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Y-box-binding protein 1 inhibits apoptosis and upregulates EGFR in colon cancer

SACHIKO NAGASU, TOMOYA SUDO, TETSUSHI KINUGASA, TAKATOU YOMODA, KENJI FUJIYOSHI, TAKAHIRO SHIGAKI and YOSHITO AKAGI

Department of Surgery, Kurume University School of Medicine, Fukuoka 830-0011, Japan

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Abstract. Y-box-binding protein 1 (YB-1) is a DNA/RNA-binding protein and an important transcription and translation factor in carcinogenesis. However, the biological function and molecular correlation of YB-1 in colorectal cancer are not fully understood. The aim of the present study was to determine the significance of YB-1 expression and its biological role in colorectal cancer. Cell proliferation, migration and apoptosis were examined upon knockdown of YB-1 expression in different colon cancer cell lines that had different genetic backgrounds. Since the properties of different colon cancer cell lines with specific RAS/RAF gene mutations downstream epidermal growth factor receptor (EGFR) may differ from wild-type colorectal cancer, it is critical to study the role of YB-1 with respect to the mutational status of RAS. The results indicated that the suppression of YB-1 decreased cell proliferation ($P<0.05$) and migration ($P<0.05$) regardless of the status of *RAS/RAF* in the HT29, HCT116 and CaCo2 cell lines. In contrast, YB-1 knockdown altered the expression of apoptosis-related genes and the expression of EGFR was detected in the cell lines expressing wild-type *RAS/RAF* but not in those expressing mutated *RAS/RAF*. These results indicated that YB-1 plays an important role in cell proliferation, migration, apoptosis and EGFR expression in colorectal cancer. Furthermore, apoptosis and EGFR expression may be affected by the mutational status of *RAS/RAF* and controlled through YB-1.

Introduction

The 5-year survival rate for colorectal cancer is undeniably increasing in many developed countries; for patients diagnosed with colon cancer and rectal cancer in 22 countries worldwide, it was 50% in the past (1995-1999) and has now reached over 60%, according to data from 2005 to 2009 (1). Improvements in surgical techniques for resectable cases of colorectal cancer and the development of multidisciplinary treatments, particularly the introduction of chemotherapy with molecular targeted therapeutic agents against progressive or recurrent unresectable colorectal cancer, have contributed to the improved survival rates. In recent years, efforts have been actively made in the field of colorectal cancer toward developing precision medicine to further improve the prognosis, search for and identify novel biomarkers, and develop new medicines including molecular targeted therapeutic drugs. In particular, in recent years, elucidation of the genetic mutations responsible for the development of colorectal cancer has been achieved. However, with respect to RAS (KRAS/NRAS)/RAF mutation, the positive effect of the anti-EGFR antibody could not be determined (2).

Based on these findings, it is indispensable to perform genetic analysis of RAS/RAF as a biomarker for predicting the effect of anti-EGFR antibody drugs, when performing systemic chemotherapy for unresectable and recurrent colorectal cancer.

Y-box-binding protein 1 (YB-1) is a transcription factor that contains a cold shock domain highly conserved across species, and binds to the Y-box (CCAAT) region in the promoter domain of the major histocompatibility complex II gene HLA-DRα (3) and the gene encoding the EGFR enhancer (4). It has been reported that YB-1 plays a crucial role in cell proliferation, invasion, metastasis and drug resistance of cancer cells through the actions of transcription, translational control and DNA repair (5).

Previous studies have reported that YB-1 expression is a poor prognostic factor in gastric (6), cervical (7), non-small cell lung (8) and breast cancer (9). In a previous study, we also demonstrated that the nuclear expression of YB-1 in colorectal tumor cells led to a poor prognosis (10).

A significant correlation between HER2 expression and YB-1 expression exists in estrogen receptor (ER)-positive breast cancer, with the two elements being involved in tumor growth and drug resistance. As a result, YB-1 expression in breast cancer is reported to be a prognostic factor for a worse outcome (11). Furthermore, the co-expression of EGFR and YB-1 can modulate the effect of the anti-EGFR antibody as a treatment in non-small cell lung cancer (*NSCLC*) (8). Even

Correspondence to: Dr Sachiko Nagasu, Department of Surgery, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
E-mail: shiraiwa_sachiko@med.kurume-u.ac.jp

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in colorectal cancer, YB-1 expression has been correlated with poor prognosis (12,13); however, the biological function and molecular correlation of YB-1 in colorectal cancer are not fully understood. The objective of the present study was to elucidate the importance and biological role of YB-1 in colorectal cancer at the molecular level.

Materials and methods

Cell culture. Five human colon cancer cell lines, T84, HT29, HCT116, CaCo2 and SW480 were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Next, the expression of YB-1 and the mutational status of RAS/RAF were examined. Based on these results, three cell lines, namely CaCo2, HCT116 and HT29 were selected and used for further experiments. All cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium supplemented with 10% fetal bovine serum (FBS) (Fujifilm Wako Pure Chemical Industries, Ltd., Osaka, Japan), 100 U/ml penicillin and 100 mg/l streptomycin. The cells were maintained in a humidified incubator at 37˚C with 5% CO_2 (Sanyo Electric Biomedical Co., Osaka, Japan).

