MYBL2 in synergy with *CDC20* promotes the proliferation and inhibits apoptosis of gastric cancer cells

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Abstract

Background. Gastric cancer (GC) is a common malignant tumor with a high morbidity and mortality worldwide. It has been reported that V-Myb avian myeloblastosis viral oncogene homolog-like 2 (*MYBL2*) could be a promising prognostic biomarker for GC. However, the specific role of *MYBL2* in GC progression remains unclear.

Objectives. To examine the role of *MYBL2* in GC progression and investigate the underlying mechanisms.

Materials and methods. The mRNA levels of target genes were detected using quantitative real-time polymerase chain reaction (RT-qPCR) and protein expression was measured with western blot analysis. Cell Counting Kit-8 (CCK-8) and colony formation assays were employed to inspect HGC-27 cell proliferation, and cellular apoptosis was determined with TUNEL staining. Finally, the interaction of *MYBL2* and cell division cycle 20 (*CDC20*) was verified with immunoprecipitation.

Results. *MYBL2* was confirmed to be overexpressed in GC cells. *MYBL2* knockdown inhibited HGC-27 cell proliferation and promoted cellular apoptosis, and these effects were reversed by *CDC20* overexpression. Interestingly, *MYBL2* interacted with *CDC20* and regulated its expression. *MYBL2* knockdown also inhibited activation of the Wnt/β-catenin signaling pathway, while *CDC20* overexpression showed the opposite effect.

Conclusions. In summary, the synergy between *MYBL2* and *CDC20* induced the proliferation of GC cells and inhibited cell apoptosis; these effects may have involved the Wnt/β-catenin signaling pathway. Thus, *MYBL2* may be a promising target for GC treatment.

Key words: apoptosis, gastric cancer, proliferation, cell division cycle 20 (*CDC20*), V-Myb avian myeloblastosis viral oncogene homolog-like 2 (*MYBL2*)

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Background

Gastric cancer (GC) is the 5th most common malignant tumor and the associated mortality rate is 3rd globally.[1,2] There are more than one million new GC cases diagnosed worldwide each year and this disease has become a major burden on human health.[3] Since patients with early GC have no obvious symptoms, the majority are already at an advanced stage at the time of diagnosis.[4,5] While great progress has been achieved through the development of new diagnostic methods and treatment strategies, the five-year survival rate in advanced patients has not exceeded 15%.[6] Due to the complex molecular mechanisms involved in the onset and development of GC, the specifics of GC pathogenesis are still unclear. Hence, it is important to explore novel and effective biomarkers for the diagnosis, treatment and prognosis of GC.

V-Myb avian myeloblastosis viral oncogene homolog-like 2 (*MYBL2*) is a member of the MYB family of transcription factors and is involved in the regulation of infinite replicative potential, evasion from apoptosis, tissue infiltration, and metastasis.[7] Expression of *MYBL2* is ubiquitous and can be observed in almost every proliferating cell.[8] Overexpression of this gene is also associated with a poor prognosis in multiple cancers.[7] Upregulation of *MYBL2*, a key downstream effect of Akt/FoxM1 signaling, facilitates the progression of glioma.[9] Recently, *MYBL2* overexpression has been also observed in malignant tumors including colorectal cancer,[10] acute myeloid leukemia[11] and breast cancer,[12] suggesting that this gene plays an essential role in tumor cell growth and carcinogenesis. A search of the CCLE database (https://portals.broadinstitute.org/ccle) suggests that *MYBL2* is generally upregulated in numerous tumor cell lines. Interestingly, it has been reported that *MYBL2* is relevant to cancer cell differentiation and lymph node metastasis. Studies have shown that its expression is negatively correlated with the survival rate of GC patients, suggesting that this gene could be a promising prognostic biomarker for gastric adenocarcinoma.[6] However, the specific role of *MYBL2* in the occurrence and progression of GC remains unclear.

Cell division cycle 20 (*CDC20*), a gene first discovered in yeast, plays an essential role in the progress of cell cycle.[13] *CDC20* is an indispensable developmental gene as its inhibition in mice leads to chromosome condensation and embryonic death, partly due to abnormal mitosis.[14] *CDC20* ablation can also effectively inhibit the invasiveness of mouse skin tumors, mainly due to increased apoptosis.[15] In addition, a retrospective study identified *CDC20* expression as a useful biomarker for the prognosis of pancreatic cancer.[16] This gene has also been studied in a great diversity of other tumors.[17]

Objectives

The results cited above suggest that *MYBL2* is very promising as a biomarker for the prognosis of GC. Thus, the aim of this study is to verify the role of *MYBL2* in GC progression, and more importantly, to investigate the underlying mechanisms.

Materials and methods

Cell culture and transfection

Human GC cell lines, including MKN-45, MKN-74, AGS and HGC-27, and a normal gastric GES-1 cell line were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). All of these cell lines were cultured in RPMI-1640 medium (Gibco, Thermo Fisher Scientific, Waltham, USA) containing 10% fetal bovine serum (FBS; Thermo Fisher Scientific), 100 U/mL of penicillin-G, and 100 µg/mL of streptomycin. The cells were maintained in a 37°C humidified atmosphere with 5% CO_2.

