — Detection of cell apoptosis by flow cytometry. [The miR-20a-5p group had a significantly lower apoptosis rate than those of control and miR-NC groups, while no significant difference was found in the rate between control and miR-NC groups.*P <0.05 *vs*. miR-NC group. NC, Negative control]

Through promoting the deacetylation of histone deacetylase and binding specific transcription factors, BRMS1L, a homologous gene of BRMS1, can regulate the expressions of related genes and suppress the proliferation and metastasis of various tumor cells. Cao *et al.*[19] reported that BRMS1L suppressed the

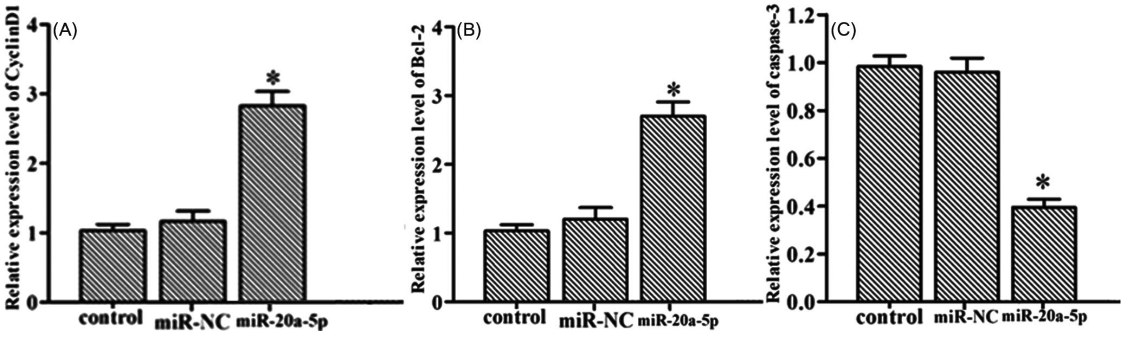

Fig. 9 — Detection of expression levels of cyclin D1, Bcl-2 and caspase-3 by Western blotting. [The miR-20a-5p group had significantly higher expression levels of cyclin D1 and Bcl-2 and lower expression of caspase-3 than those of control and miR-NC groups, while the expressions of these proteins had no significant differences between control and miR-NC groups. *$P < 0.05$ *vs*. miR-NC group. NC, Negative control]

invasion and metastasis of ovarian cancer cells by inhibiting the Wnt/β-catenin signaling pathway. Besides, Lv *et al.*[20] found that BRMS1L suppressed the invasion of neuroblastoma, thus acting as a tumor suppressor. Chen *et al.*[21] showed that miR-17-5p facilitated the proliferation, invasion, migration and apoptosis of nasopharyngeal carcinoma CNE2 cells by downregulating the expression of BRMS1L. In this study, the binding sites between miR-20a-5p and BRMS1L were predicted by the online database TargetScanHuman, and the targeted negative regulation of miR-20a-5p on BRMS1L was validated by dual-luciferase reporter assay. Additionally, Human Protein Atlas and GEPIA databases displayed that the expression of BRMS1L in gastric cancer tissues remarkably rose and the survival time was shorter in patients who had a lower expression of BRMS1L, suggesting that miR-20a-5p negatively regulated BRMS1L expression, thereby suppressing the proliferation of gastric cancer cells and resulting in poor prognosis.

Tumor growth is closely associated with cell proliferation and apoptosis, and tumor progression alters when the expressions of proliferation- and apoptosis-related genes affect tumor growth[22]. Cyclin D1, as a pro-proliferation gene, is involved in regulating cell cycle, and its coding products allow the progression of cell cycle and enhance cell viability through binding corresponding kinases. As an inhibitor of apoptosis, Bcl-2 antagonizes pro-apoptosis gene Bax. Caspase-3 is a pro-apoptotic gene which can be activated by the mitochondrial pathway and death receptor pathway, thus inducing cell apoptosis. Xu *et al.*[23] reported that cucurbitacin decreased the expression of cyclin D1 in gastric cancer cells and effectively inhibited cell proliferation. Moreover, Song *et al.*[24] found that the overexpression of miR-494 down-regulated the expression of cyclin D1, consequently suppressing the proliferation and cell cycle progression of gastric cancer cells. In the study conducted by Khashab *et al.*[25], atorvastatin increased the expression of caspase-3 and suppressed that of Bcl-2, thus affecting the proliferation, cycle and apoptosis of gastric cancer cells. Similarly, in this study, the expressions of cyclin D1 and Bcl-2 significantly increased and that of caspase-3 reduced in the miR-20a-5p group, suggesting that miR-20a-5p induced the proliferation and inhibited the apoptosis of cancer cells by modulating the expressions of cyclinD1, Bcl-2 and caspase-3. However, the mechanism by which miR-20a-5p up-regulates the expressions of cyclin D1 and Bcl-2 and down-regulates that of caspase-3 still needs further research.

Conclusion

In summary, the expressions of miR-20a-5p and BRMS1L increased in gastric cancer tissues and cells, and the overexpression of miR-20a-5p facilitated the proliferation and suppressed the apoptosis of gastric cancer cells. The results provide theoretical and experimental bases for the treatment of gastric cancer by inhibiting the expression of miR-20a-5p. Regardless, this study is limited. Further, we need to study the correlation between biomarkers with disease severity and outcomes in a longitudinal setting to find biomarkers.

Conflict of interest

Authors declare no competing interests.

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