Yin Yang 1 regulates ITGAV and ITGB1, contributing to improved prognosis of colorectal cancer

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Abstract. Yin Yang 1 (YY1) is a multifunctional transcription factor with critical roles in carcinogenesis and metastasis. However, its biological role and clinical impact in colorectal cancer (CRC) remain unclear. In the present study, the function and underlying molecular mechanisms of YY1 in CRC progression were investigated. The immunohistochemistry (IHC) of 143 CRC tissues revealed a significant correlation of low YY1 expression with aggressive clinicopathological features, increased metastasis and recurrence and poor patient survival. Multivariate analysis identified low YY1 expression as an independent poor prognostic factor. Subsequently, the IHC of 66 paired CRC primary tumor and liver metastasis tissues revealed that low YY1 expression in the primary CRC was significantly associated with multiple liver metastases, major hepatectomy, extrahepatic metastasis and poor prognosis. *In vitro* experiments revealed that YY1 knockdown promoted the migration and invasion of CRC cells. To examine the downstream genes of YY1, a cDNA microarray assay was conducted and the differentially expressed genes between the YY1-knockdown and control cells were compared. Integrin alpha V (ITGAV) and integrin beta 1 (ITGB1) were identified as upregulated hub genes using gene enrichment analysis and protein-protein interaction analyses. Western blotting and IHC confirmed YY1 expression to be negatively correlated with ITGAV and ITGB1 expression. In summary, it was revealed that YY1, as a tumor-suppressor in CRC, contributes to the survival of patients with CRC. Low YY1 expression was associated with the poor prognosis of the patients with primary CRC and liver metastases. YY1 suppressed the expression of ITGAV and ITGB1, and this transcriptional regulation may lead to the suppression of CRC cell migration and invasion.

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Abbreviations: CA19-9, carbohydrate antigen 19-9; CEA, carcinoembryonic antigen; CRC, colorectal cancer; CSS, cancer-specific survival; DFS, disease-free survival; IHC, immunohistochemistry; ITGAV, integrin alpha V; ITGB1, integrin beta 1; TSF, time to surgical failure; YY1, Yin Yang 1

Key words: colorectal cancer, liver metastasis, YY1, ITGAV, ITGB1

Introduction

Colorectal cancer (CRC), one of the most common cancers, was reported as having the third-highest incidence and the second-highest number of cancer-related deaths among all the cancers worldwide in 2020 (1). A total of ~35% of CRC patients are diagnosed with metastasis and 20-50% of non-metastatic CRC patients develop metastasis during their disease (2,3). Although extensive efforts have been made to elucidate the molecular pathways associated with CRC progression, the treatment of metastatic CRC remains challenging. Therefore, an improved understanding of the molecular mechanisms underlying CRC metastasis is essential.

The transcription factor Yin Yang 1 (YY1) is a member of the GLI-Krüppel family of zinc finger DNA-binding proteins, which is ubiquitously expressed in various tissues (4,5). YY1 participates in various biological functions, such as cell proliferation (6-8), cell cycle (9), apoptosis (10), invasion (11-13), migration (7,13), drug resistance (14-16), and epithelial-mesenchymal transition (17,18). Therefore, YY1 is critical for tumor progression, and increasing evidence suggests a close association between YY1 and cancer.

However, the association between YY1 and the prognosis of patients with cancer is controversial. Certain studies have demonstrated YY1 expression to be associated with favorable outcomes (9-11,13,19,20), whereas others have demonstrated detrimental outcomes (21-26). These findings suggested that YY1 can activate or suppress target gene expression, depending on the interactions between the cellular environment, tissues and cofactors.

The present study aimed to elucidate the oncological role of YY1 in CRC. The correlation between YY1 expression and clinicopathological features and outcomes was evaluated in the patients with CRC. The *in vitro* experiments investigated the functions of YY1 in the CRC cells. Furthermore, the underlying mechanisms of clinical outcomes and *in vitro*

data were explored by investigating the downstream molecules under YY1.

Materials and methods

Patients and tissue samples. The clinical samples and data were obtained from 143 consecutive patients who underwent surgical resection for CRC between January 2012 and December 2013. Of these 143 patients, 12 patients underwent resection of liver metastases. Additionally, 66 pairs of CRC and liver metastatic tissues were collected after resection between January 2005 and December 2014. The patients who underwent both surgical resection for a primary tumor and initial hepatectomy at Chiba University Hospital (Chiba, Japan) were included. The patients who underwent repeat hepatectomy or two-stage hepatectomy were excluded. The resection for CRC was performed at the Department of Frontier Surgery, Chiba University Hospital, and the resection for liver metastasis was performed at the Department of General Surgery of the same hospital. The present study was approved (approval no. 2405) by the Ethics Committee of Chiba University Hospital and written informed consent was obtained from each patient before surgery.

Immunohistochemistry (IHC). Briefly, the paraffin-embedded tissue blocks were cut into 4-μm thick sections and deparaffinized with xylene and rehydrated with descending ethanol series. The slides were microwave-treated with 10 mmol/l citrate buffer (pH 6) for 25 min for antigen retrieval. The endogenous peroxidase activity was blocked at room temperature (21-26˚C) using 3% H_2O_2 in methanol for 15 min. After blocking the non-specific protein binding with 5% skimmed milk at room temperature (21-26˚C) for 10 min, the tissues were incubated overnight at 4˚C with primary antibodies against YY1 (1:500; product code ab109228), integrin alpha V (ITGAV; 1:500; product code ab179475) and integrin beta 1 (ITGB1; 1:100; product code ab52971; all from Abcam). The slides were washed three times with phosphate-buffered saline and treated with biotinylated secondary antibody (EnVision™ kit; cat. no. K4003; Dako; Agilent Technologies, Inc.) for 1 h at 37˚C and visualized using 0.01% 3,3-diaminobenzidine, both used according to the manufacturer's instructions. Finally, the sections were counterstained for 1 min at room temperature (21-26˚C) with hematoxylin and then rehydrated and sealed.

IHC evaluation of YY1, ITGAV and ITGB1. Using an inverted light microscope (BX40; Olympus, Inc.), intranuclear YY1 expression was assessed as the percentage of positively stained nuclei in the tumor cells relative to the total number of malignant cells in three positive high-power fields (magnification, x400). The expression of ITGAV and ITGB1 was observed in the cytoplasm of the tumor cells and the staining intensity varied in each sample. Therefore, the expression was assessed in three positive high-power fields based on the staining intensity and the percentage of positively stained cells using the following scoring system: The staining intensity was scored (0, negative; 1, weak; 2, moderate; 3, strong staining) and the percentage of positively stained cells was scored (0, 0; 1, 1-25; 2, 26-50; 3, 51-75; 4, 76-100% positively stained cells) and the final score was obtained by multiplying the scores together. Protein expression was independently assessed by two researchers with a pathologist who was blinded to the clinical information of the patient. In case of disagreement, the slides were re-examined until a final consensus was reached.