Small interfering (si)-*MYBL2* (si-MYBL2-1 5′-CCAAGAGCACACCTGTTAA-3′; si-MYBL2-2 5′-CCAGAAACATGCTGCGTTT-3′), and the scramble siRNA (si-NC, 5′-ACGTGACACGTTCGGAGAATT-3′) as a negative control (NC), were obtained from Shanghai GenePharma Co., Ltd. (Shanghai, China). The si-*MYBL2* (50 nM) and si-NC (50 nM) were transfected into HGC-27 cells (5×10^5 cells/well) using Lipofectamine 2000 (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. In addition, *CDC20* transcript cDNA was inserted into the pCDNA3.1 by Lederer Biological Technology (Guangdong, China), and then transfected into HGC-27 cells (20 µg) to achieve *CDC20* overexpression (Ov-*CDC20*). An empty vector without *CDC20* sequence was used as the negative control (OV-NC).

RT-qPCR

Total RNA from HGC-27 cells was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific) following the manufacturer's protocol. Complementary DNA (cDNA) was synthesized using a Reverse Transcription kit (Thermo Fisher Scientific) according to manufacturer's instructions. Real-time quantitative polymerase chain reaction (RT-qPCR) was performed using Roche SYBR Green PCR kits (Roche Diagnostics, Basel, Switzerland) and carried out using the Opticon Real-Time PCR Detection System (ABI 7500; Life Technologies, Carlsbad, USA). The *GAPDH* gene was used as an internal gene for normalization. The cycling conditions were as follows: 1 cycle of 95°C for 2 min and 40 cycles of 95°C for 15 s, with a final extension at 60°C for 60 s. The relative mRNA quantity was calculated using the $2^{-\Delta\Delta Cq}$ method.[18] The primer sequences were as follows: *MYBL2* forward, 5'-AAAACAGTGAGGAGGAAC-3' and reverse, 5'-CAGGGAGGTCAAATTTAC-3'; *CDC20* forward, 5'-GGCACCAGTGATCGACACATTCGCAT-3' and reverse, 5'-GCCATAGCCTCAGGGTCTCATCTGCT-3'; and *GAPDH* forward, 5'-CTGGGCTACACTGAGCACC-3' and reverse, 5'-AAGTGGTCGTTGAGGGCAATG-3'.

Western blot analysis

The cells were washed with cold phosphate-buffered saline (PBS) and then lysed in a lysis buffer supplemented with phenylmethylsulfonyl fluoride (1 mM), trypsin (10 μg/mL), aprotinin (10 μg/mL), and leupeptin (10 μg/mL). A bicinchoninic acid (BCA) protein assay was used to quantify the protein concentration. Proteins (25 μg/lane) were separated in a 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto polyvinylidene difluoride PVDF membranes. The membranes were then blocked with skim milk for 2 h at room temperature and subsequently incubated with primary antibodies against *MYBL2* (#PA5-79713; 1:1000), PCNA (#13-3900; 1:1000), Ki-67 (#14-5698-82; 1:1000), Bcl-2 (#MA5-11757; 1:1000), Bax (#33-6400; 1:1000), cleaved caspase-3 (#ab2302; 1:500; Abcam, Cambridge, UK), caspase-3 (#MA5-11521; 1:1000), *CDC20* (#PA5-63103; 1:1000), c-Myc (#MA1-980; 1:1000), β-catenin (#MA1-301; 1:1000), p-GSK-3β (#MA5-14873; 1:500), and GSK-3β (#39-9500; 1:1000) at 37°C overnight. *GAPDH* (#39-8600; 1:1000) was used as a loading control. After washing with PBS 3 times, the polyvinylidene difluoride (PVDF) membranes were incubated with horseradish peroxidase (HRP)-goat anti-rabbit secondary antibody (#G-21234; 1:50000; Invitrogen) for 2 h at room temperature, and the intensities of the bands were analyzed using ImageJ software v. 1.6 (National Institutes of Health, Bethesda, USA). Antibodies that are not branded were obtained from Thermo Fisher Scientific.

CCK-8 assay

A Cell Counting Kit-8 (CCK-8) assay (Beyotime Institute of Biotechnology, Haimen, China) was used to investigate the effects of *MYBL2* knockdown and *CDC20* overexpression on the viability of HGC-27 cells. Cells were seeded in 3 independent 96-well plates (5×10^3 cells/well) and incubated for 24 and 48 h at 37°C. Following this, CCK-8 reagent (10 μL) was added into each well and the plates were subsequently incubated at 37°C for another 2 h. The absorbance at 450 nm was detected using an enzyme-linked immunosorbent assay (ELISA) plate reader (Bio-Rad, Hercules, USA).

Colony formation assay

The colony formation assay was performed to detect the effect of *MYBL2* knockdown and *CDC20* overexpression on cell proliferation. After transfection, cells (1×10^3/well) were seeded in a 35-mm petri dish and incubated for 10 days at 37°C to form colonies. Subsequently, HGC-27 cells were fixed with 4% paraformaldehyde for 5 min and stained with 0.1% crystal violet solution for 20 min at room temperature. The number of colonies (diameters >0.5 mm) within a field was counted using a digital camera (Nikon Corp., Tokyo, Japan).

TUNEL staining

HGC-27 cells (1×10^5 cells/mL) were seeded in six-well plates and then fixed in 4% paraformaldehyde for 5 min at room temperature. After permeabilization with 0.1% Triton X-100 (Sigma-Aldrich, Merck KGaA, St. Louis, USA) for 5 min, HGC-27 cells were stained according to the protocol of the ApopTag Fluorescein In Situ Apoptosis Detection kit (Chemicon International Inc., Temecula, USA). Nuclei were labeled with 4′,6-diamidino-2-phenylindole (DAPI) and quantified under a fluorescence microscope at ×200 magnification (Leica Microsystems GmbH, Wetzlar, Germany).