Mutation analysis. For the detection of *KRAS*, *NRAS* and *BRAF* mutations, genomic DNA was isolated from the cell lines, using an AllPrep DNA/RNA/Protein Mini kit (Qiagen, Inc., Valencia, CA, USA) according to the manufacturer's protocol. The genomic DNA was subjected to polymerase chain reaction (PCR) amplification using the primers listed in Table I. The primer sets were designed to amplify exons 2, 3 and 4 of *KRAS* and *NRAS* and exon 15 of *BRAF*. The PCR products were treated with ExoSap-IT PCR Product Cleanup reagent (Thermo Fisher Scientific, Inc., Waltham, MA, USA) according to the manufacturer's protocol to inactivate the free primers and dNTPs, and then were subjected to sequencing using the forward primers and BigDye® Terminator v3.1 (Thermo Fisher Scientific, Inc.). Each 20-μl PCR reaction consisted of 2 μl 10X LA PCR buffer II, 2 μl 10 mmol/l dNTPs, 0.1 μl AmpliTaq Gold (Thermo Fisher Scientific, Inc.), 2 μl of genomic DNA, 1 μl 100 pmol/μl forward primer, 1 μl 100 pmol/μl reverse primer and 12 μl H_2O. The cycling conditions were 95˚C for 12 min; 10 cycles at 94˚C for 15 sec, 55˚C for 15 sec, and 72˚C for 30 sec; 25 cycles at 89˚C for 15 sec, 55˚C for 15 sec, 72˚C for 30 sec; and a final extension at 72˚C for 10 min. The PCR products were evaluated by electrophoresis using 2% agarose gels. Sequencing was carried out using an ABI 3100 Genetic Analyzer (Thermo Fisher Scientific, Inc.) according to the manufacturer's protocol. The sequences obtained were aligned to the consensus reference-sequences from the University of California, Santa Cruz (UCSC) Genome Bioinformatics website (http://genome.ucsc.edu/index.html) using the ClustalW program (https://www.genome.jp/tools-bin/clustalw) to identify nucleotide differences.

YB-1 knockdown. The small interfering RNAs (siRNAs) for YB-1 (siRNA-1: Sense, 5'-GUAAAAUGGUUCAAUGUA Att-3' and antisense, 5'-UUACAUUGAACCAUUUUACtg-3'; and siRNA2: Sense, 5'-CGAAGGUUUUGGGAACAGUtt-3' and antisense, 5'-ACUGUUCCCAAAACCUUCGtt-3') and the negative control siRNAs (siCtr) were purchased from Life Technologies Corp. (Thermo Fisher Scientific, Inc.). The siRNAs were transfected into the cells, using Lipofectamine RNAiMAX Transfection reagent and Opti-MEM medium (Invitrogen Life Technologies; Thermo Fisher Scientific, Inc.) according to the manufacturer's recommendations. A total of $1x10^4$ cells/well were seeded in 96-well plates (Iwaki Co., Ltd., Tokyo, Japan) and cultured for 24 h before transfection. The adjusted amounts of siRNAs were added to each well. Reverse transcription PCR (RT-PCR) and western blotting were performed 72 h post-transfection, and MTT and migration assays were performed 48 h post-transfection.

Quantitative real-time PCR (RT-qPCR). Total RNA was extracted using an AllPrep DNA/RNA/Protein Mini kit (Qiagen, Inc.) according to the manufacturer's protocol and reverse-transcribed into complementary DNA (cDNA), using SuperScript IV Reverse Transcriptase (Promega Corporation, Madison, WI, USA). RT-qPCR was performed with TaqMan Gene Expression Assays (Applied Biosystems; Thermo Fisher Scientific, Inc.) for *YB-1* (Hs00358903_g1), *GAPDH* (Hs02758991_g1), *Bax* (Hs00180269_m1) and *Bcl-2* (Hs00608023_m1) using the TaqMan Gene Expression Master Mix (Applied Biosystems; Thermo Fisher Scientific, Inc.) on a StepOnePlus Real-Time PCR system (Applied Biosystems; Thermo Fisher Scientific, Inc.). The levels of transcripts of the indicated genes were standardized to the corresponding *GAPDH* transcript levels. All values reported represent the average of at least three independent experiments.

Western blotting. Total cell proteins were extracted using an AllPrep DNA/RNA/Protein Mini kit (Qiagen, Inc.) according to the manufacturer's protocol. Protein determination method is BCA. Mass of protein loaded per lane is 20 μg. Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis SDS-PAGE (4-12%) and transferred to polyvinylidene difluoride (PVDF) membranes. After blocking with 5% milk in Tris-buffered saline containing Tween-20 (TBST) buffer, the membranes were incubated at 4˚C overnight with an anti-YB-1 (dilution 1:1,000; rabbit; cat. no. ab12148; Abcam, Cambridge, MA, USA) or anti-EGFR (dilution 1:1,000; rabbit; cat. no. 4267; Cell Signaling Technology, Inc., Danvers, MA, USA) primary antibody. Subsequently, the membranes were incubated for 1 h at room temperature with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG secondary antibody (dilution 1:5,000; cat. no. 7074; Cell Signaling Technology, Inc.). The immunoreactive bands were visualized using Fusion-SL7-400 (Vilber Lourmat, Marne Le-Vallée, France). Immunostaining using an anti-β-actin primary antibody (dilution 1:5,000; mouse; cat. no. ab6276; Abcam) was used as a loading control.