Cell culture. Human colon cancer cell lines, DLD-1 (ATCC no. CCL-221) and SW48 (ATCC no. CCL-231), were obtained from the American Type Culture Collection. DLD-1 cells were cultured at 37˚C in the RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS (Thermo Fisher Scientific, Inc.), and the SW48 cells were cultured in Leibovitz's L-15 medium (Gibco; Thermo Fisher Scientific, Inc.) containing 10% FBS.

RNAi transfection. The sequences of the double-stranded small interfering (si)RNAs used to knock down YY1 were as follows: siRNA1: Hs_YY1_1, cat. no. SI00051912 (target sequence: 5'-GACGACGACTACATTGAACAA-3'), and siRNA2: Hs_YY1_3, cat. no. SI00051926 (target sequence: 5'-ATGCCTCTCCTTTGTATATTA-3') (both from Qiagen, Inc.). The control cells were treated with negative control siRNA (AllStars negative control siRNA; cat. no. SI1027280; Qiagen, Inc.). These siRNAs (final concentration, 5 nmol/l) were transfected into the DLD-1 and SW48 cells using Lipofectamine® RNAiMAX (Invitrogen; Thermo Fisher Scientific, Inc.) at 37˚C. The cells were transfected with siRNAs 24 h before each assay and the knockdown efficiency was assessed by western blotting 72 h after transfection.

Western blot analysis. The whole-cell proteins were purified from the cultured cell lines using the radioimmunoprecipitation assay (RIPA) buffer (Sigma-Aldrich; Merck KGaA). The proteins (20 μg) determined using bicinchoninic acid were loaded onto 5-12.5% XV PANTERA Gels (cat. no. NXV-2E4HP; DRC Co., Ltd.) and transferred onto a polyvinylidene difluoride membrane. The membranes were blocked in 5% skimmed milk in 0.1% Tris-buffered saline with Tween-20 (TBS-T) at a temperature of 21-26˚C for 60 min and incubated at 4˚C overnight with the following primary antibodies: YY1 (1:10,000), ITGAV (1:5,000), ITGB1 (1:10,000) and β-actin (1:5,000; cat. no. 5125S; Cell Signaling Technology, Inc.). After three washes with 0.1% TBS-T, the membranes were incubated with anti-rabbit IgG horseradish peroxidase-conjugated secondary antibody (1:2,000; cat. no. sc-2305; Santa Cruz Biotechnology, Inc.) at 37˚C for 1 h. The protein bands were detected using an enhanced chemiluminescence detection reagent (Chemi-Lumi One Ultra; cat. no. 11644; Nacalai Tesque, Inc.) and developed using a LAS-4000UV mini luminescent image analyzer (FUJIFILM Wako Pure Chemical Corporation). The band intensities from the western blot were quantified using densitometry and normalized to β-actin using the Adobe Photoshop version 7.0 (Adobe Systems, Inc.).

Cell proliferation assay. Quantification of the living cells was performed using Cell Count Reagent SF (cat. no. 07553; Nacalai Tesque, Inc.) according to the manufacturer's protocol. The DLD-1 and SW48 cells, which were transfected with siYY1 or siControl, were seeded at the rate of 1,000 and 3,000 cells/well, respectively, in 96-well plates. After pre-incubation at 37˚C,

the 10-μg/well of cell count reagent was added to each well at 0, 24, 48, 72 and 96 h. After 2 h of incubation, the absorbance at 450 nm was measured using a microplate reader.

Gap closure assay. The DLD-1 and SW48 cells were transfected using siYY1 and siControl 24 h before the gap closure assay. Cells of appropriate density ($2x10^4$ cells/well for DLD-1 and $15x10^4$ cells/well for SW48) and 100% confluence in the monolayer were seeded into each well of a culture insert (cat. no. 81176; Culture-Insert 2 Well in μ-Dish; Ibidi GmbH). After 24 h of incubation at 37˚C, the culture insert was removed and the dish was filled with complete medium. Images of the cell-free gaps were captured using an inverted light microscope (Axio Observer Z1; Carl Zeiss AG). The images were captured in three fields per well at each point in time (DLD-1, 24 h; and SW48, 96 h after removing the culture-insert). The cell-free gaps were measured using ImageJ software version 1.53k (National Institutes of Health) and the percentage of cell-free gaps was compared with that at 0 h.

Transwell migration and Matrigel invasion assay. For the Transwell migration assay, the DLD-1 and SW48 cells were transfected with siYY1 and siControl 24 h before the assay. Following overnight starvation, the cells of appropriate density ($1x10^5$ cells/well for DLD-1 and $3x10^5$ cells/well for SW48 in the RPMI-1640 and L-15 medium containing 0.1% FBS, respectively), were seeded in the upper chamber of the culture inserts with an 8-μm pore-size polyester membrane (Corning, Inc.). A total of 500 μl of RPMI-1640 or L-15 medium containing 10% FBS was added to the lower chamber as a chemoattractant. Following incubation at 37˚C for 48 h, the non-migrating cells on the top of the insert membrane were carefully removed and the migrating cells on the bottom of the membrane were stained at 37˚C for 10 min with a dye solution containing 0.1% crystal violet and 20% methanol. A total of 10 images of each membrane were captured and the migratory cells were counted. For the Transwell invasion assay, the Cell Biolabs CytoSelect™ 24-well cell invasion assay kit (cat. no. CBA-110; Cell Biolabs, Inc.) utilizing basement membrane-coated inserts was used according to the manufacturer's protocol. The experimental procedure for the invasion assay was similar to that described for the Transwell migration assay.

RNA preparation and microarray analysis. Total RNA was isolated from the negative control siRNA-transfected cells and the siRNA1-transfected cells in two cell lines, DLD-1 and SW48, using the QIAGEN RNeasy Mini kit (cat. no. 74104; Qiagen, Inc.). The total RNA quantity and quality were evaluated and verified using NanoDrop 2000 (Thermo Fisher Scientific, Inc.) and Bioanalyzer 2100 (Agilent Technologies, Inc.). The microarray analysis was performed by Macrogen Japan Corp. Sample labeling and microarray hybridization were performed according to the Affymetrix Human Clariom™-S Assay standard protocols. Briefly, cDNA was synthesized using the GeneChip WT Amplification kit (Thermo Fisher Scientific, Inc.) as described by the manufacturer. The sense cDNA was then fragmented and biotin-labeled with (TdT) using the GeneChip WT Terminal labeling kit (Thermo Fisher Scientific, Inc.). Approximately 5.5 μg of labeled DNA target was hybridized to the Affymetrix GeneChip Array at 45˚C for 16 h. The hybridized arrays were washed and stained on a GeneChip Fluidics Station 450 and scanned on a GCS3000 Scanner (Affymetrix; Thermo Fisher Scientific, Inc.). The probe cell intensity data were computed using the Affymetrix® GeneChip Command Console® software. The differentially expressed genes (DEGs) that were upregulated and downregulated in the siYY1 cells compared with the siControl cells were defined as a cut-off criterion with fold change ≥1.5.