Immunoprecipitation

Cells were collected and lysed with immunoprecipitation (IP) lysis buffer containing protease inhibitors. After centrifugation at 12,000 × g at 4°C, *MYBL2* antibody (1 μg) was added into the supernatant and the samples were placed on a rotating platform overnight at 4°C. Subsequently, 50 μL of SureBeads™ protein G magnetic beads (No. 1614023; Bio-Rad) were added into the above mixture at 4°C with gentle rotation for 4 h. The pellets were dissolved in 60 μL ×1 electrophoresis sample buffer and boiled for 5 min. Samples (30 μL) were analyzed using western blot analysis as outlined above.

Statistical analyses

Data are presented as the mean ± standard error of mean (SEM) of at least 3 experiments. Statistical analyses was performed using SPSS v. 17.0 software (SPSS Inc., Chicago, USA). Analysis of variance (ANOVA) followed by Bonferroni's post hoc test were used to determine the differences in the means among multiple groups. P-value <0.05 was considered to indicate a statistically significant difference.

Results

MYBL2 is highly expressed in GC cells

A search of the CCLE database suggested that *MYBL2* is generally upregulated in numerous tumor cell lines, including GC cell lines (Fig. 1A). To explore the effect of *MYBL2* on GC progression, the mRNA and protein expression of *MYBL2* were detected using RT-qPCR and western blot analysis, respectively. The mRNA and protein expression of *MYBL2* is significantly upregulated in GC cell lines, including MKN-45, MKN-74, AGS, and HGC-27, compared to the GES-1 cell line, suggesting that *MYBL2* may play an oncogenic role in the onset and development of GC (Fig. 1B,C).