MTT assay. For the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) tetrazolium (MTT) reduction assays, the three colorectal cell lines were used as either untreated (CaCo2, HT29 and HCT116), treated with siYB-1 (CaCo2-siYB-1, HT29-siYB-1 and HCT116-siYB-1), or treated with siCtr (CaCo2-siCtr, HT29-siCr and HCT116-siCtr). The cells were added to 96-well plates ($1x10^4$ cells/well) and incubated for 2 days in a humidified incubator at 37˚C with 5% CO_2. MTT Cell Proliferation Assay Reagent (Cell Biolabs, Inc., San Diego,

Table I. Primer sequences for the quantitative polymerase chain reaction.

	Primers	Expected size (bp)
KRAS-ex2-F	5'-TTAACCTTATGTGTGACATGTTCTAA-3'	225
KRAS-ex2-R	5'-AGAATGGTCCTGCACCAGTAA-3'	
KRAS-ex3-F	5'-CCAGACTGTGTTTCTCCCTTC-3'	286
KRAS-ex3-R	5'-TGCATGGCATTAGCAAAGAC-3'	
KRAS-ex4-F	5'-AAGGACTCTGAAGATGTACCTATGG-3'	
KRAS-ex4-R	5'-AAGAAGCAATGCCCTCTCAA-3'	294
NRAS-ex2-F	5'-GATGTGGCTCGCCAATTAAC-3'	220
NRAS-ex2-R	5'-CCGACAAGTGAGAGACAGGA-3'	
NRAS-ex3-F	5'-CCCCTTACCCTCCACACC-3'	243
NRAS-ex3-R	5'-CACAAAGATCATCCTTTCAGAGAA-3'	
NRAS-ex4-F	5'-AGCCACTGTACCCAGCCTAA-3'	250
NRAS-ex4-R	5'-TGCACAAATGCTGAAAGCTG-3'	
BRAF-ex15-F	5'-TGCTTGCTCTGATAGGAAAATG-3'	228
BRAF-ex15-R	5'-AGCATCTCAGGGCCAAAAAT-3'	

CA USA) was added (10 μl) to each well, and the cells were incubated for 3 h at 37˚C. Thereafter, the detergent solution provided with the assay kit was added to each well (100 μl) and the cells were incubated for 3 h at room temperature while being protected from the light. The absorbance of each sample for each well was measured at 540 nm using a microplate reader (VersaMax ELISA; Molecular Devices Japan K.K., Tokyo, Japan).

Migration assay. A total of 1x10^4 CaCo2, CaCo2-siYB-1, CaCo2-siCtr, HT29, HT29-siYB-1, HT29-siCtr, HCT116, HCT116-siYB-1, or HCT116-siCtr cells/well were seeded into 96-well Collagen I coated plates (Platypus Technologies, LCC, Fitchburg, WI, USA) and incubated at 37˚C to allow for cell attachment. After 24 h, all silicon stoppers at the center of each well were removed, and any unattached cells were removed by washing with 100 μl of sterile phosphate-buffered saline (PBS). After 72 h, the number of migrated cells was quantified using ImageJ software (ver. 1.51, NIH; National Institutes of Health, Bethesda, MD, USA).

Statistical analysis. Each assay was independently performed three times. The data were analyzed using JMP software (version 13.0.0; SAS Institute Inc., Cary, NC, USA). The values were presented as the means ± standard deviation (SD). The comparisons between groups were analyzed using the Kruskal-Wallis test. P<0.05 was considered to indicate a statistically significant difference.

Results

KRAS/NRAS/BRAF mutation profiles and expression of YB-1 in human colon cancer cell lines. In colorectal cancer, the mutational status of *RAS* markedly affects the characteristics of the cancer cells. Therefore, in order to confirm the molecular background of each of the five colon-cancer cell lines, we determined the genetic mutation of *RAS*/*RAF* by direct sequence evaluation. A summary of the *KRAS*/*NRAS*/*BRAF* gene mutations is presented in Table II. The detected *KRAS* mutations were G12V (GGT→GTT) and G13D (GGC→GAC), and the *BRAF* mutation was V600E (GTG→GAG). The genotypes of the mutant *KRAS* and mutant *BRAF* are displayed in Fig. 1A.

Table II. KRAS/NRAS/BRAF mutation profiles of cancer cell lines.