Gene annotation enrichment analysis, protein-protein interaction (PPI) network analysis and identification of hub genes. The gene lists of the upregulated and downregulated DEGs were uploaded to Metascape (http://metascape.org), and enrichment for Gene Ontology (27) (http://geneontology.org) and Kyoto Encyclopedia of Genes and Genomes pathways (28) (https://www.genome.jp/kegg/pathway.html) were analyzed. Metascape is a gene annotation and analysis tool that updates monthly information and the last update was on February 1, 2021. The PPI network analysis and identification of significant candidate genes were performed using the Cytoscape software version 3.8.2 (http://cytoscape.org). DEGs were imported into the STRING database (http://string-db.org), and a PPI network was constructed. The results of the PPI network analysis were downloaded and visualized using Cytoscape. Finally, the network analyzer application version 4.4.6 (https://apps.cytoscape.org/apps/networkanalyzer) was used to calculate the node degree, and the top 10 genes of degree centrality were identified as the hub genes.

The cancer genome atlas (TCGA) analysis. Kaplan-Meier survival analysis was performed using R2 (http://r2platform.com/), which is a web-based platform for genomics analysis and visualization. TCGA dataset, including 174 colon adenocarcinoma samples, was analyzed. The scanned cut-off value was used as the threshold to distinguish between the high and low expression of YY1.

Identification of the ITGAV and ITGB1 promoter sequences and YY1-specific binding site. The promoter sequences of *ITGAV* and *ITGB1* were obtained using the database of transcriptional start sites, DBTSS 10.1 (https://dbtss.hgc.jp). In order to identify the YY1 specific binding site in each promoter region, the sequence was inserted into JASPAR 2020 (https://www.jaspar.jp) software, which is an open access database for transcription factor binding sites.

Statistical analysis. The survival curves were calculated using the Kaplan-Meier method and the significance of differences was analyzed using the log-rank test. Cancer-specific survival (CSS) was calculated as the duration from the date of surgery to the date of death from CRC. Patients were censored if they succumbed from other causes or if the patients were alive at the time of the final observation. Disease-free survival (DFS) was calculated from the date of surgery to the date of recurrence. The time to surgical failure (TSF) was defined as the period between the date of surgery and the date of appearance of unresectable recurrence. Multivariate analysis for survival was performed using the Cox proportional hazards model, and the odds ratio for distant metastasis was analyzed using the logistic regression analysis. The correlation between YY1 and

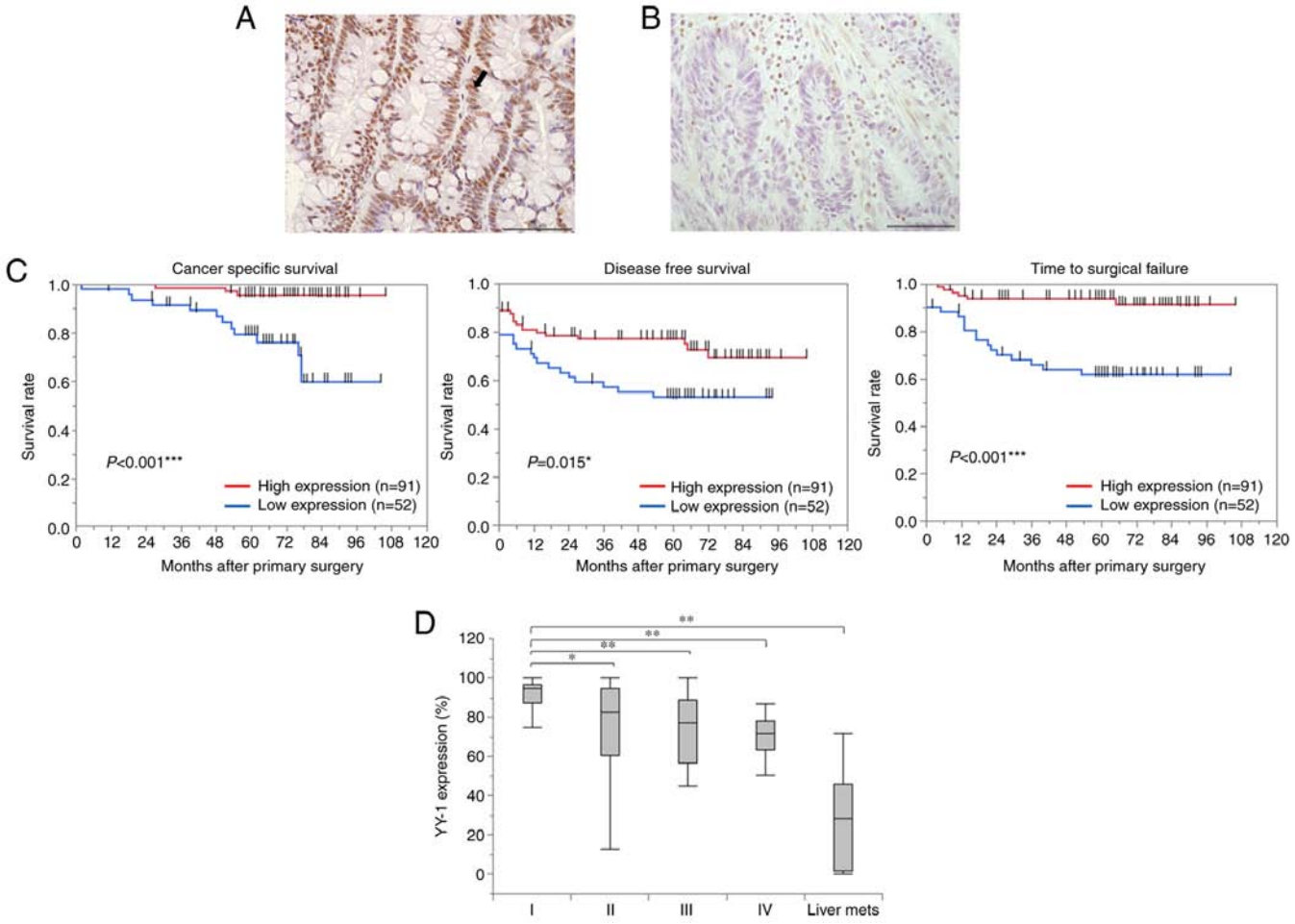

Figure 1. Immunohistochemistry of colorectal cancer tissues. (A and B) YY1 protein was predominantly expressed in the nucleus of cancer cells (arrow). (A) High YY1 expression and (B) low YY1 expression (magnification, x400; scale bar, 100 μm). (C) Kaplan-Meier analysis was based on YY1 expression. Low YY1 expression was significantly associated with shorter cancer-specific survival (P<0.001), disease-free survival (P=0.015), and time to surgical failure (P<0.001). (D) The YY1 positive rate significantly decreased from stage I to IV and that of liver metastatic tissues was the lowest. *P<0.05 and **P<0.01. YY1, Yin Yang 1.