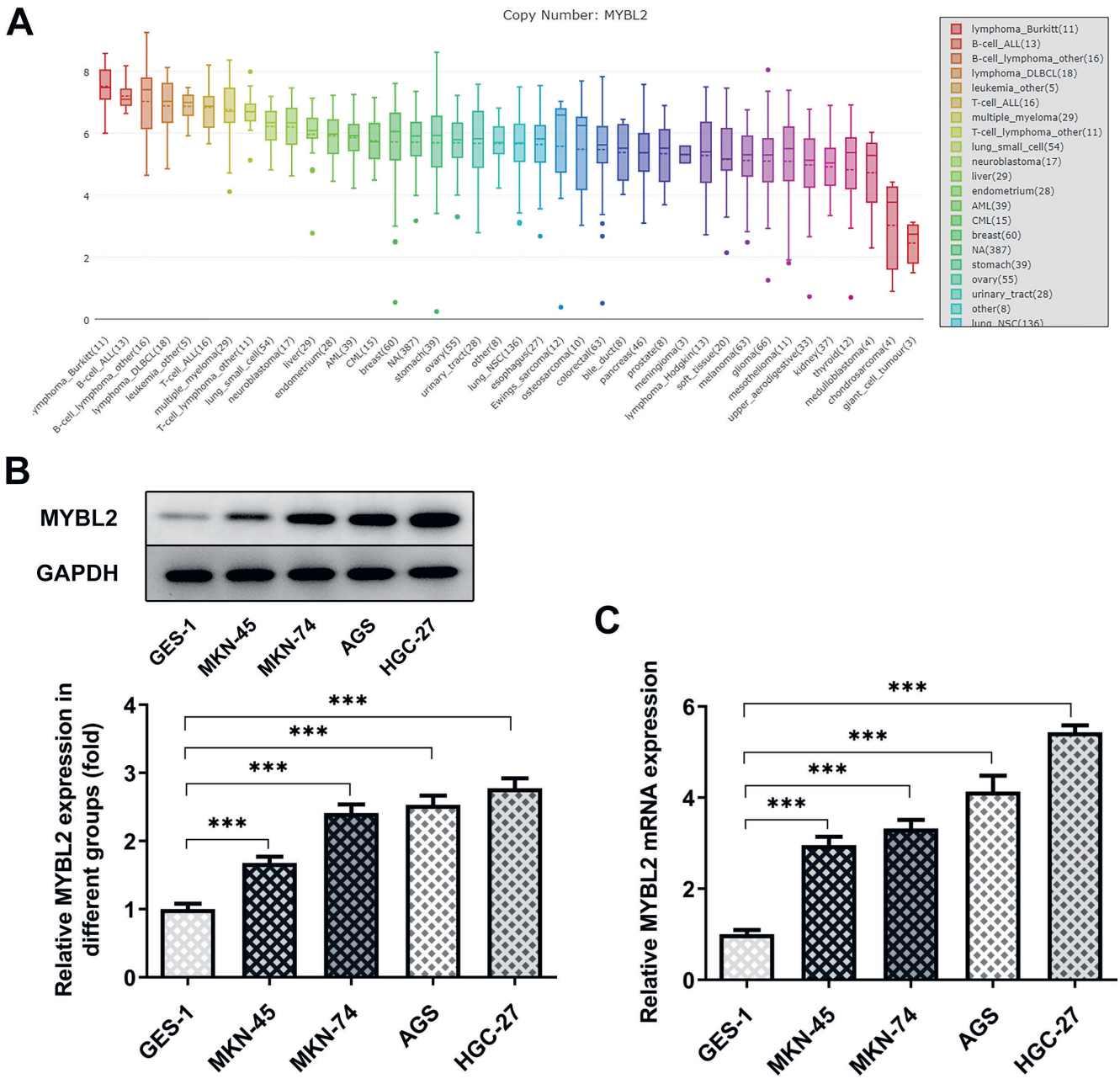

Fig. 1. *MYBL2* is highly expressed in gastric cancer (GC) cells. A. The *MYBL2* expression in multiple cancer cell lines in the CCLE database; B. The MYBL2 protein expression was determined with western blot analysis and quantification; C. The *MYBL2* mRNA level was analyzed with quantitative real-time polymerase chain reaction (RT-qPCR). Error bars represent the mean ±standard error of mean (SEM) from 3 independent experiments

***$p < 0.001$.

MYBL2 silencing inhibits HGC-27 cell growth

To examine the specific effect of *MYBL2* on GC progression, cell growth was analyzed with CCK-8 and colony formation assays. The si-*MYBL2* was used to achieve *MYBL2* knockdown. As shown in Fig. 2A, si-*MYBL2* caused a significant reduction in the *MYBL2* mRNA level, especially in the si-MYBL2-1 group. Hence, si-MYBL2-1 was selected for the subsequent experiments. Moreover, the results of the CCK-8 assay showed that viability was significantly depressed by *MYBL2* knockdown at the indicated time (24 h and 48 h), as compared to the control (Fig. 2B). The results of the western blot analysis demonstrated that the expression levels of proliferative markers, including proliferating cell nuclear antigen (PCNA) and Ki-67, were decreased in HGC-27 cells from the si-*MYBL2* group (Fig. 2C,D). Finally, the results of the colony formation assay showed that *MYBL2* silencing remarkably suppressed the proliferation of HGC-27 cells (Fig. 2E). These results indicate that *MYBL2* silencing inhibits HGC-27 cell growth.

