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Original Article

MYBL2 is an independent prognostic marker that has tumor-promoting functions in colorectal cancer

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Abstract: The MYBL2 gene plays an important role in the genesis and progression of tumors; however, few studies to date have defined the role of this gene in colorectal cancer (CRC). The aim of this study was to determine the relationship between MYBL2 and the prognosis of patients with CRC and to determine the possible effect of MYBL2 on colorectal carcinogenesis. Solid CRC tissues (n=180) preserved with RNAlater were collected to examine the mRNA levels of MYBL2 by real-time quantitative PCR (RT-qPCR). Formalin-fixed, paraffin-embedded (FFPE) blocks of CRC tissues (n=97) and adjacent noncancerous tissues (ANCTs, n=104) were obtained to detect MYBL2 protein levels by immunohistochemistry (IHC). siRNA was used to downregulate MYBL2 expression in the SW480 cell line to detect changes in proliferation, cell cycle progression, apoptosis, migration and invasion. The protein levels of MYBL2 were significantly higher in CRC tissues compared with ANCTs ($P<0.05$). Kaplan-Meier survival curves indicated that disease-free survival (DFS) was significantly worse in CRC patients in whom MYBL2 was overexpressed (at both the mRNA and protein levels) compared with patients not overexpressing MYBL2. Cox multivariate analysis revealed MYBL2 overexpression as an independent prognostic factor for poor patient survival. In addition, siRNA downregulation of MYBL2 suppressed SW480 cell proliferation, delayed cell cycle progression and induced apoptosis; however, changes in cell migration were minor. Western blot analysis demonstrated an association between MYBL2 expression and that of MMP9, Vimentin, and E-cadherin. MYBL2 is overexpressed in CRC and may therefore play an important role in tumourigenesis.

Keywords: MYBL2, colorectal carcinoma, prognosis, proliferation, cell cycle, apoptosis, epithelial to mesenchymal transition

Introduction

Colorectal cancer (CRC) is a common malignancy worldwide. The development of CRC is a complicated process that includes the activation of multiple oncogenes and the inactivation of tumor suppressor genes.

The MYBL2 gene, which is also known as B-MYB, is a member of the MYB family that includes A-MYB and C-MYB. C-MYB, which was identified first, is expressed in haematopoietic stem cells [1], the brain [2] and the colon[3]. A-MYB is expressed predominantly in the testis and is expressed at extremely low levels in the ovaries, spleen and brain [4]. MYBL2 is generally expressed in proliferative cells [5], is crucial for the regulation of proliferation and differentiation, and has a vital role in guiding cell cycle progression[6]. Some studies have found that the downregulation of MYBL2 results in the inhibition of cell cycle progression [7, 8]. MYBL2 also suppresses apoptosis [9, 10] through multiple pathways [11, 12] and is associated with cellular aging [13, 14]. In addition, as the MYBL2 gene is reportedly associated with a stem cell-like phenotype, this gene may function in maintaining pluripotent stem cell characteristics, such as self-renewal and differentiation [6, 15, 16].

MYBL2 is overexpressed in many cancers, including hepatocellular carcinoma [9], breast cancer [17], lung cancer [18] and others [19]. MYBL2 is also associated with cancer patient prognosis [20-22]. However, few studies to date have elucidated the role of the MYBL2 gene in CRC. Therefore, this study examined the expression of MYBL2 mRNA and protein and assessed the effects of the MYBL2 gene in CRC cell lines to explore its possible mechanisms of action.

Materials and methods

Patient samples

All samples, along with available clinical-pathological data, were obtained from Fudan University Shanghai Cancer Center. Fresh tissues from CRC patients were collected between 2007 and 2009 and preserved in RNAlater (n=180). Formalin-fixed, paraffin-embedded (FFPE) blocks of CRC tissues (n=97) and adjacent noncancerous tissues (ANCTs) (n=104) were obtained between 2005 and 2008. The inclusion criteria were as follows: no preoperative chemotherapy or radiotherapy and the presence of primary sporadic tumors. The tumors were assessed according to the American Joint Committee on Cancer classification (seventh edition) by two academic gastrointestinal pathologists. The clinicopathological data for parameters were collected from pathology reports.

Cell culture

Leibovitz L-15 medium, penicillin, streptomycin, foetal bovine serum (FBS), trypsin-EDTA (ethylenediaminetetraacetic acid) and phosphate-buffered saline (PBS) were purchased from Gibco BRL (Carlsbad, CA, USA). The human CRC cell line SW480 (highly differentiated) was purchased from American Type Culture Collection (Manassas, VA, USA). SW480 cells were cultured in L15 supplemented with 10% FBS, 100 units/mL penicillin, and 100 μg/mL streptomycin. All cells were cultured in a 5% CO_2 incubator at 37°C.