	Cell line	Mutation
1	SW480	G12V
2	HCT116	G13D
3	T84	G13D
4	CaCo2	Wild-type
5	HT29	V600E

Subsequently, western blot analysis was performed to investigate the levels of protein expression of YB-1 in the five colon cancer cell lines. As revealed in Fig. 1B, the expression of YB-1 was confirmed in all five cell lines. HCT116 demonstrated the highest level of YB-1 expression among the cell lines. Notably, the expression level of YB-1 tended to be higher in the cell lines harboring the *RAS*/*RAF* mutation compared to that in the cell line harboring wild-type *RAS*. To clarify the biological function of YB-1 in colorectal cancer with respect to the mutational status of the *RAS*, the CaCo2, HCT116 and HT29 cell lines were selected for further examination.

Knockdown of YB-1 in HCT16, HT29 and CaCo2 cell lines inhibits cell proliferation and migration. Two siRNAs (siRNA-1 and siRNA-2) were transfected into the cell lines HCT116, HT29 and CaCo2 in order to knockdown YB-1. The suppression of YB-1 mRNA levels and protein levels were confirmed by RT-qPCR and western blot analyses, respectively. As revealed in Fig. 2A, the expression level of

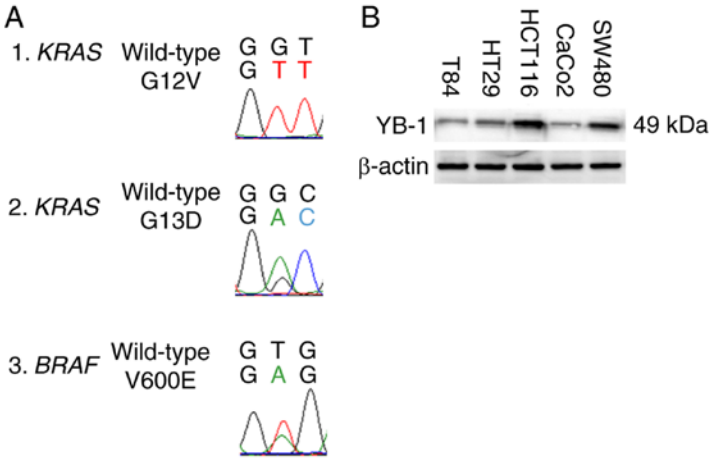

Figure 1. *KRAS/NRAS/BRAF* mutation profiles and expression of YB-1 in human colon cancer cell lines. (A) Representative sequencing results revealing *KRAS* and *BRAF* genotypes. The numbers 1, 2 and 3 represent mutant *KRAS* (codon 12 and codon 13) and *BRAF* (V600), respectively. (B) The expression of total YB-1 protein was determined by western blotting using protein lysates extracted from the indicated cell lines. YB-1, Y-box-binding protein 1.

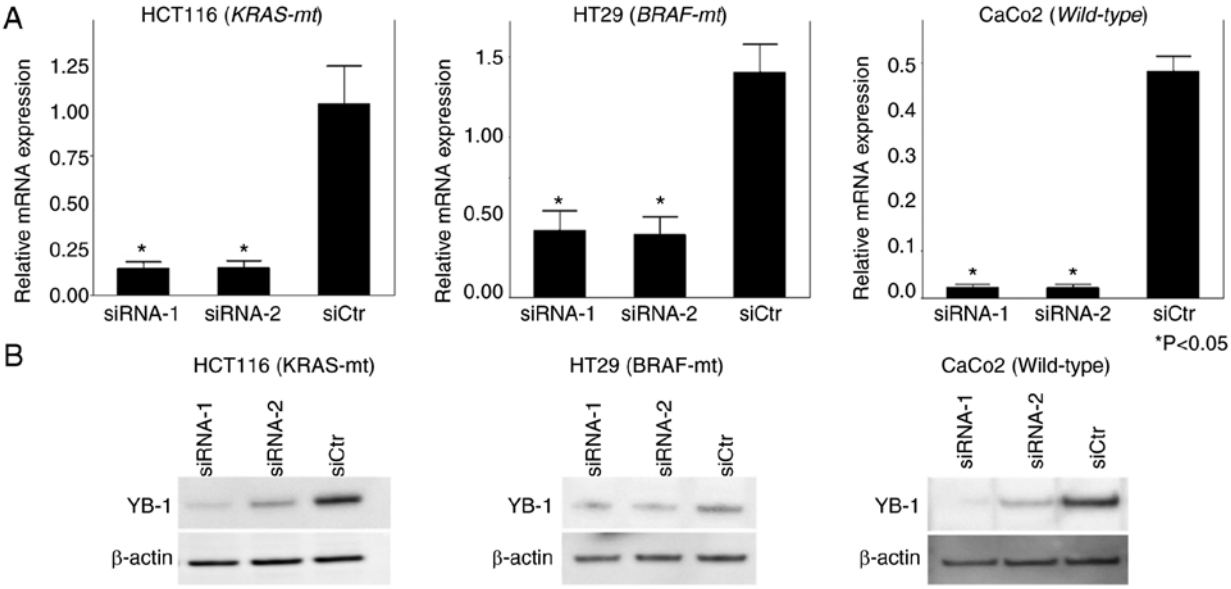

Figure 2. Effect of YB-1 knockdown on human colon carcinoma cell lines HCT116, HT29 and CaCo2. (A) mRNA expression levels of YB-1 were reduced by different small interfering RNAs (siRNA-1 and siRNA-2). (B) Western blot analysis of YB-1 knockdown efficiency. *P<0.05. YB-1, Y-box-binding protein 1.