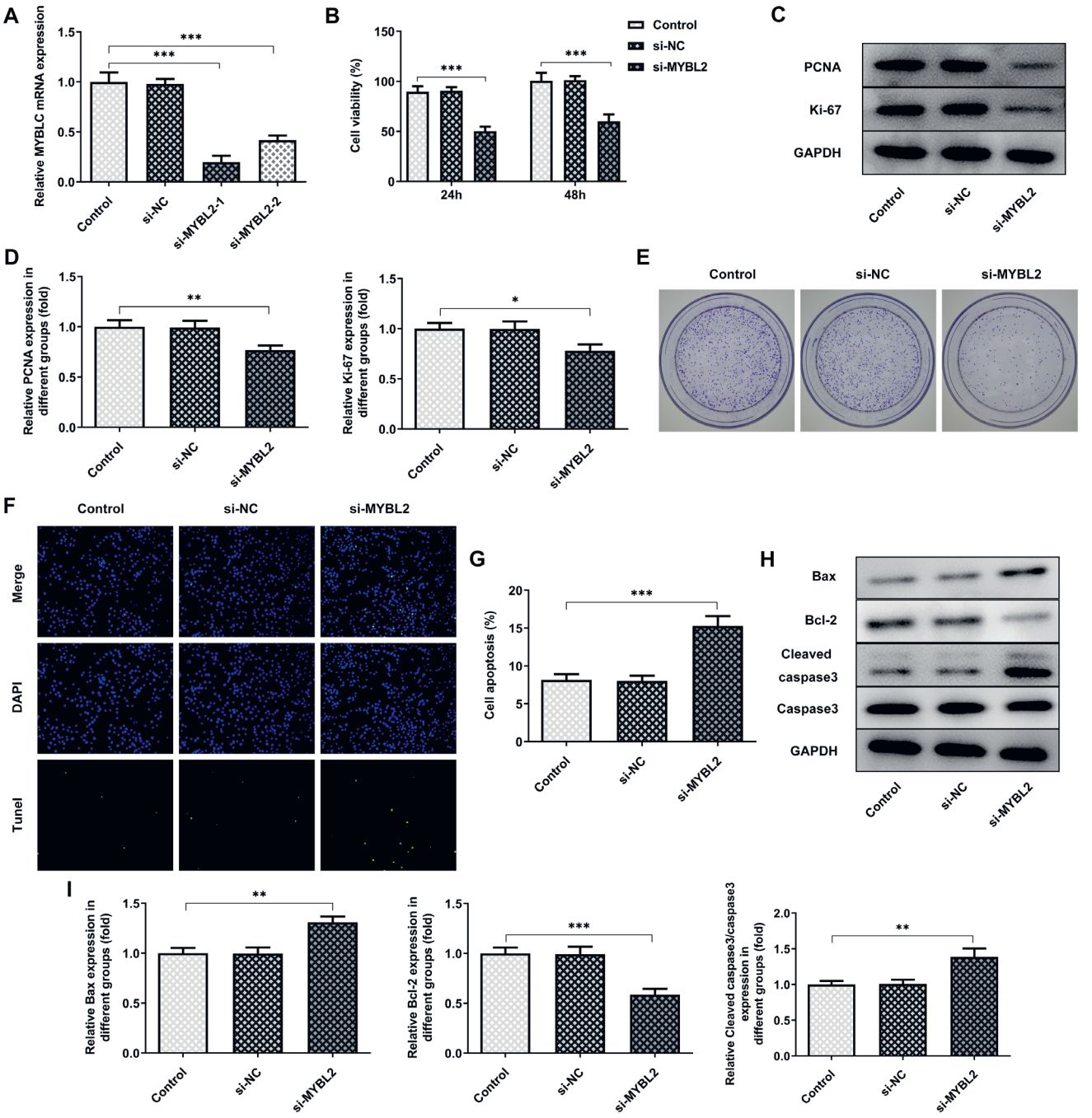

Fig. 2. *MYBL2* silencing inhibits cells growth and promotes cell apoptosis of HGC-27 cells. A. The *MYBL2* mRNA level was analyzed with quantitative real-time polymerase chain reaction (RT-qPCR); B. The HGC-27 cell viability was detected using the Cell Counting Kit-8 (CCK-8) assay; C and D. The proliferating cell nuclear antigen (PCNA) and Ki-67 protein expressions were determined with western blot analysis and quantification; E. HGC-27 cell proliferation was analyzed using colony formation assay; F and G. The HGC-27 cell apoptosis was determined with TUNEL staining; H and I. Bax, Bcl-2, cleaved caspase-3, and caspase-3 protein expressions were determined using western blot analysis and quantification. Error bars represent the mean ±standard error of mean (SEM) from 3 independent experiments

*p < 0.05; **p < 0.01; ***p < 0.001.

MYBL2 silencing promotes the apoptosis of HGC-27 cells

Apoptosis was analyzed by TUNEL staining to detect the specific effect of *MYBL2* on GC progression. The HGC-27 cell apoptosis rate in the si-MYBL2-1 group was higher than that in the control group (Fig. 2F,G). Additionally, the expression levels of Bax (pro-apoptotic), Bcl-2 (anti-apoptotic) and cleaved caspase-3/caspase-3 were assessed using western blot analysis. *MYBL2* silencing led to the loss of Bcl-2 and upregulation of Bax and cleaved caspase-3 (Fig. 2H,I). These results imply that *MYBL2* silencing promotes the apoptosis of HGC-27 cells.

MYBL2 interacts with *CDC20* and regulates its expression

To investigate the underlying mechanism of *MYBL2* in GC progression, studies were carried out to explore its downstream targets. Based on the LinkedOmics website (www.linkedomics.org), the correlation between the *MYBL2* and *CDC20* in GC was analyzed. It was found that *MYBL2* expression is positively correlated with most genes (Fig. 3A,B), and is highly correlated with *CDC20* ($p < 0.0001$; Fig. 3C). Furthermore, the co-expression of *MYBL2* and *CDC20* in the Gene Expression Omnibus (GEO) database was analyzed using COEXPEDIA (https://www.coexpedia.org/), as shown in Fig. 3D.

The role of *CDC20* in GC progression and its expression in GC cells was assessed by RT-qPCR and western blot analysis. The mRNA and protein expression levels of *CDC20* were significantly increased in GC cell lines, including MKN-45, MKN-74, AGS, and HGC-27, as compared to the GES-1 cell line (Fig. 3E,F). Moreover, *MYBL2* knockdown inhibited protein and mRNA expression of *CDC20* (Fig. 3G,H). Consistently with the search results outlined above, the IP assay showed that endogenous *CDC20* and *MYBL2* formed a complex in HGC-27 cells (Fig. 3I). Collectively, these results suggest that *MYBL2* interacts with *CDC20* in vitro.

MYBL2 knockdown inhibits the proliferation and promotes apoptosis of HGC-27 cells through the regulation of *CDC20* expression

To confirm the mechanism by which *MYBL2* regulates GC cell growth, the biological significance of the interaction between *MYBL2* and *CDC20* was examined. First, an Ov-*CDC20* plasmid was constructed, and its transfection efficiency was confirmed using RT-qPCR and western blot analysis (Fig. 4A,B). The results from the CCK-8 assay demonstrated that *CDC20* overexpression partly abolished the inhibitive effects of *MYBL2* knockdown on HGC-27 cell viability (Fig. 4C) and proliferative markers expressions (Fig. 4D). Consistent with these observations, the results from the colony formation assay showed that *CDC20* overexpression reversed the inhibitive effect of *MYBL2* silencing on HGC-27 cell proliferation (Fig. 4E). Furthermore, the results from TUNEL staining showed that the cell apoptosis rate was significantly decreased in HGC-27 cells co-transfected with si-*MYBL2* and Ov-*CDC20* compared to cells transfected with si-*MYBL2* alone (Fig. 4F,G). The Bcl-2 expression level in HGC-27 cells from the si-*MYBL2*+Ov-*CDC20* group was higher, while the expression levels of Bax and cleaved caspase-3 was lower, than that in si-*MYBL2* group (Fig. 4H,I). These results indicate that *MYBL2* knockdown inhibits the proliferation and promotes the apoptosis of HGC-27 cells through the regulation of *CDC20* expression.

Effects of *MYBL2* on the Wnt/β-catenin signaling pathway

To further clarify the underlying mechanisms of *MYBL2* in GC progression, the effects of *MYBL2* on the Wnt/β-catenin signaling pathway were analyzed. As shown in Fig. 5, *MYBL2* knockdown led to a reduction in the expression of β-catenin, p-GSK-3β and mc-Myc, which was reversed by *CDC20* overexpression. These results suggest that *MYBL2* knockdown induces inactivation of Wnt/β-catenin signaling pathway.