RNA extraction and quantitative real-time PCR (RT-qPCR)

Total RNA was extracted from 180 CRC tissues and cultured cells using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. First-strand cDNA synthesis was performed using PrimeScript RT Master Mix (TaKaRa Biotechnology Co., Ltd., Dalian, China). GAPDH was used as an endogenous control. The cycling conditions for GAPDH and MYBL2 were as follows: one cycle of 95°C for 2 minutes, followed by 40 cycles of 95°C for 15 seconds, with a final extension at 60°C for 60 seconds. The amplification specificity was validated by the presence of a single peak in the melting curves. The MYBL2 gene was amplified using the following primers: 5'-AAAACAGTGAGGAGGAAC-3' (forward) and 5'-CAGGGAGGTCAAATTTAC-3' (reverse). The endogenous GAPDH gene was amplified using the following primers: 5'-GCTGAACGGGAAGCTCACTG-3' (forward) and 5'-GTGCTCAGTGTAGCCCAGGA-3' (reverse). Standard curves for GAPDH were generated using serial dilutions. Each RT-qPCR cycle was repeated three times to confirm the results. Gene expression in the 180 CRC tissues and cultured cells was calculated using the relative quantification method, and the relative expression of the target gene was determined using the $2^{(-\Delta\Delta ct)}$ method [23]. The level of MYBL2 mRNA from the 180 CRC tissues was divided into two groups according to the receiver operating characteristic curve (ROC curve) only for the Kaplan-Meier survival curves.

Immunohistochemistry (IHC) analysis

FFPE blocks from 97 CRC tissues and from 104 ANCTs were collected for tissue microarrays. Two cores from the same patient's FFPE blocks were arranged on a recipient paraffin block (with a 1 mm core per specimen). Five-micrometre-thick paraffin sections were deparaffinised in xylene, rehydrated in a graded alcohol series, boiled with EDTA (pH 9) for 10 minutes, treated with 3% H_2O_2 for 15 minutes, and pre-incubated in blocking solution (10% normal goat serum) for 30 minutes at room temperature. The steps were performed using the Envision two-step method. An EnVision DAB colour kit was purchased from Gene Tech Company Limited (Shanghai, China). A MYBL2 rabbit anti-human polyclonal antibody (Sigma-Aldrich, St. Louis, MO, USA) was used at a 1:200 dilution. Slides from CRC tissues that were not incubated with the primary antibody were used as negative controls [24]. The tissue microarray slides were evaluated by two of the authors independently. Immunostaining of the cell nucleus and cytoplasm was scored [25].

Briefly, the staining score was considered the product of the intensity score (0, no staining; 1, faint/equivocal; 2, moderate; and 3, strong) and the distribution score (0, 0-10%; 1, 10%-24%; 2, 25%-49%; 3, 50%-74%; and 4, 75%-100% of cells). A staining score over 5 was defined as high staining [26].

Transient transfection with siRNAs

A small interfering RNA (siRNA) for the specific inhibition of MYBL2 expression and a negative control siRNA were synthesised by GenePharma Co., Ltd. (Shanghai, China). SW480 cells were plated in a 6-well plate (Crystalgen, NY, USA) or in a 96-well plate (Corning, NY, USA) 1 day before transfection. After 70% confluence was achieved, 100 pmol siRNA was diluted with 100 μL serum-free medium using the Lipofectamine 2000 transfection reagent (Life Technologies, Carlsbad, CA, USA) according to the manufacturer's instructions.

Cell proliferation assay

To detect cell proliferation, a cell counting kit (CCK8) purchased from Dojindo (Kumamoto, Japan) was used. The cells were seeded in 96-well plates and transfected with siRNA. The absorbance value (OD) of each well was measured at 0, 1, 2 and 3 days after applying the CCK8 reagent according to the manufacturer's instructions. The experiments were performed in triplicate.

Cell cycle analysis

Harvested cells (10^6) were suspended in 300 μL PBS and mixed with cold ethanol (700 μL), and the mixture was incubated at 4°C overnight. After centrifugation, the pellet was washed with cold PBS, resuspended in 500 μL PBS, and incubated with 50 μL RNase (at a final concentration of 20 μg/mL) for 30 minutes. The cells were incubated with propidium iodide (at a final concentration of 50 μg/mL) for 30 minutes in the dark. The cell cycle distribution was determined using a FACSAria flow cytometer (Beckman Coulter, Brea, CA, USA).

Apoptosis analysis

Apoptosis was analysed by flow cytometry using an annexin V-FITC apoptosis detection kit according to the manufacturer's instructions. Briefly, 3×10^5 cells were harvested, washed in PBS, and incubated with annexin V and propidium iodide in binding buffer at room temperature for 10 minutes in the dark. The stained cells were analysed using a FACSAria flow cytometer.