ITGAV or ITGB1 expression was analyzed using the Pearson's correlation coefficient. Each *in vitro* experiment was independently performed at least thrice. The statistical significance of the results was determined by the unpaired Student's t-test, Chi-square test, or Fisher's exact test. P<0.05 was considered to indicate a statistically significant difference. Data are expressed as the median ± standard deviation or the mean ± standard error of the mean. The statistical analyses were performed using JMP PRO 15 software (SAS Institute, Inc.).

Results

Low YY1 expression in the primary tumor is associated with a poor prognosis. The expression of YY1 was assessed using IHC in 143 primary tumors. YY1 expression was predominantly localized in the nucleus (Fig. 1A and B). Based on receiver operating characteristic (ROC) analysis in accordance with CSS, all tissues were categorized into two groups (cut-off value, 75.2%; AUC, 0.727; P=0.096). Comparison of the clinicopathological features between the two groups (Table I) revealed that the low YY1 expression group (<75.2% YY1-positive cells) was significantly associated with elevated CEA levels (P=0.048) and CA19-9 levels (P=0.018). The proportion of T4 (P=0.043), Ly 2-3 (P=0.045), V 2-3 (P=0.014), and lymph node metastasis (P=0.013) was significantly higher in the low YY1 group. In addition, the low YY1 group had a lower proportion of stage I and a higher proportion of stage IV than the high YY1 group (P=0.004). Furthermore, the distant metastases in all patients (P<0.001) and recurrence after curative resection in patients with Stage I-III disease (P=0.012) occurred more frequently in the low YY1 expression group. The Kaplan-Meier analysis revealed that patients with low YY1 expression had significantly shorter CSS (P<0.001), DFS (P=0.015), and TSF (P<0.001) (Fig. 1C). Examining the correlation between TNM stage and YY1 expression in primary tumors revealed that the YY1 positive rate was significantly lower from stage I to IV. In addition, YY1 expression in the 12 liver metastases that occurred in 143 patients was the lowest of any of them (stage I, 89.3±4; stage II, 74±3; stage III, 72.1±3.4; stage IV, 68.5±4.7; and liver metastasis, 27.5±6.2%) (Fig. 1D). The multivariate analyses revealed a significant association between low YY1 expression and CSS (HR, 4.54; 95% CI, 1.22-16.88; P=0.024; Table II). Furthermore, low YY1 expression was an independent risk factor for distant metastases (odds ratio, 3.09; 95% CI, 1.20-7.95; P=0.020; Table III). To validate our data, Kaplan-Meier survival analysis was performed

Table I. Associations between YY1 expression and clinicopathological features of patients with colorectal cancer.

Clinicopathological feature, unit	Expression level of YY-1		P-value
	High (n=91)	Low (n=52)	
Age at primary surgery, years	70 (29-91)	68 (27-91)	0.309
Sex			0.717
Male	60	32	
Female	31	20	
CEA, ng/ml	10±5.4	27.9±7.2	0.048[a]
CA19-9, U/ml	24.9±47.5	214.2±62.8	0.018[a]
Site of tumor			0.121
Right	5	7	
Left	86	45	
Neoadjuvant therapy			0.052
+	6	9	
-	85	43	
Size of tumor, mm	38.5±2.1	43.9±2.8	0.126
T stage[c]			0.043[a]
1-3	74	34	
4	17	18	
Degree of differentiation			0.135
tub, pap	89	48	
por, muc	1	3	
Ly			0.045[a]
0-1	82	41	
2-3	8	11	
V			0.014[a]
0-1	69	29	
2-3	21	23	
Lymph node metastasis			0.013[a]
+	29	28	
-	62	24	
TNM stage[c]			0.004[a]
I	26	3	
II	33	19	
III	23	18	
IV	9	12	
RAS mutation			>0.999
Wild	15	14	
Mutant	9	8	
BRAF mutation			>0.999
Wild	23	21	
Mutant	1	1	
Adjuvant chemotherapy			0.018[a]
+	24	24	
-	67	28	
Occurrence of distant metastasis (Stage I-IV)			<0.001[b]
+	16	23	
-	75	29	
Recurrence after primary surgery (Stage I-IV)			0.012[a]
+	7	11	
-	75	29	

[a]P<0.05 and [b]P<0.01. [c]Union for International Cancer Control 8th edition. YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma.

Table II. Univariate and multivariate analysis for cancer-specific survival in patients with colorectal cancer.

		Univariate		Multivariate	
Clinicopathological feature, unit	n	5-year survival rate (%)	P-value	HR (95% CI)	P-value
Age at primary surgery, years					
<65	44	92.3	0.201		
≧65	99	84.3			
Sex			0.102		
Male	93	84.1			
Female	51	100			
CEA, ng/ml			0.106		
≧5	58	83.9			
<5	86	93.3			
CA19-9, U/ml			0.094		
≧37	26	74.5			
<37	118	92.6			
Site of primary tumor			0.846		
Left	132	89.7			
Right	12	88.9			
Neoadjuvant chemotherapy before primary surgery			0.140		
+	16	73.3			
-	128	91.4			
T stage[a]			0.354		
4	35	87.5			
1-3	109	90.2			
Degree of differentiation			0.049[b]	3.36 (0.64-17.57)	0.151
por, muc	4	50			
tub, pap	137	90.8			
Ly			<0.001[c]	4.51 (1.31-15.53)	0.017[b]
2-3	19	58.4			
0-1	124	94.6			
V			0.044[b]	1.05 (0.36-3.09)	0.932
2-3	44	81.1			
0-1	99	93.5			
Lymph node metastasis			<0.001[c]	3.17 (0.58-17.33)	0.184
+	57	79.5			
-	87	96.8			
RAS mutation			0.380		
Mutant	17	61.9			
Wild	29	74.1			
Adjuvant chemotherapy after primary surgery			0.002[d]	1.7 (0.48-5.96)	0.409
+	48	79.6			
-	96	95.6			
Expression of YY-1 in primary tumors			<0.001[b]	4.54 (1.22-16.88)	0.024[b]
Low	51	79.7			
High	91	95.6			

[a]Union for International Cancer Control 8th edition. [b]P<0.05, [c]P<0.001 and [d]P<0.01. YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; HR, hazard ratio; CI, confidence interval.

Table III. Univariate and multivariate analysis for distant metastasis in patients with colorectal cancer.