Discussion

Gastric cancer is a common malignant tumor with a high morbidity and mortality globally. Nowadays, due to risk factors such as changes in diet and lifestyle, the incidence of GC is increasing.[19] It is a disease that is highly heterogeneous in terms of molecular and cellular phenotype, and is diagnosed histologically through endoscopic biopsy. Endoscopic resection is mainly used for early-stage GC, and surgery is mainly used for advanced GC.[1] Despite the advances that have been achieved in diagnosis and therapy, the outcome for GC patients remains poor. There are great limitations in the understanding of the etiology of GC, which involves a multifaceted process and complex molecular mechanisms. Hence, it is important to explore promising targets for GC treatment.

The MYB family of proteins contains numerous subtypes with diverse functions, the majority of which act as transcription factors and have different numbers of MYB domain repeats. This latter feature endows these proteins with the ability to bind DNA.[20] Rapidly accumulating evidence now suggests that the MYB family regulates the cell cycle to maintain DNA replication, cell survival and proliferation.[9,21,22] It has also been reported that *MYBL2* expression is significantly increased in numerous cancer tissues as compared to adjacent tissue, and is negatively associated with the survival rate of cancer patients.[7] In addition, *MYBL2* downregulation inhibits the proliferation and DNA replication of gallbladder cancer cells in vitro,[23] which is consistent with the current findings. Specifically, in the current study, it was observed that *MYBL2* is overexpressed in GC cell lines (MKN-45, MKN-74, AGS, and HGC-27) compared to a normal gastric cell line (GES-1). Hence, *MYBL2* may be an oncogene in GC progression. To confirm the role of *MYBL2* in GC progression, *MYBL2* downregulation was induced by the transfection of si-*MYBL2*, and the survival and apoptosis of HGC-27 cells were detected using CCK-8, colony formation and TUNEL assays. The results suggested that *MYBL2* downregulation inhibits the proliferation and promotes the apoptosis of HGC-27 cells.

In order to explore the underlying mechanisms by which *MYBL2* regulates GC progression, the molecules that can