Wound healing assay

Cells were seeded in 6-well plates and cultured. Artificial wounds were gently made using a micropipette tip, and the cells were washed with PBS to remove floating cells and debris. The cells were then incubated in serum-free medium. Representative images of cells migrating into the wounds were captured at 0 and 24 hours in the same wounded region using an inverted microscope (Olympus, Hamburg, Germany). The wound coverage percentage was calculated from the images using ImageJ 1.47 software.

Transwell migration assay

For migration assays, siRNA-transfected cells were seeded in the upper chamber of a transwell device at 2×10^5 cells per well in serum-free medium; medium with 1% FBS was added to the bottom chamber. After 24 hours, the cells on the upper surface of the filter were removed using a cotton swab, and the chambers were fixed in formaldehyde and stained with 0.1% crystal violet. Sixteen high-power fields of each chamber were selected, and the number of cells was counted using an inverted microscope.

Western blot analysis

Whole-cell lysates were generated using RIPA lysis buffer (Abcam, Cambridge, UK). Total proteins were separated using 10% SDS-PAGE and then transferred onto a nitrocellulose membrane. The membrane was incubated with the primary antibody at 4°C overnight, followed by a horseradish peroxidase-conjugated secondary antibody the next day for 2 hours at room temperature. Primary antibodies against the following proteins were used: MYBL2, which was purchased from Sigma-Aldrich (St. Louis, MO, USA); GAPDH and E-cadherin, which were purchased from Epitomics (Cambridge, UK); and MMP9, Bcl-2 and Vimentin, which were purchased from Abcam (Cambridge, UK). The secondary antibodies were purchased from Cell Signaling Technology (Danvers, MA, USA).