	Univariate		Multivariate	
Clinicopathological feature, unit	n	P-value	Odds ratio (95% CI)	P-value
Age at primary surgery, years		0.075		
<65	44			
≧65	99			
Sex		0.395		
Male	93			
Female	51			
CEA, ng/ml		<0.001[a]	5.29 (1.92-14.56)	0.001[b]
≧5	58			
<5	86			
CA19-9, U/ml		0.026[b]	1.33 (0.42-4.28)	0.629
≧37	26			
<37	118			
Site of primary tumor		0.279		
Left	132			
Right	12			
Neoadjuvant chemotherapy before primary surgery		0.146		
+	16			
-	128			
T stage[d]		0.008[c]	1.63 (0.61-4.43)	0.332
4	35			
1-3	109			
Degree of differentiation		0.338		
por, muc	4			
tub, pap	137			
Ly		<0.001[a]	3.97 (1.1-14.35)	0.036[b]
2-3	19			
0-1	124			
V		0.024[b]	1.55 (0.56-4.25)	0.398
2-3	44			
0-1	99			
Lymph node metastasis		<0.001[a]	3.09 (1.15-8.33)	0.027[b]
+	57			
-	87			
RAS mutation		0.213		
Mutant	17			
Wild	29			
Adjuvant chemotherapy after primary surgery		0.069		
+	48			
-	96			
Expression of YY-1 in primary tumors		<0.001[a]	3.09 (1.2-7.95)	0.020[b]
Low	51			
High	91			

[a]P<0.001, [b]P<0.05 and [c]P<0.01. [d]Union for International Cancer Control 8th edition. YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; CI, confidence interval.

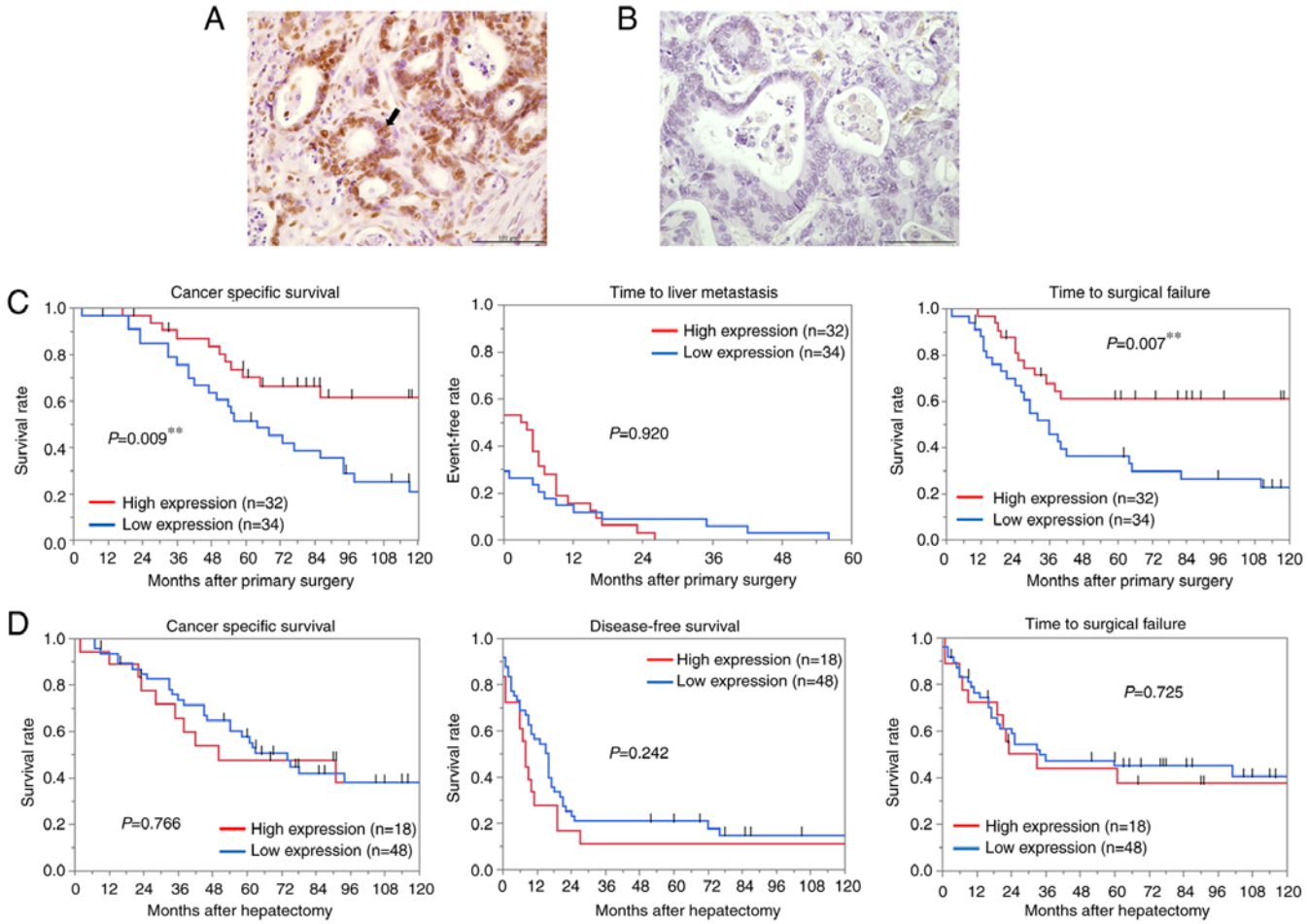

Figure 2. Immunohistochemistry of primary colorectal cancer tissues and liver metastasis. (A and B) YY1 protein was predominantly expressed in the nucleus of liver metastatic cells (arrow). (A) High YY1 expression and (B) low YY1 expression (magnification, x400; scale bar, 100 μm). (C) Low YY1 expression in the primary tumors was significantly associated with shorter cancer-specific survival (P=0.009) and time to surgical failure (P=0.007). (D) No correlation was observed between YY1 expression in liver metastatic tissues and the survival of patients after hepatectomy. YY1, Yin Yang 1.

on TCGA 174 colon adenocarcinoma dataset using the R2 Platform. Analysis from the TCGA dataset also revealed that the patients with low YY1 expression tended to have shorter survival (P=0.068; Fig. S1).

Low YY1 expression in the primary tumors with liver metastases is associated with poor prognosis. YY1 protein expression in 66 paired tissues of CRC and liver metastases was examined by IHC. YY1 expression in liver metastases was predominantly localized in the nucleus as well as in primary CRC (Fig. 2A and B). The YY1 positive rate of the nucleus was calculated using the same protocol as aforementioned, and patients were divided into two groups (cut-off value, 52.9%; AUC, 0.703; P=0.006). Analysis of the association between YY1 expression in primary tumors and clinicopathological features (Table IV) revealed that low YY1 expression (<52.9% YY1-positive cells) was significantly associated with elevated CEA levels (P=0.045), multiple liver metastases (P=0.004), and major hepatectomy (P=0.013). In addition, the rate of extrahepatic metastases was also significantly higher in the patients with low YY1 expression (P=0.024). Low YY1 expression was significantly associated with shorter CSS (P=0.009) and TSF (P=0.007) (Fig. 2C). Multivariate analysis revealed that low YY1 expression was significantly associated with CSS (HR, 2.40; 95% CI, 1.09-5.31; P=0.030; Table V). The IHC of the metastatic liver tissues revealed no significant correlation between YY1 and the clinicopathological features, in contrast to the results in the primary tumors (Table VI). Furthermore, no significant relationship was observed between YY1 expression and patient survival after hepatectomy (Fig. 2D).