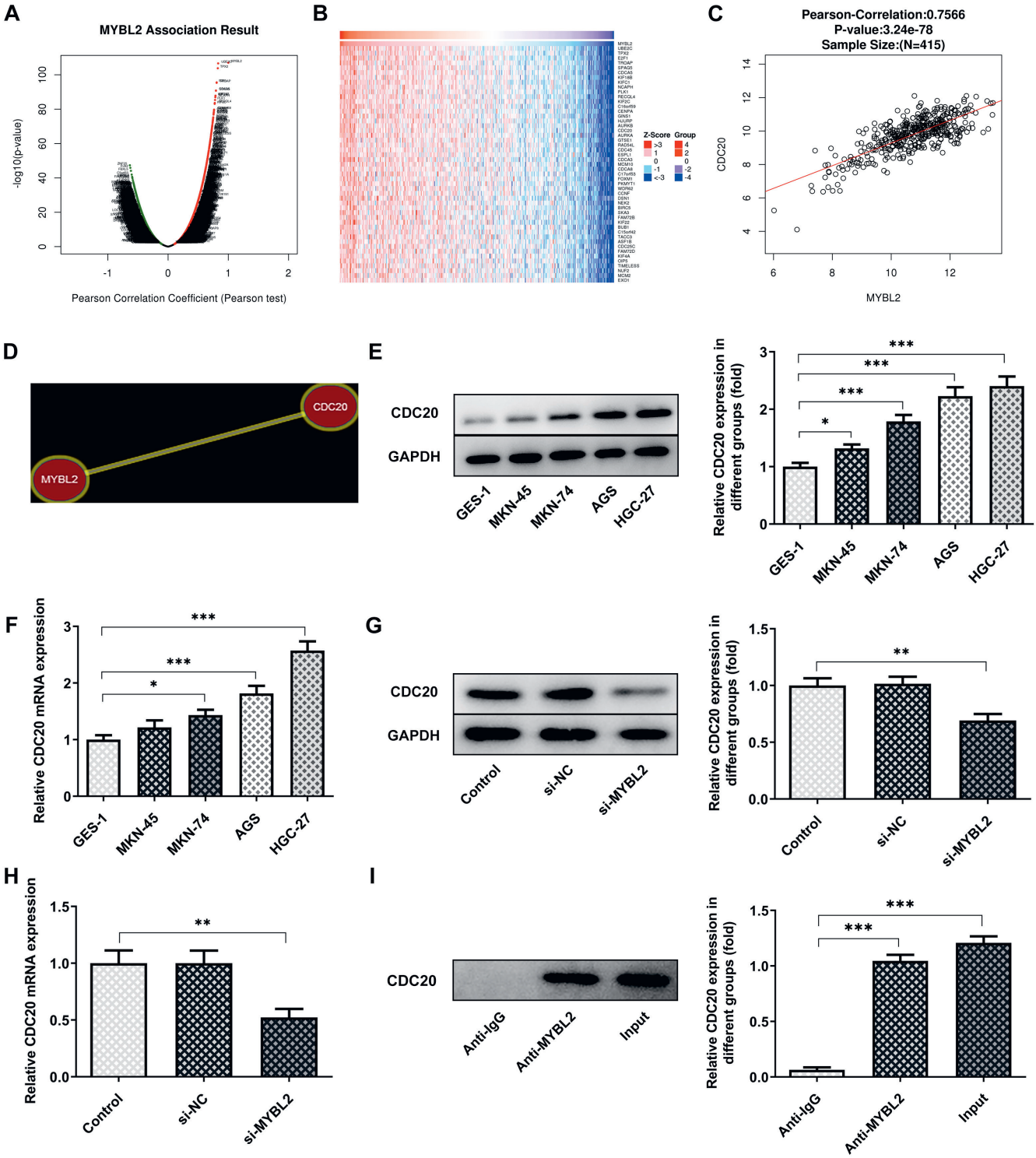

Fig. 3. *MYBL2* interacts with *CDC20* and regulates its expression. A–C. The correlation between *MYBL2* and *CDC20* in gastric cancer (GC) was analyzed using the LinkedOmics website; D. The co-expression of *MYBL2* and *CDC20* in the Gene Expression Omnibus (GEO) database was analyzed using the COEXPEDIA website; E and G. The CDC20 protein expression was determined with western blot analysis and quantification; F and H. The *CDC20* mRNA level was analyzed with quantitative real-time polymerase chain reaction (RT-qPCR); I. The interaction between *MYBL2* and *CDC20* was determined using IP. Error bars represent the mean ±standard error of mean (SEM) from 3 independent experiments

*p < 0.05; **p < 0.01; ***p < 0.001.

interact with this protein were searched on the LinkedOmics and COEXPEDIA websites. The results suggested that *MYBL2* expression is positively correlated with *CDC20*. Consistent with these search results, an IP assay indicated that *MYBL2* interacts with *CDC20* in vitro. It has been reported that *CDC20* possesses regulatory potential at multiple points of the cell cycle and plays a carcinogenic role in various types of tumor.[24] For example, 445 breast