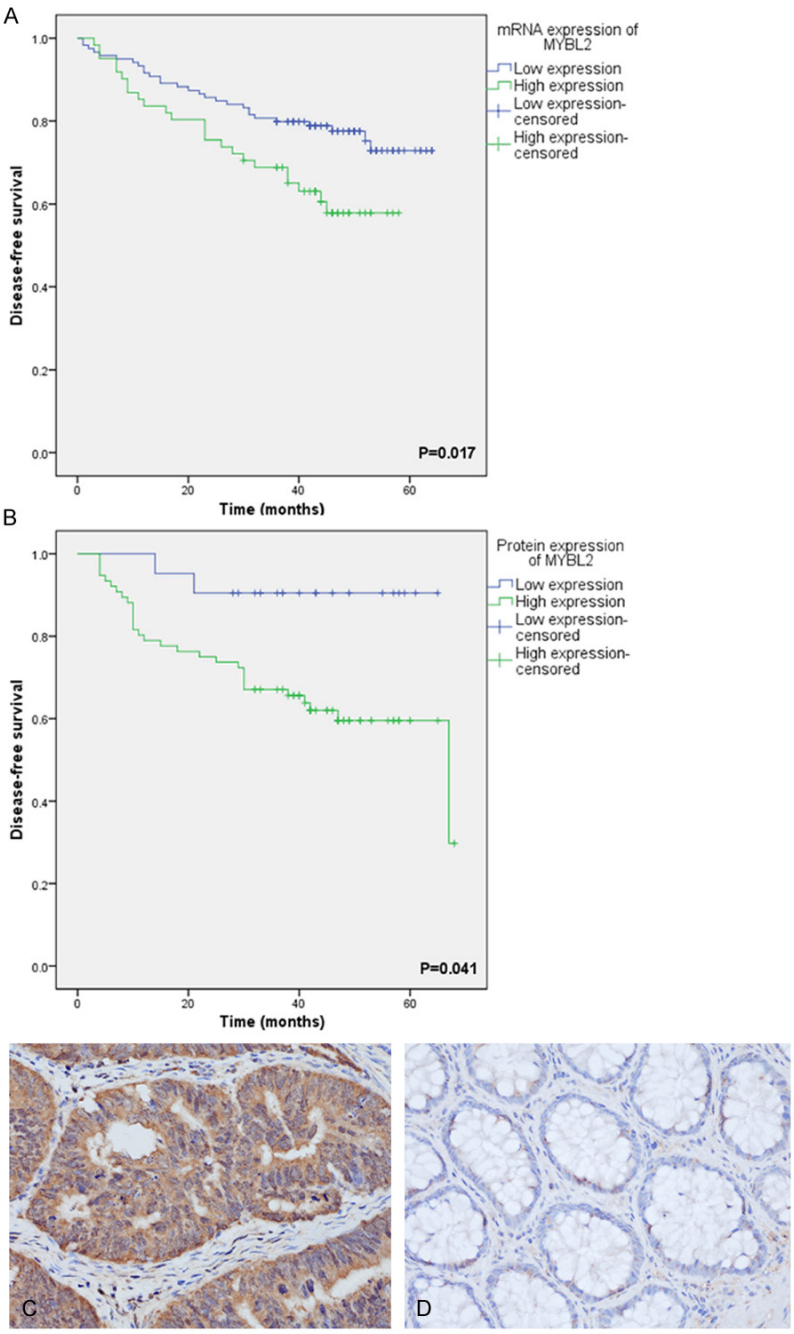

Figure 1. MYBL2 overexpression predicted poorer DFS and MYBL2 protein immunostaining. A. Relationship between MYBL2 mRNA expression and DFS. B. Relationship between MYBL2 protein expression and DFS. C. Representative immunostaining of MYBL2 in tissue microarrays (EnVision ×400). The MYBL2 protein shows strong staining in CRC tissues. D. ANCTs are negative for the MYBL2 protein (EnVision ×400).

The immunoreactive bands were visualised using enhanced chemiluminescence (ECL) reagents (Pierce, Rockford, IL, USA) [27]. An anti-GAPDH antibody was used as a loading control.

Statistical analysis

Statistical significance between different groups was determined using t-tests for the MYBL2 mRNA level analysis and cell assay. The difference in MYBL2 protein expression between CRC tissues and ANCTs was determined using X^2 tests. Kaplan-Meier survival curves were used to compare survival rates. Univariate and multivariate Cox proportional hazard models were used to explore the associations between patient characteristics and biomarkers with outcomes. Statistical significance was defined as a *P*-value less than 0.05.

Results

Quantitative analysis of MYBL2 mRNA expression

MYBL2 mRNA levels were found to be related to several clinicopathological features in CRC patients. Specifically, MYBL2 mRNA expression was higher in larger tumors (tumor size ≥5 cm vs tumor size <5 cm, P=0.048) and in stage III patients (stage III vs stage II, P=0.014), while MYBL2 mRNA expression was not significantly related to patient gender, age, or tumor differentiation degree, gross type, location, nerve invasion, or vascular invasion. The Kaplan-Meier survival curves and log-rank test demonstrated that disease-free survival (DFS) was significantly worse in MYBL2-overexpressing CRC patients compared to patients not overexpressing MYBL2 (**Figure 1A**, the cases were divided into two groups only

Table 1. Univariate Cox regression model for the survival of 180 CRC patients

Characteristics	N=180	Univariate*	
		P value	HR (95%CI)
Sex			
Male/female (ref)	105/75	0.640	0.878 (0.509-1.515)
Age (years)			
>60/<60 (ref)	76/104	0.815	0.936 (0.538-1.629)
Gross type			
Massive/ulcerative/invasive (ref)	32/144/4		
Tumor size (cm)			
>5/<5 (ref)	54/126	0.781	0.917 (0.497-1.692)
Location			
Colon/rectum (ref)	70/110	0.533	0.834 (0.471-1.476)
Differentiation			
Well/moderate/poor (ref)	4/139/37		
Nerve invasion			
Yes/no (ref)	38/142	0.035	1.890 (1.047-3.410)
Vascular invasion			
Yes/no (ref)	51/129	0.001	2.567 (1.486-4.435)
TNM stage			
III/II (ref)	96/84	0.000	4.106 (2.103-8.017)
MYBL2 mRNA expression			
Continuous variable	180	0.001	1.014 (1.005-1.022)

**P* values and HR were not calculated when there were <5 samples in either group. Abbreviations: Ref (reference).

Table 2. Multivariate Cox regression model for the survival of 180 CRC patients

Characteristics*	N=180	Multivariate	
		P value	HR (95% CI)
Nerve invasion			
Yes/ no (ref)	38/142		
Vascular invasion			
Yes/ no (ref)	51/129		
TNM stage			
III/ II (ref)	96/84	0.000	3.831 (1.954-7.510)
MYBL2 mRNA expression			
Continuous variable	180	0.010	1.011 (1.003-1.020)

*Only those characteristics having significance (P<0.05) in the univariate cox regression model were included in the multivariate cox regression model.

for Kaplan-Meier survival curves and the log-rank test, as mentioned in the Methods section). A univariate Cox regression analysis of prognostic parameters for DFS was also performed. As shown in **Table 1**, overexpression of MYBL2 was significantly associated with a poorer DFS (P=0.001). Additionally, vascular invasion, nerve invasion, and higher TNM stage also predicted a poorer DFS. A multivariate analysis was performed using the Cox regression model, and MYBL2 mRNA expression (P=0.010) and tumor stage (P=0.000) were found to be independent prognostic factors for CRC patients (**Table 2**).