Knockdown of YY1 promotes cell migration and invasion. The *in vitro* experiments were performed to elucidate the effect of YY1 on the migration and invasion abilities of CRC cells since the clinical data indicated that YY1 may play a critical role in CRC metastasis. YY1 protein expression was knocked down using siRNAs, as revealed in Fig. 3A.

The wound healing assays demonstrated that the cell-free gaps in the YY1-knockdown cells were significantly reduced compared with those in the control cells (Fig. 3B). The Transwell migration assays demonstrated that YY1 knockdown significantly increased the number of migratory cells in both cell lines (Fig. 3C). The Matrigel invasion assays demonstrated that YY1 knockdown significantly increased the number of invasive cells in both cell lines (Fig. 3D).

Table IV. Associations between YY1 expression in primary tumors and clinicopathological features of patients who developed liver metastasis.

Clinicopathological feature, unit	Expression level of YY-1 in primary tumor		P-value
	High (n=32)	Low (n=34)	
Age at primary surgery, years	69 (46-81)	69 (50-82)	0.925
Sex			0.797
Male	22	22	
Female	10	12	
CEA before primary surgery, ng/ml	39.9±180.5	553.5±175.1	0.045[a]
CA19-9 before primary surgery, U/ml	51.8±394.6	885.5±382.8	0.134
Site of tumor			0.057
Right	13	6	
Left	19	28	
Neoadjuvant therapy before primary surgery			0.493
+	0	2	
-	32	32	
T stage[c]			0.145
1-3	21	16	
4	11	18	
Degree of differentiation			>0.999
tub, pap	30	32	
por, muc	2	2	
Ly			0.748
0-1	26	29	
2-3	6	5	
V			0.631
0-1	14	17	
2-3	18	17	
Lymph node metastasis			0.624
+	20	19	
-	12	15	
TNM stage[c]			0.146
I	1	0	
II	7	8	
III	7	2	
IV	17	24	
RAS mutation			0.128
Wild	7	11	
Mutant	8	3	
BRAF mutation			>0.999
Wild	14	11	
Mutant	1	0	
Adjuvant chemotherapy			>0.999
+	5	5	
-	27	29	
Interval to liver metastasis, months	5.5±1.9	5.6±1.8	0.973
Timing of metastasis			0.079
Synchronous	15	24	
Metachronous	17	10	

Table IV. Continued.

Clinicopathological feature, unit	Expression level of YY-1 in primary tumor		P-value
	High (n=32)	Low (n=34)	
Number of liver metastatic tumors	2.5±0.5	3.3±0.5	0.252
Solitary/Multiple	17/15	6/28	0.004[b]
Size of largest liver metastatic tumor, cm	3.5±0.4	4±0.4	0.461
Site of liver metastasis			0.145
Unilateral	21	16	
Bilateral	11	18	
Hepatectomy			0.013[a]
Minor	31	25	
Major	1	9	
H factor			0.082
H1	25	18	
H2	7	15	
H3	0	1	
Metastasis other than liver			0.024[a]
+	2	10	
-	30	24	

[a]P<0.05 and [b]P<0.01. [c]Union for International Cancer Control 8th edition. YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma.

Knockdown of YY1 does not alter the cell proliferation. Subsequently, the cell proliferation assays were performed. The assays revealed that cell proliferation was not different between the YY1-knockdown cells and control cells (Fig. 4).

Gene enrichment analysis and identification of the key downstream genes regulated by YY1. To investigate downstream genes that may be regulated by YY1, a cDNA microarray assay was performed. DEGs between the YY1-knockdown and control cells are shown in Fig. 5A and B. A total of 241 genes were revealed to be commonly upregulated, and 254 genes to be commonly downregulated in DLD-1 and SW48 cell lines (Fig. 5C).

Among the upregulated DEGs, the genes involved in the 'MAPK signaling pathway', 'cell-substrate adhesion', 'extracellular matrix binding', 'regulation of cell adhesion', 'positive regulation of cellular protein localization', 'positive regulation of protein kinase activity', 'adherens junction', 'pathways in cancer' and the 'mTOR signaling pathway' were significantly enriched (Fig. 5D and E). In downregulated DEGs, genes involved in the 'nucleobase biosynthetic process', the 'metabolic processes of water-soluble vitamins', 'phosphatidylserine', 'valine', 'cholesterol', 'glycerophospholipids' and 'pyrimidine' were significantly enriched (Fig. 5F and G). The list of genes contained in each term is presented in Tables SI and SII.

The upregulated DEGs in the siYY1 cells were investigated since they were expected to be more relevant to the results of the *in vitro* experiments than the downregulated DEGs. A PPI network of upregulated DEGs was created using the STRING App and they were visualized using Cytoscape. As revealed in Fig. 6, the PPI network contained 234 nodes and 175 edges. The top 10 genes of degree centrality calculated by the network analyzer were identified as the hub genes: *TLR4, IL1B, FGFR2, ITGB1, CCR7, FOXO1, JAG1, SELL, ITGAV* and *PIK3R2*. Among these genes, focus was addressed on the integrin family genes *ITGAV* and *ITGB1,* which are strongly associated with cell adhesion (29), migration (30) and invasion (31).

ITGAV and ITGB1 expression is negatively correlated with YY1 expression in the CRC cell lines and primary CRC tumors. To verify the association between the YY1 knockdown and the expression of ITGAV and ITGB1, western blot and IHC analyses were performed. The western blot analysis revealed that ITGAV and ITGB1 expression was significantly increased in the YY1-knockdown cell lines (Fig. 7). IHC in 143 primary tumors demonstrated that YY1 expression in the primary CRC tumors was negatively correlated with both ITGAV (R=-0.247; P=0.003) and ITGB1 expression (R=-0.299; P<0.001; Fig. 8).

The promoters of ITGAV and ITGB1 have a YY1-specific binding site. The sequences of the transcription factor binding sites of ITGAV and ITGB1 were examined to investigate the possibility that YY1 binds directly to the respective promoters. The promoter sequences of *ITGAV* and *ITGB1* obtained using DBTSS were inserted into JASPAR 2020 software to identify

Table V. Univariate and multivariate analysis for cancer-specific survival in patients with liver metastases.