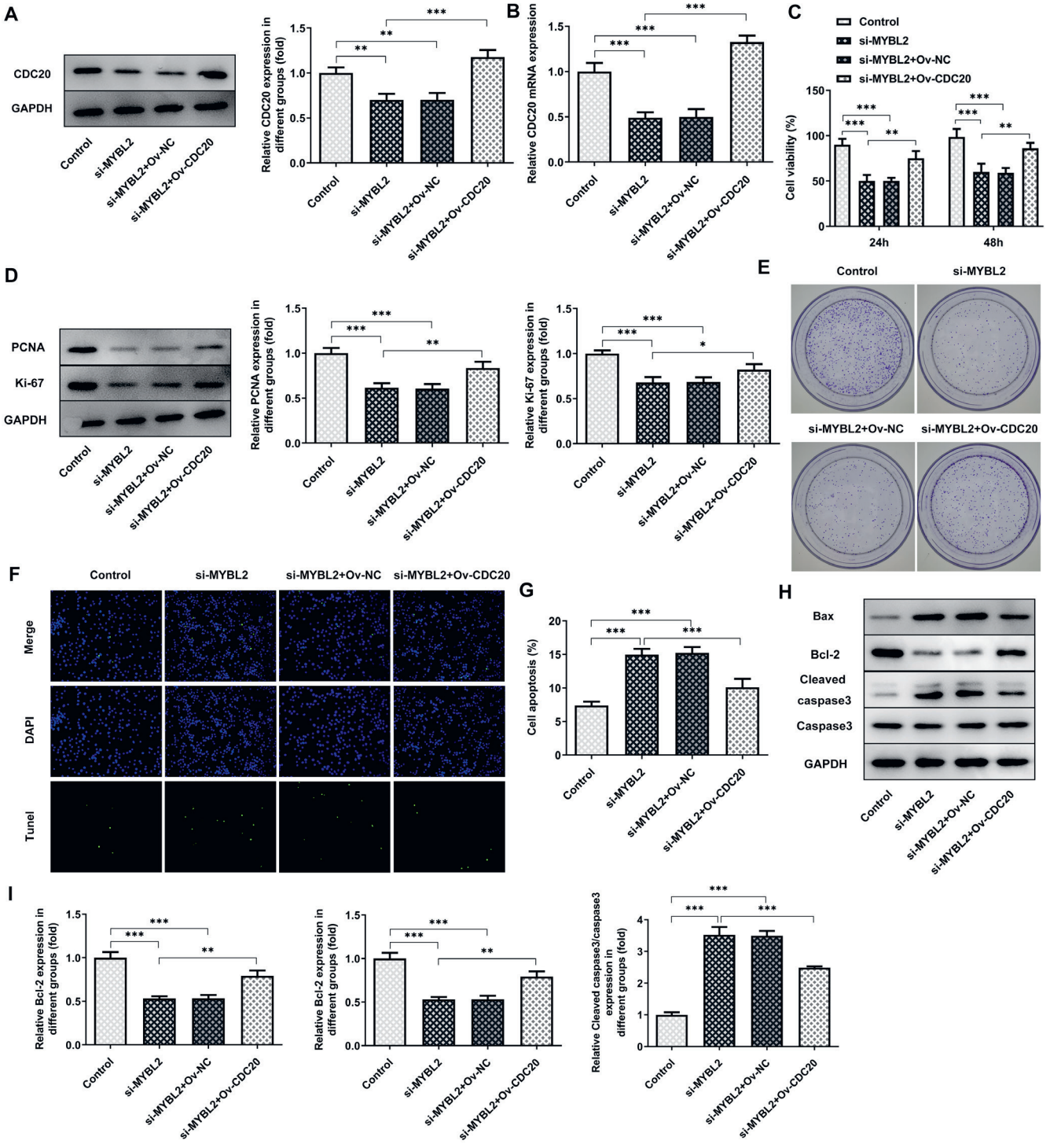

Fig. 4. *MYBL2* knockdown inhibits the proliferation and promotes apoptosis of HGC-27 cells via the regulation of *CDC20* expression. A. The CDC20 protein expression was determined with western blot analysis and quantification; B. The *CDC20* mRNA level was analyzed with quantitative real-time polymerase chain reaction (RT-qPCR); C. HGC-27 cell viability was detected using the Cell Counting Kit-8 (CCK-8); D. The proliferating cell nuclear antigen (PCNA) and Ki-67 protein expressions were determined with western blot analysis and quantification; E. HGC-27 cell proliferation was analyzed with the colony formation assay; F and G. HGC-27 cell apoptosis was determined with TUNEL staining; H and I. Bax, Bcl-2, cleaved caspase-3, and caspase-3, and protein expressions were determined using western blot analysis and quantification. Error bars represent the mean ±standard error of mean (SEM) from 3 independent experiments

**p < 0.01; ***p < 0.001.

cancer patients were followed up for 20 years to detect the expression of *CDC20*, which verified that *CDC20* is highly expressed in breast cancer patients. In addition, the overexpression of *CDC20* was related to the aggressive course of breast cancer.[25] *CDC20* is also overexpressed in colorectal cancer cell lines and primary cancer tissues. It is worth noting that the expression of *CDC20* is relevant to clinical stage, metastasis and short-term overall survival,

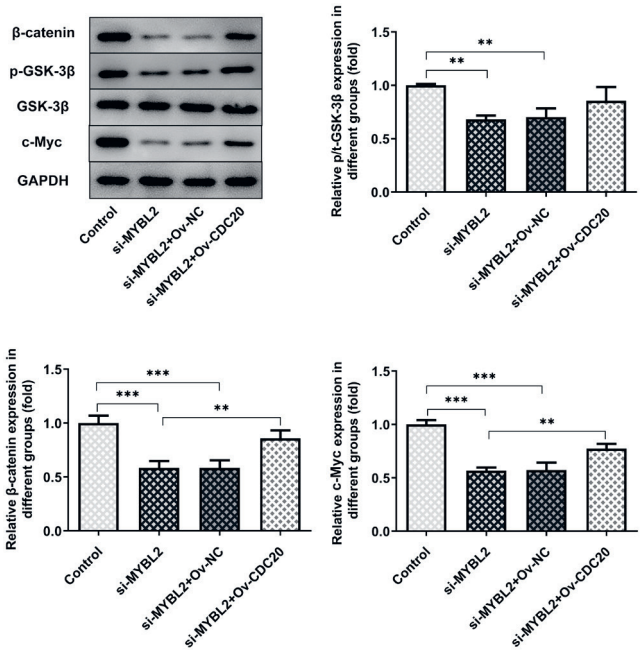

Fig. 5. The effect of *MYBL2* on the Wnt/β-catenin signaling pathway. The protein levels of β-catenin, p-GSK-3β, GSK-3β, and c-Myc were assessed with western blot analysis and quantification. Error bars represent the mean ±standard error of mean (SEM) from 3 independent experiments

**p < 0.01; ***p < 0.001.

which indicates that *CDC20* can be regarded as an independent prognostic biomarker for human colorectal cancer.[26] Importantly, a previous study has demonstrated that *CDC20* expression is significantly increased in GC tumor tissues compared noncancerous tissues, and its overexpression is closely related to aggressive progression and poor prognosis in GC patients.[27] On the basis of the result that *MYBL2* silencing depressed *CDC20* expression, the biological significance of the interaction between *MYBL2* and *CDC20* was subsequently examined. The findings revealed that *CDC20* overexpression partly abolished the effect of *MYBL2* downregulation on HGC-27 cell proliferation and apoptosis, suggesting that *MYBL2* works in synergy with *CDC20* to promote the proliferation and inhibit the apoptosis of GC cells.

The Wnt signal transduction cascade is a key driving factor for a variety of tissue stem cells. The Wnt pathway can participate in and can cause a variety of growth-related pathologies and cancers.[28] In breast, lung and hematopoietic malignancies, activation of the Wnt/β-catenin signaling pathway has been found and it mediates tumor recurrence.[29,30] It has been reported that *CDC20* silencing not only suppresses prostate cancer growth, but also enhances chemosensitivity to docetaxel via inhibition of Wnt/β-catenin signaling.[31] In this study, it was found that *MYBL2* knockdown induced inactivation of the Wnt/β-catenin signaling pathway, while *CDC20* upregulation had the opposite effect. Thus, *MYBL2*, in synergy with *CDC20*, promotes the proliferation and inhibits the apoptosis of GC cells, and these effects may involve the Wnt/β-catenin signaling pathway.

The purpose of this study was to investigate the underlying mechanisms of *MYBL2* in the progression of GC. However, the current experiments were carried out in vitro and thus require further investigation in vivo.

Conclusions

Taken together, the current results indicate that the synergy between *MYBL2* and *CDC20* induces the proliferation of GC cells and inhibits cell apoptosis, and that these processes may involve the Wnt/β-catenin signaling pathway. Thus, *MYBL2*, as a promising target, is of great significance for advancing the treatment of GC.

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