IHC analysis of MYBL2 protein expression

The MYBL2 protein was found to be primarily expressed in the cytoplasm and nucleus (**Figure 1C**, **1D**), and expression in CRC tissues was much higher compared with ANCTs (P=0.000). X^2 tests were used to analyse the relationship between MYBL2 protein expression and the clinicopathological features of CRC patients, and no significant associations with patient gender, age, tumor differentiation degree, gross type, tumor size, location, or TNM stage were found. DFS was significantly worse in CRC patients with MYBL2 overexpression compared with patients not overexpressing MYBL2 (P=0.041, **Figure 1B**, **Table 3**). A higher TNM stage also predicted a worse DFS (P=0.012, **Table 3**). Cox multivariate regression analysis revealed MYBL2 protein expression (P=0.032, **Table 4**) and tumor stage (P=0.008) as independent prognostic factors for CRC patients.

Downregulation of MYBL2 expression suppressed cell proliferation, restricted cell cycle progression and induced apoptosis

To investigate the function of MYBL2, we transfected colon epithelial cells (SW480 cells) with

Table 3. Univariate Cox regression model for the survival of 97 CRC patients

Characteristics	N=97	Univariate*	
		P value	HR (95% CI)
Sex			
Male/female (ref)	53/44	0.865	0.942 (0.470-1.886)
Age (years)			
≥60/<60 (ref)	41/56	0.259	1.492 (0.745-2.990)
Gross type			
Massive/ invasive (ref)	35/5	0.081	0.306 (0.081-1.157)
Ulcerative/ invasive (ref)	57/5	0.283	0.515 (0.153-1.730)
Tumor size (cm)			
≥5/<5 (ref)	38/59	0.796	0.907 (0.434-1.896)
Location			
Colon/ rectum (ref)	60/37	0.992	0.996 (0.482-2.062)
Differentiation			
Well/ moderate/ poor	2/74/21		
TNM stage			
III/ II (ref)	48/49	0.012	2.631 (1.237-5.596)
MYBL2 protein expression			
High/ low (ref)	76/21	0.041	4.452 (1.062-18.663)

**P* values and HR were not calculated when there were <5 samples in either group.

Table 4. Multivariate Cox regression model for the survival of 97 CRC patients

Characteristics*	N=97	Multivariate	
		P value	HR (95% CI)
TNM stage			
III/ II (ref)	48/49	0.008	2.786 (1.309-5.929)
MYBL2 protein expression			
High/low (ref)	76/21	0.032	4.816 (1.147-20.212)

*Only those characteristics having significance (P<0.05) in the univariate cox regression model were included in the multivariate cox regression model.

a MYBL2-specific siRNA and successfully down-regulated MYBL2 mRNA expression in these cells (**Figure 2A**). We then used CCK8 to assay the proliferation of SW480 cells transfected with siRNAs. We found that the growth of the MYBL2-specific siRNA-transfected cells was slower compared to the negative control cells. Notably, the proliferation difference reached statistical significance at day 2 (**Figure 2B**).

We conducted a cell cycle analysis as described in the Methods section to study the mechanisms by which MYBL2 regulates cell proliferation. A greater number of MYBL2-specific siRNA-transfected cells transitioned into the S and G2/M phases of the cell cycle compared to the negative control cells (only the G2/M phase showed significance, P=0.043, **Figure 2C**). These data indicated that MYBL2 suppression might delay the progression of the cell cycle, particularly at the G2/M phase.

To determine whether MYBL2 is associated with apoptosis, SW-480 cells were transfected with MYBL2-specific siRNA, as described above, and the number of apoptotic cells was assessed using an annexin V-FITC apoptosis detection kit. The transfection of SW480 cells with MYBL2-specific siRNA resulted in a significant increase in apoptotic cells (P= 0.003, **Figure 2D**), which indicated that the MYBL2-specific siRNA could effectively induce apoptosis in this CRC cell line.

MYBL2 slightly promoted migration and altered invasion-related protein expression

SW480 cells were transfected with the MYBL2-specific siRNA and a negative control siRNA to determine whether MYBL2 affects the migration and invasion abilities of CRC cells. The results of the wound healing assay demonstrated that the migratory ability of MYBL2-specific siRNA-transfected cells was lower compared with negative control siRNA-transfected cells (P=0.021, **Figure 3A**, **3B**). The results of the Matrigel Transwell migration assay demonstrated that MYBL2-specific siRNA transfection significantly reduced the migration ability of the cells (P=0.034, **Figure 3C-E**), which was consistent with the results of the wound healing assay. Although these results suggested that MYBL2 could promote the migration ability of CRC cells in vitro, the difference between the two groups was small. Additionally, we conducted western blotting to analyse the levels of several proteins, including MMP9, Vimentin and E-cadherin. The MYBL2-specific siRNA-transfected cells showed a significant reduction in the protein levels of MMP9 and vimentin and a significant increase in the protein level of E-cadherin (**Figure 3F**). This