			Univariate	Multivariate	
Clinicopathological feature, unit	n	5-year survival (%)	P-value	HR (95% CI)	P-value
Age at primary surgery, years					
<65	22		0.823		
≧65	44				
Sex			0.676		
Male	22	54.6			
Female	44	63.9			
CEA before primary surgery, ng/ml			0.242		
≧5	48	58.8			
<5	18	65.5			
CA19-9 before primary surgery, U/ml			0.296		
≧37	23	52.6			
<37	43	64.5			
Site of primary tumor			0.033[a]	1.54 (0.65-3.65)	0.332
Left	47	53.5	0.033[a]	1.54 (0.65-3.65)	0.332
Right	19	78.6			
T stage[c]			0.558		
4	29	54.6			
1-3	37	64.9			
Degree of differentiation			0.695		
tub, pap	62	59.6			
por, muc	4	75			
Ly			0.870		
2-3	11	71.6			
0-1	55	58.6			
V			0.120		
2-3	35	50.6			
0-1	31	71			
Lymph node metastasis			0.004[b]	3.21 (1.4-7.35)	0.006[b]
+	39	51.3			
-	27	75.4			
RAS mutation			0.843		
Mutant	11	50.5			
Wild	18	55.6			
Adjuvant chemotherapy after primary surgery			0.240		
+	10	50			
-	56	62.5			
Timing of liver metastasis			0.221		
Synchronous	39	56.9			
Metachronous	27	65.8			
Metastasis other than liver					
+	12	41.7	0.063		
-	54	65			
Number of liver metastasis			0.225		
Multiple	43	54.8			
Solitary	23	71.2			
Size of largest liver metastatic tumor, cm			0.308		
≧5	13	50.4			
<5	53	62.9			

Table V. Continued.

Clinicopathological feature, unit	n	5-year survival (%)	Univariate P-value	Multivariate HR (95% CI)	Multivariate P-value
Hepatectomy			0.071		
Major	10	44.4			
Minor	56	63.2			
Surgical margin of liver metastasis			0.049[a]	1.20 (0.6-2.43)	0.606
R1-2	31	52			
R0	35	67.9			
H factor			0.023[a]	1.62 (0.81-3.24)	0.171
H2-H3	23	38.6			
H1	43	71.6			
Expression of YY-1 in primary tumors			0.009[b]	2.4 (1.09-5.31)	0.030[a]
	34	51.6			
	32	72.3			

[a]P<0.05 and [b]P<0.01. [c]Union for International Cancer Control 8th edition. YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma; HR, hazard ratio; CI, confidence interval.

the binding site. The analysis identified one YY1-specific putative binding site on each of the promoter sequences (*ITGAV*, CAAGAGGGCTGA; *ITGB1*, CATGATGGCTCT; Fig. S2).

Discussion

The present study revealed that low YY1 expression in primary CRC tumors was significantly associated with a poor prognosis. Our *in vitro* experiments demonstrated that YY1 suppressed CRC cellular migration and invasion. Furthermore, the microarray analysis revealed that YY1 may play an important role as a tumor suppressor by regulating the members of the integrin family, ITGAV and ITGB1.

There has been conflicting evidence regarding the role of YY1 in CRC biology. Chinnappan *et al* (32) reported that low YY1 expression levels in colon cancer tended to be associated with shorter survival. It was suggested that YY1 may be inactivated and could be a candidate as a tumor suppressor gene in colon cancer. The aforementioned study supported the present data in demonstrating the tumor-suppressive role of YY1. Whereas, Zhang *et al* (33) revealed that YY1 promotes colon cancer growth by inhibiting p53 and promoting the Wnt signaling pathways, leading to poor clinical outcomes. Similarly, certain reports suggested that YY1 plays a tumor promoting role (8,34-36). This discrepancy may be due to the different stages of cancer progression being explored indicating that the function of YY1 is context-dependent. To better understand the diversity of YY1 function by carcinogenic stage, YY1 expression was compared between normal mucosa and CRC primary tumors in 143 tissues. YY1 expression was significantly higher in tumors than in normal mucosa (positive rate, 44.4±2% and 75.8±1.9%; P<0.001, data not shown). A similar result was demonstrated in a previous study investigating the function of YY1 in pancreatic cancer (11). The aforementioned study demonstrated the tumor-suppressive role of YY1, revealing that YY1 expression was high in PDAC tissues but low in normal pancreatic tissues. It was theorized that YY1 is not involved in carcinogenesis but plays a tumor-suppressive role once cancer has developed. Collectively, it is considered that YY1 plays a tumor-suppressive role in inhibiting cancer progression that leads to favorable prognosis of CRC patients but cannot suppress carcinogenesis.

The present data demonstrated that low YY1 expression in primary tumors was significantly associated with lymphatic and vascular invasion, lymph node metastasis, distant metastasis, advanced TNM stage and postoperative recurrence. Since distant metastasis and postoperative recurrence are known to be the main causes of death in colon cancer (37,38), patients with low YY1 expression may have shorter survival due to these factors. Based on these findings in our clinical data, it was hypothesized that YY1 plays a tumor-suppressive role in the metastatic process. To verify this hypothesis, *in vitro* experiments were conducted and the molecular mechanisms underlying our clinical data were investigated.

In *in vitro* experiments, YY1 knockdown promoted cell migration and invasion but did not alter cell proliferation, which was consistent with the clinical data showing a significant association between YY1 expression and T stage defined by the depth of tumor invasion, and no association between YY1 expression and tumor size of the primary tumors and liver metastases. Although the association between YY1 function and cell migration and invasion properties has been reported in pancreatic cancer (11,13,39), gastric cancer (40) and CRC (34,36), it remains elusive as to whether YY1 promotes or suppresses these abilities. Particularly in CRC, a previous study revealed that YY1 promotes cell migration and invasion and miR-215 regulates these properties through YY1 (36). Another study

Table VI. Associations between YY1 expression in liver metastasis and clinicopathological features.

	Expression level of YY-1 in liver metastasis		
Clinicopathological feature, unit	High (n=18)	Low (n=48)	P-value
Age at primary surgery, years	67 (46-81)	69 (48-84)	0.989
Sex			0.770
Male	13	31	
Female	5	17	
CEA before hepatectomy, ng/ml	80.5±294.3	357±180.2	0.426
CA19-9 before hepatectomy, U/ml	281.9±839.1	637.6±513.9	0.692
Timing of metastasis			0.167
Synchronous	8	31	
Metachronous	10	17	
Interval to liver metastasis, months	4.6±2.5	5.9±1.5	0.664
Neoadjuvant chemotherapy before hepatectomy			0.751
+	5	11	
-	13	1	
Number of liver metastatic tumors	2.6±0.6	3.1±0.4	0.499
Solitary/Multiple	8/10	15/33	0.388
Size of largest liver metastatic tumor, cm	3.3±0.6	3.9±0.4	0.419
H factor			>0.999
H1	12	31	
H2	6	16	
H3	0	1	
Metastasis other than liver			0.722
+	4	8	
-	14	40	
Hepatectomy			0.264
Minor	17	39	
Major	1	9	
Resection margin			0.758
R0	9	26	
R1	5	15	
R2	4	7	
Adjuvant chemotherapy after hepatectomy			0.528
+	12	37	
-	6	11	
Recurrence after hepatectomy (all organs)			0.488
+	16	38	
-	2	10	
Intrahepatic recurrence after hepatectomy			0.586
+	11	25	
-	7	23	
Number of recurrent liver tumors	2.8±0.7	2.8±0.5	0.948
Recurrence in multiple organs			>0.999
+	2	6	
-	16	42	
Repeat resection (all organs)			0.243
+	11	19	
-	5	19	
Repeat hepatectomy			>0.999
+	5	10	
-	6	15	

YY1, Yin Yang 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; pap, papillary adenocarcinoma; tub, tubular adenocarcinoma; por, poorly differentiated adenocarcinoma; muc, mucinous adenocarcinoma.