YB-1 was significantly decreased in both siRNA-1-transfected and siRNA-2-transfected cells compared to that in siCtr-transfected cells (P<0.05). According to the RT-qPCR analysis, the reduced level of YB-1 expression was not significantly different between the siRNA-1-transfected and siRNA-2-transfected groups. However, western blot analysis revealed that the reduced level of YB-1 expression was higher in the siRNA-1-transfected group compared to that in the siRNA-2-transfected group (Fig. 2B). Based on these results, siRNA-1 was used in subsequent *in vitro* experiments.

To investigate the role of YB-1 in cell proliferation and migration, MTT assays and migration assays were performed, respectively. In the MTT assays, proliferation potency was significantly suppressed in all the cell lines transfected with siRNA-1 compared to that in the siCtr-transfected group (Fig. 3A). Moreover, in the migration assays, the number of cells transfected with si-RNA-1 that migrated into the center of the well was significantly less than that of the siCtr-transfected group (Fig. 3B). Similar results were obtained from siRNA knockdown experiments using the other cell lines. Overall, the effects of YB-1 on cell proliferation and cell migration were not affected by the presence or absence of mutations in *KRAS* or *BRAF*.

Knockdown of YB-1 induces apoptosis-related genes in human colon cancer. Since there are few studies regarding the relationship between YB-1 and apoptosis, the effect of YB-1 on apoptosis was analyzed by evaluating alterations in the expression of apoptosis-related genes *Bax* and *Bcl-2* in siRNA-1-treated cells compared to that in siCtr-treated cells, by RT-qPCR analysis. There were no significant gene expression differences between the siRNA-1-treated and siCtr-treated groups for either HCT116 or HT29 cells. In contrast, *Bcl-2*