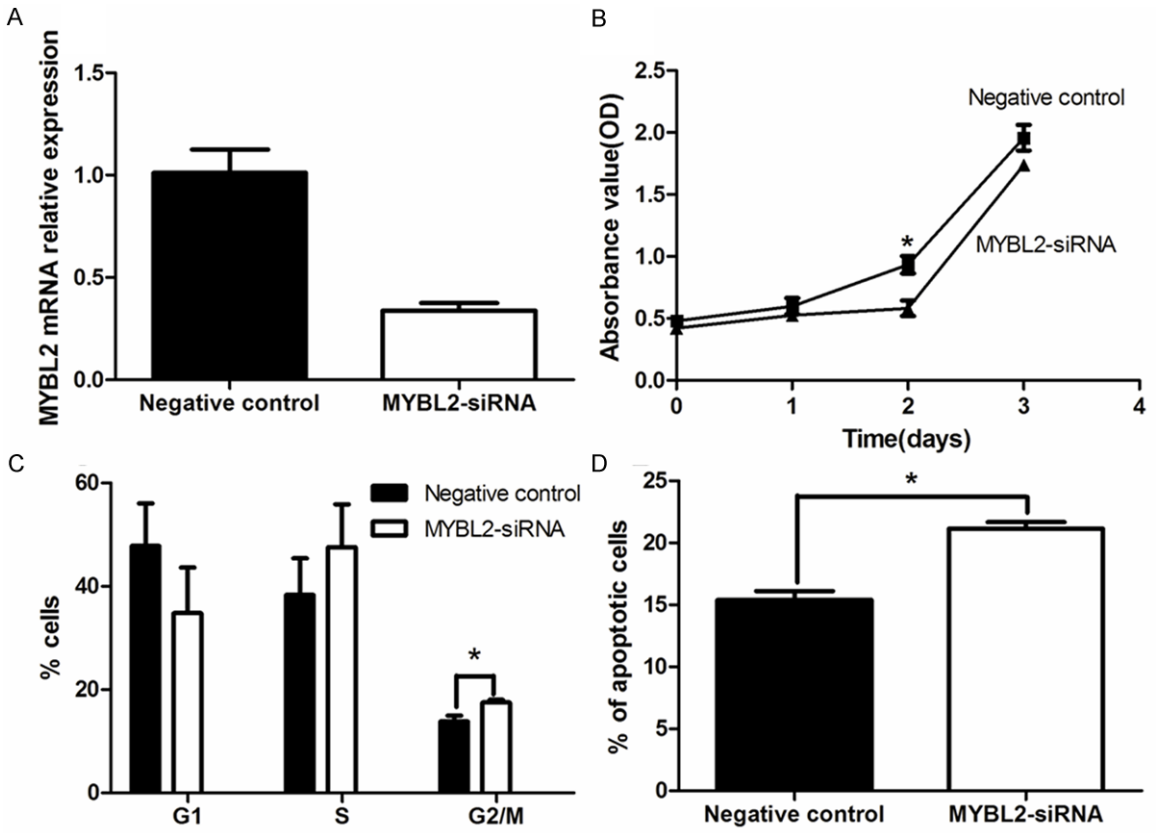

Figure 2. Downregulation of MYBL2 expression suppressed cell proliferation, restricted cell cycle progression and induced apoptosis. A. SW480 cells were transfected with a MYBL2-specific siRNA (MYBL2-siRNA group) or negative control siRNA (negative control group) for 48 hours. The relative mRNA expression levels were measured, revealing that MYBL2-siRNA could successfully downregulate the expression of MYBL2. B. Transiently reducing the expression of MYBL2 with siRNA inhibited the proliferation of SW480 cells. C. The percentage of cells in S and G2/M phases was increased after transfection with MYBL2-siRNA. D. The percentage of apoptotic cells was increased after transfection with MYBL2-siRNA. *P values <0.05.

result indicated that MYBL2 might influence the invasion and metastasis abilities of CRC cells.