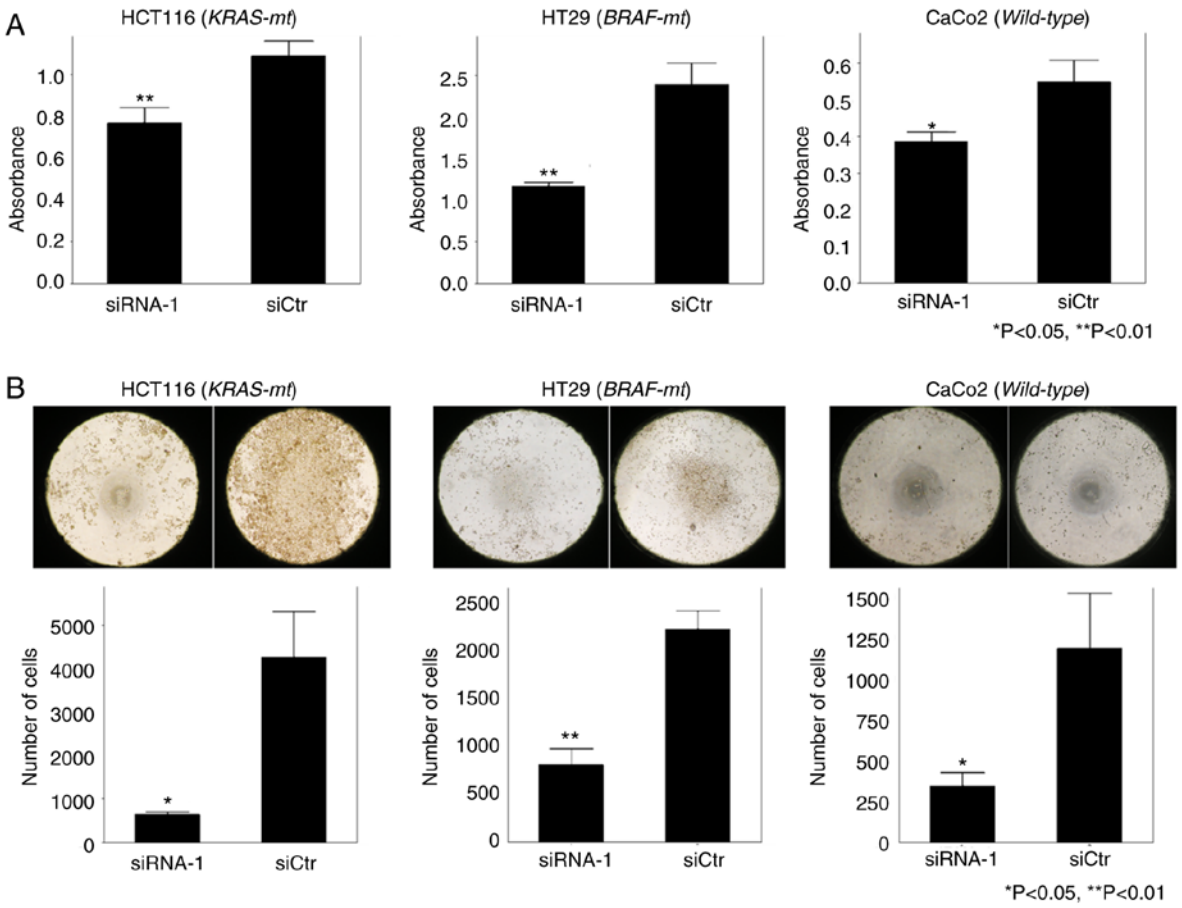

Figure 3. Knockdown of YB-1 inhibits cell proliferation and migration in human colon carcinoma cell lines HCT16, HT29 and CaCo2. (A) Effects of knockdown of YB-1 on cell proliferation as demonstrated by MTT assays. *P<0.05, **P<0.01. (B) Effects of knockdown of YB-1 on cell invasion as demonstrated by migration assays. *P<0.05, **P<0.01. YB-1, Y-box-binding protein 1.

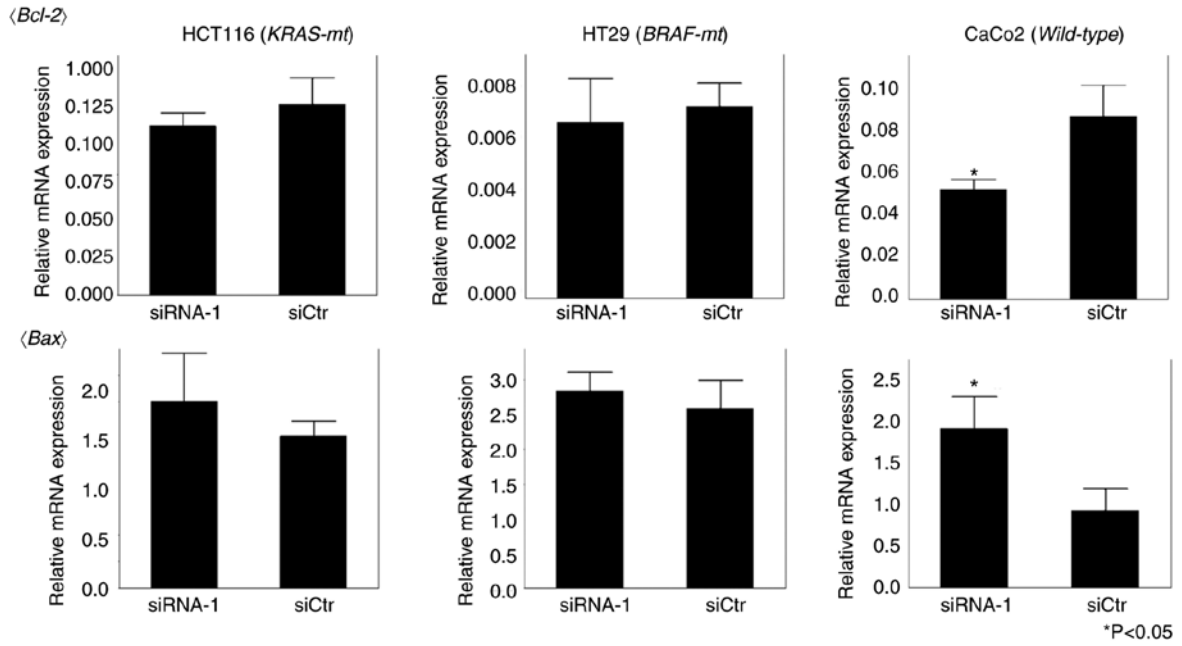

Figure 4. Knockdown of YB-1 induces expression of apoptosis-related genes in human colon carcinoma cell lines HCT16, HT29 and CaCo2. Effects of silencing of YB-1 on the expression of apoptosis-related genes. YB-1, Y-box-binding protein 1. *P<0.05.

expression levels were significantly decreased (P=0.018) and *Bax* levels were significantly increased (P=0.021) in siRNA-1-treated CaCo2 cells compared to that in siCtr-treated CaCo2 cells (Fig. 4).

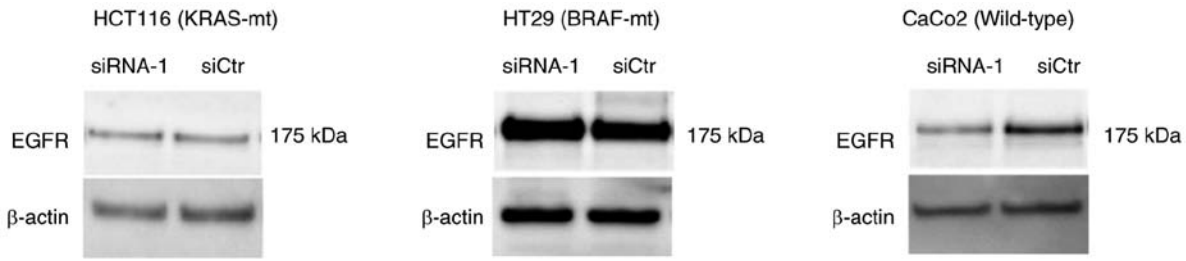

Figure 5. Knockdown of YB-1 reduces the expression of EGFR in human colon carcinoma cell lines HCT16, HT29 and CaCo2. Effects of knockdown of YB-1 on the expression of EGFR as determined by western blotting. YB-1, Y-box-binding protein 1; EGFR, epidermal growth factor receptor.

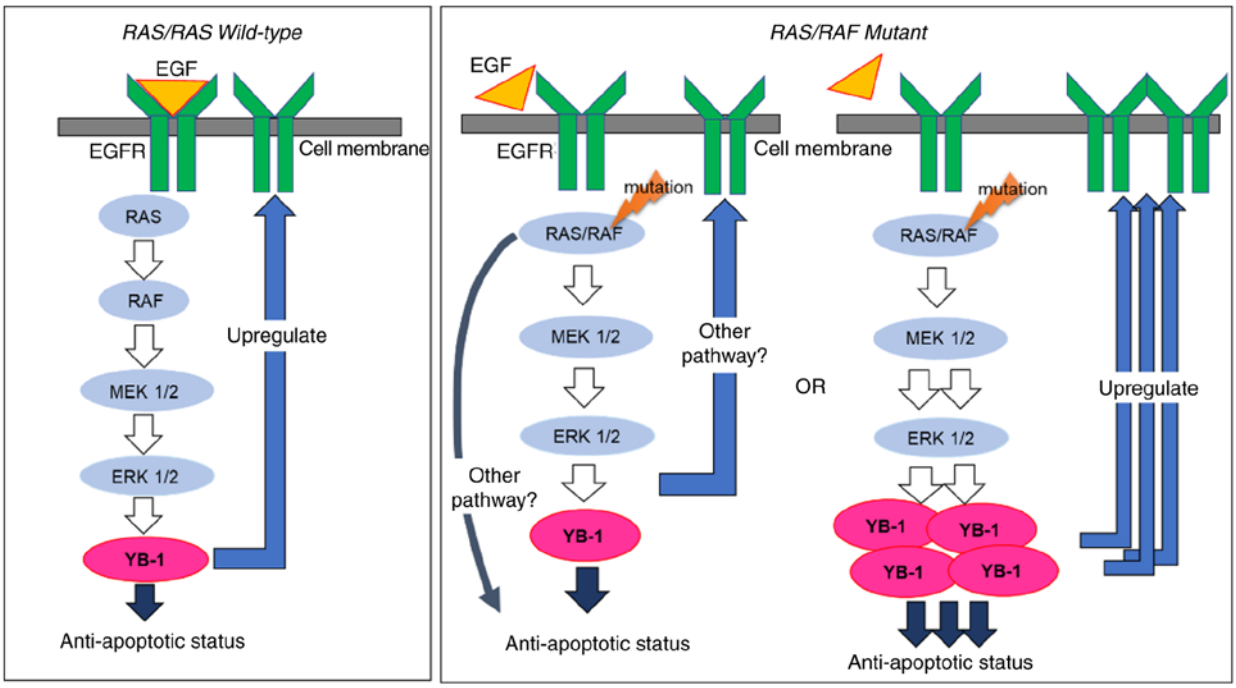

Figure 6. In colorectal cancer cell lines expressing wild-type *RAS/RAF*, YB-1 induced suppression of apoptosis via the MAPK pathway and upregulated the expression of EGFR. In colorectal cancer cell lines expressing mutated *RAS/RAF*, there was another pathway that was not regulated by YB-1. Alternatively, excessive YB-1 expression may have been activated via the MAPK pathway and promoted apoptosis inhibition and EGFR expression regardless of the knockdown of YB-1. YB-1, Y-box-binding protein 1; MAPK, mitogen-activated protein kinase; EGFR, epidermal growth factor receptor.

Knockdown of YB-1 reduces the expression of EGFR in human colon cancer. EGFR protein expression was compared between the siRNA-1-treated and siCtr-treated groups by western blot analysis. There were no significant differences in EGFR expression between the siRNA-1-treated and siCtr-treated groups for either HCT116 or HT29 cells. However, EGFR expression was significantly decreased in the siRNA-1-treated group of CaCo2 cells compared to that in siCtr-treated CaCo2 cells (Fig. 5).

Discussion

Findings from the present study suggest that Y-box-binding protein 1 (YB-1) was involved in promoting the malignancy potential of colon cancer through the enhancement of cell proliferation and -migration properties. Furthermore, YB-1 also contributed to the suppression of apoptosis and epidermal growth factor receptor (EGFR) expression in colorectal cancer cells. The alteration of apoptosis-related genes and EGFR expression by YB-1 knockdown was confirmed by our results to occur only in the cell lines expressing wild-type *RAS/RAF* and not in those expressing mutated *RAS/RAF*. These results suggest that the presence of the *RAS/RAF* mutation may affect the suppression of apoptosis and promotion of EGFR expression by YB-1 in colorectal cancer.

Previous studies have reported that there is a clear association between YB-1 expression and cell proliferation, and an increase in the expression levels of YB-1 was correlated with the expression of proliferation markers (14). In addition, there is a possible relationship between YB-1 expression and hyperplasia (15).