Discussion

CRC carcinogenesis involves the combined actions of multiple oncogenes and tumor suppressor genes [28]. MYBL2, which is a positive growth control gene, participates in cell cycle progression and apoptosis. Previous studies have indicated that MYBL2 is overexpressed in many types of cancers, such as hepatocellular carcinoma [29] and breast cancer [17]. Additionally, MYBL2 overexpression indicates poor prognosis in patients with breast cancer [20], neuroblastoma [21] and acute myeloid leukaemia [22]. The role of MYBL2 in CRC was investigated in the present study. First, we examined the levels of mRNA and protein expression in CRC tissues and found that the amount of mRNA expression negatively correlated with patient survival. Koga Y et al. detected MYBL2 mRNA expression in colonocytes from faeces. The results showed that MYBL2 expression was significantly higher in CRC patients compared to healthy volunteers [30], which suggested that MYBL2 mRNA expression may indicate malignance. IHC analysis using tissue microarrays of samples from CRC patients confirmed the results of RT-qPCR. Therefore, we postulate that the abnormal expression of MYBL2 might be involved in the carcinogenesis of CRC and might have the potential for future use as a prognostic marker.

The MYBL2 gene is involved in cell growth, cell cycle, and apoptosis. To investigate the function of MYBL2 in CRC, we used MYBL2-specific siRNA to downregulate MYBL2 expression in

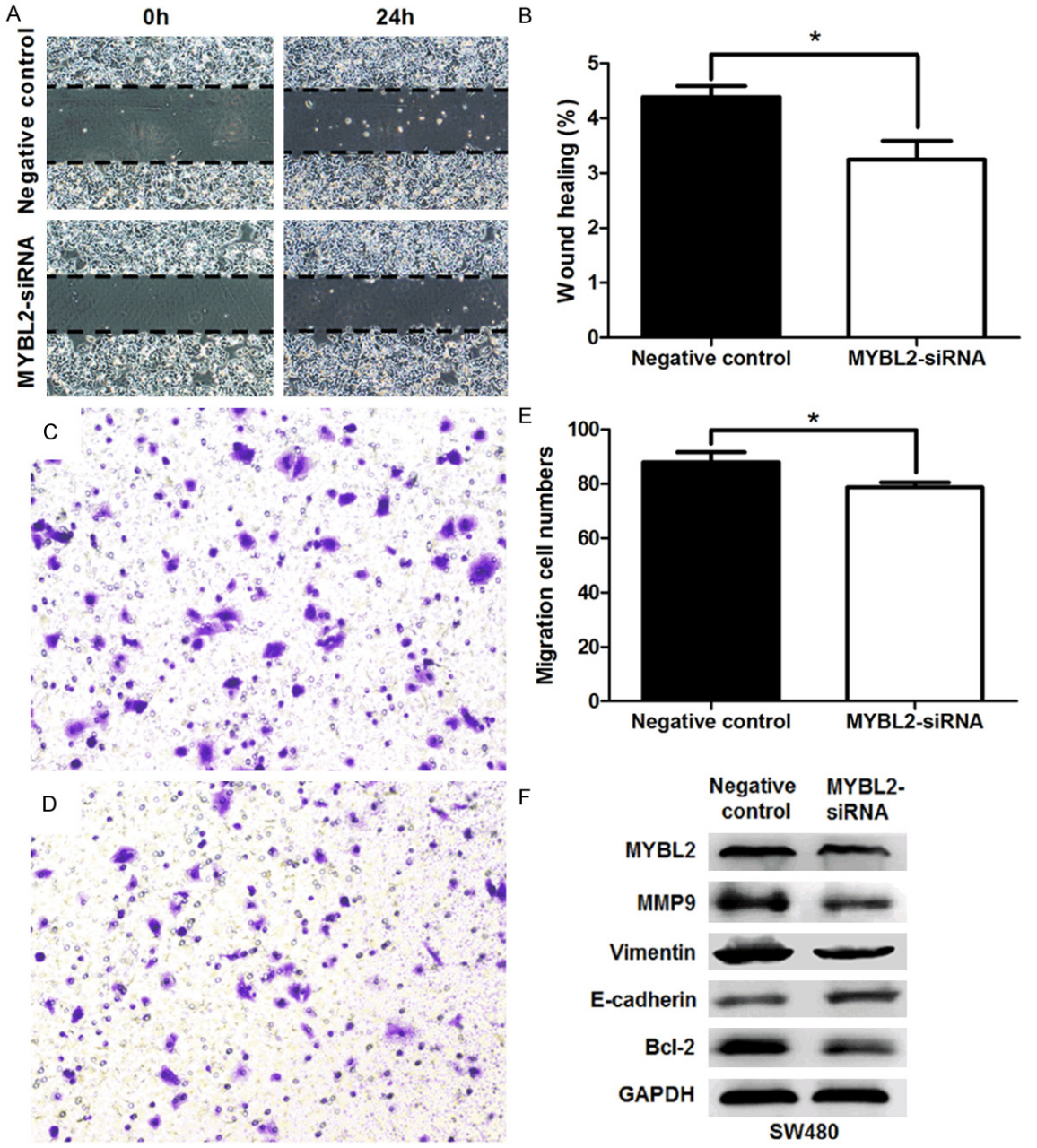

Figure 3. Wound healing, transwell migration and western blotting assays. (A, B) Representative images of the wound healing assay using SW480 cells transfected with MYBL2-siRNA and negative control siRNA. The percentage of wound coverage was calculated. (C-E) Representative images (C, negative control; D, MYBL2-siRNA) and quantification of the transwell migration assay (E) in SW480 cells transfected with MYBL2-siRNA and negative control siRNA. (F) Expression of invasion-related and apoptosis-related proteins was determined by western blot analysis. Cells transfected with MYBL2-siRNA showed a reduction in MMP9, Vimentin and Bcl-2 protein and an increase in E-cadherin protein. *P values <0.05.

CRC cell lines and performed an analysis of cell proliferation using a CCK8 assay. The growth of MYBL2-specific siRNA-transfected cells was significantly reduced compared to negative control siRNA-transfected cells, indicating that the MYBL2 gene could enhance cell proliferation. A similar result was also reported in hepatocellular carcinoma [9, 10] and neuroblastoma cell lines [31]. The qRT-PCR results in the present study indicated that MYBL2 mRNA expres-

sion was positively related to tumor size, also consistent with the CCK8 results.

The possible mechanism responsible for the induction of proliferation was explored, and we hypothesised that cell cycle regulation may be one of the primary pathways of this mechanism. According to our cell cycle analysis, MYBL2 downregulation resulted in the inhibition of cell cycle progression at the S and G2/M phase transitions. MYBL2 plays a role in cell cycle progression in multiple cell types and is involved in regulating progression through the S and G2/M phases [7, 8, 32-35]; thus, MYBL2 may regulate the cell cycle by mediating the expression levels of cell cycle genes [20, 36] and critical regulators such as CDC2 and cyclin B1 [37]. Moreover, the inhibition of apoptosis could be another pathway that explains the observed proliferation results. In the present study, interfering with MYBL2 gene expression facilitated apoptosis, indicating that this gene is anti-apoptotic. In other cancers, transfection of cells with a MYBL2-specific siRNA also facilitated apoptosis [9], whereas MYBL2 gene overexpression led to the downregulation of apoptogenic genes. MYBL2 can regulate bcl-2 [38]; furthermore, Grassilli E et al. found that a segment of the bcl-2 promoter contained a putative Myb-binding site that specifically interacted with MYBL2, and MYBL2 was also reported to stimulate promoter activity in the 5' flanking region of bcl-2 [39]. Based on the above studies, we conducted western blot analysis and found that Bcl-2 protein expression decreased with decreased MYBL2 expression, indicating that MYBL2 could enhance the expression of Bcl-2. In summary, the majority of published studies support the findings that the MYBL2 gene is able to enhance cell proliferation, regulate the cell cycle and inhibit apoptosis. Investigating the associated mechanisms in cancer cells may help in a better understanding of cancer progression.

Wound healing, transwell migration and western blotting assays were conducted to determine other functions of the MYBL2 gene. The wound healing and transwell migration assays showed that decreased MYBL2 gene expression slightly inhibited the migration of CRC cells. However, the difference in migration was small and might have been a consequence of increased apoptosis and reduced proliferation. Thus, determining whether MYBL2 in fact influenced the migration ability of the cells based on the above results is difficult. Western blotting demonstrated that MYBL2-specific siRNA-transfected cells had decreased levels of MMP9 and vimentin and a concomitant increase in the level of E-cadherin. MMP9, Vimentin and E-cadherin are important invasion/metastasis-related proteins in CRC. MMP9 degrades the extracellular matrix during the processes of migration, invasion and metastasis [40, 41]. Vimentin expression is an indication of the epithelial to mesenchymal transition (EMT) and thus serves as a mesenchymal marker [42, 43]. EMT is also accompanied by the loss of E-cadherin [44], and the loss of E-cadherin might convey signals that actively induce tumor cell invasion and metastasis, in addition to causing a loss of cell-cell adhesion [45]. We postulated that MYBL2 might be involved in mediating aggressive CRC biology and EMT. However, to better illustrate the role of MYBL2 in the EMT and invasion processes, other related proteins must be studied, and the interactions of these proteins with MYBL2 should be analysed.

Conclusions

In the present study, MYBL2 was found to be significantly overexpressed in CRC tissues compared to ANCTs. Our results suggest that the increased expression of MYBL2 may play an important role in CRC carcinogenesis and may serve as a useful prognostic marker. Downregulation of MYBL2 was able to suppress cell proliferation, regulate the cell cycle and induce apoptosis. We postulate that the MYBL2 gene may be involved in mediating aggressive CRC biology.

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Disclosure of conflict of interest

None.

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