Basaki *et al* (16) demonstrated that knockdown of YB-1 resulted in a reduction of the number of cells in the S phase for multiple types of cancer, including breast, lung cancer and leukemia. Furthermore, they indicated that downregulation of cyclin D1 and upregulation of p21 occured as a result of YB-1 knockdown. These investigators also reported that YB-1 was associated with the cell-proliferation cycle (16). In

addition, Yan *et al* (17) revealed that the downregulation of E-cadherin and the upregulation of vimentin and N-cadherin occured in colon cancer cell lines transfected with *YB-1* and resulted in cancer cell proliferation through the enhancement of epithelial-mesenchymal transition (EMT).

The present study revealed that the knockdown of YB-1 impaired cell proliferation and cell migration in colon cancer cell lines, regardless of the status of *RAS*/*RAF*. While additional studies are required, it is possible to conclude that YB-1 is strongly involved in cancer cell proliferation and mobility, independent of the type of cancer cell.

The knockdown of YB-1 in the colon cancer cell line expressing wild-type *RAS*/*RAF* resulted in the upregulation of *Bax* and downregulation of *Bcl-2,* which are both associated with apoptosis. Similar results were observed in breast (18), bladder cancer (19) and neuroblastoma (20). Our results may be explained by the molecular relationship between YB-1 and p53, which selectively bind with each other and cause the suppression of p53 function to induce an anti-apoptotic effect. The enhancement of apoptosis by YB-1 knockdown was not observed in the colorectal cancer cell lines HCT116 or HT29, which harbor mutated *RAS*/*RAF*.

In breast cancer, non-small cell lung and cervical cancer, phosphorylation of YB-1 via the mitogen-activated protein kinase (MAPK) pathway upregulated EGFR expression (7,21,22). Previously, we reported that there is a positive correlation between YB-1 expression and EGFR expression in colorectal cancer tissue and high expression of both YB-1 and EGFR were associated with poor prognosis (10).

In the present study, suppression of EGFR expression by YB-1 knockdown was observed in the CaCo2 cell line, which expresses wild-type *RAS*/*RAF*. It was considered that YB-1 regulated EGFR in colorectal cancer harboring wild-type *RAS*/*RAF*, similar to that confirmed in other types of cancer. However, the same phenomenon was not verified in the cell lines HCT116 and HT29, which harbor the mutated *RAS*/*RAF*.

Our results were consistent with the promotion of apoptosis and suppression of EGFR expression upon knockdown of YB-1 in colon cancer with wild-type *RAS*/*RAF*. In contrast, in colorectal cancer cells harboring mutated *RAS*/*RAF*, there were no significant differences in the promotion of apoptosis or suppression of EGFR expression, despite the knockdown of YB-1.

In colorectal cancer, the expression of EGFR and factors of the downstream MAPK pathway are involved in cancer proliferation, invasion, metastasis and cell survival. In *RAS*/*RAF*-mutated cell lines, the mutated proteins activate the MAPK pathway independent of stimulation by EGFR, which help to maintain cancer survival and proliferation (23). It has been reported that extracellular signal-regulated kinases 1 and 2 (ERK 1/2) are located downstream of the MAPK pathway and they suppress apoptosis (24). Furthermore, in several types of cancer, YB-1 functioned downstream of ERK 1/2 and promotes proliferation, invasion and metastasis of cancer cells, resulting in poor prognosis (25-27). Moreover, Chu *et al* (28) revealed that in colon cancer cells with KRAS mutation, the expression of YB-1 was upregulated through the MEK/Sp1/DNMT1/miR-137 pathway.

In colon cancer with wild-type *RAS*/*RAF*, it is thought that YB-1 is activated via the MAPK pathway, which then induces the suppression of apoptosis and upregulation of EGFR. On the other hand, in *RAS*/*RAF*-mutated colorectal cancer, there may be an alternative pathway not mediated by YB-1, or YB-1 may exist in abundance due to constant expression activated by the MAPK pathway. The regulation of YB-1 by siRNA may become insufficient with the induction of apoptosis and the suppression of EGFR expression not being achieved (Fig. 6). Based on these findings, YB-1 may be useful as a therapeutic target in colon cancer harboring wild-type *RAS*/*RAF*. Furthermore, there was a significant association between the high expression of YB-1 and existence of mutated *RAS*/*RAF* in colon cancer cell lines.

In conclusion, it is possible that YB-1 plays a vital role in cell proliferation, cell migration, apoptosis and EGFR expression in colon cancer. Furthermore, apoptosis and expression of EGFR mediated through the control of YB-1 may be affected by the mutational status of *RAS*/*RAF*.

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Availability of data and materials

The datasets used during the present study are available from the corresponding author upon reasonable request.

Authors' contributions

SN designed the study and wrote the initial draft of the manuscript. TSu contributed to the analysis and interpretation of the data and assisted in the preparation of the manuscript. TK, TY, KF, TSh and YA contributed to the collection and interpretation of the data and critically reviewed the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in an effort to ensure that any questions related to the accuracy or integrity of any portion of the work are appropriately addressed and resolved.

Ethics approval and consent to participate

Since the present study used only commercially-available human-derived cells, approval by the institutional ethics committee was not necessary.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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