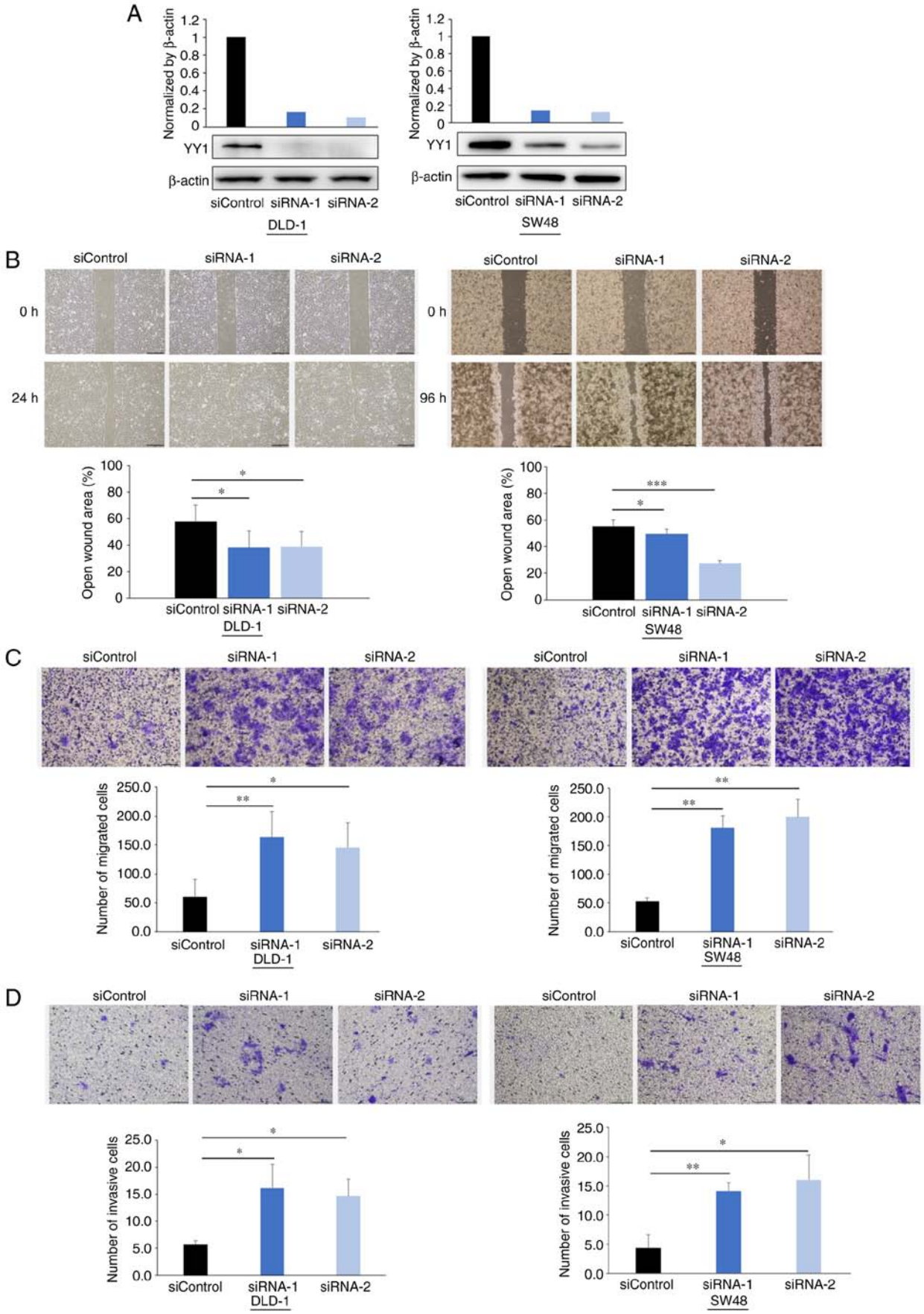

Figure 3. Knockdown of YY1 increases migration and invasion abilities in both DLD-1 and SW48 cell lines. (A) Western blotting confirmed YY1 knockdown by siRNA in both the cell lines. (B) The gap closure assay revealed that the cell-free gaps in the YY1-knockdown cells were significantly reduced compared with those in the control cells. (C) The Transwell migration assay revealed that the migrated cells were significantly increased in the YY1-knockdown cells. (D) The Transwell invasion assay showed that YY1 knockdown significantly increased the number of invasive cells in both cell lines. *P<0.05, **P<0.01 and ***P<0.001. YY1, Yin Yang 1; si, small interfering.

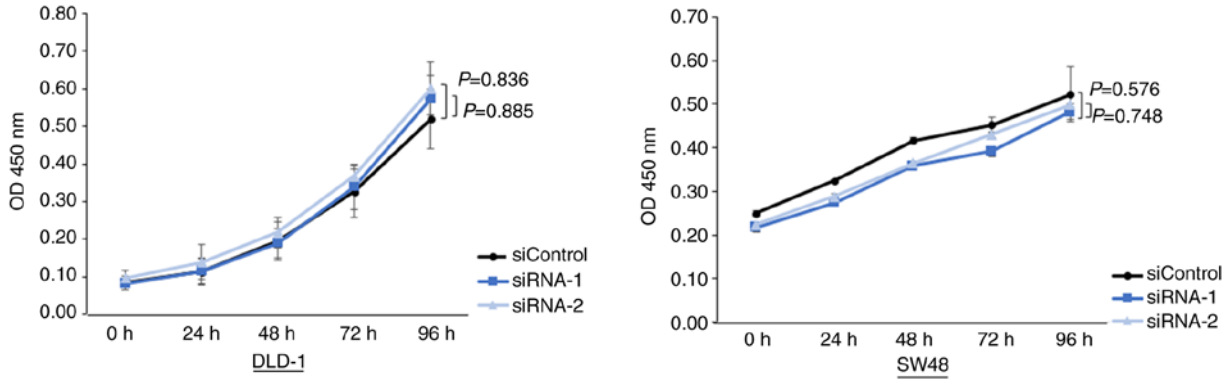

Figure 4. Proliferation assays. Knockdown of YY1 did not affect the proliferation ability of both the cell lines (Student's t-test). YY1, Yin Yang 1; si, small interfering.

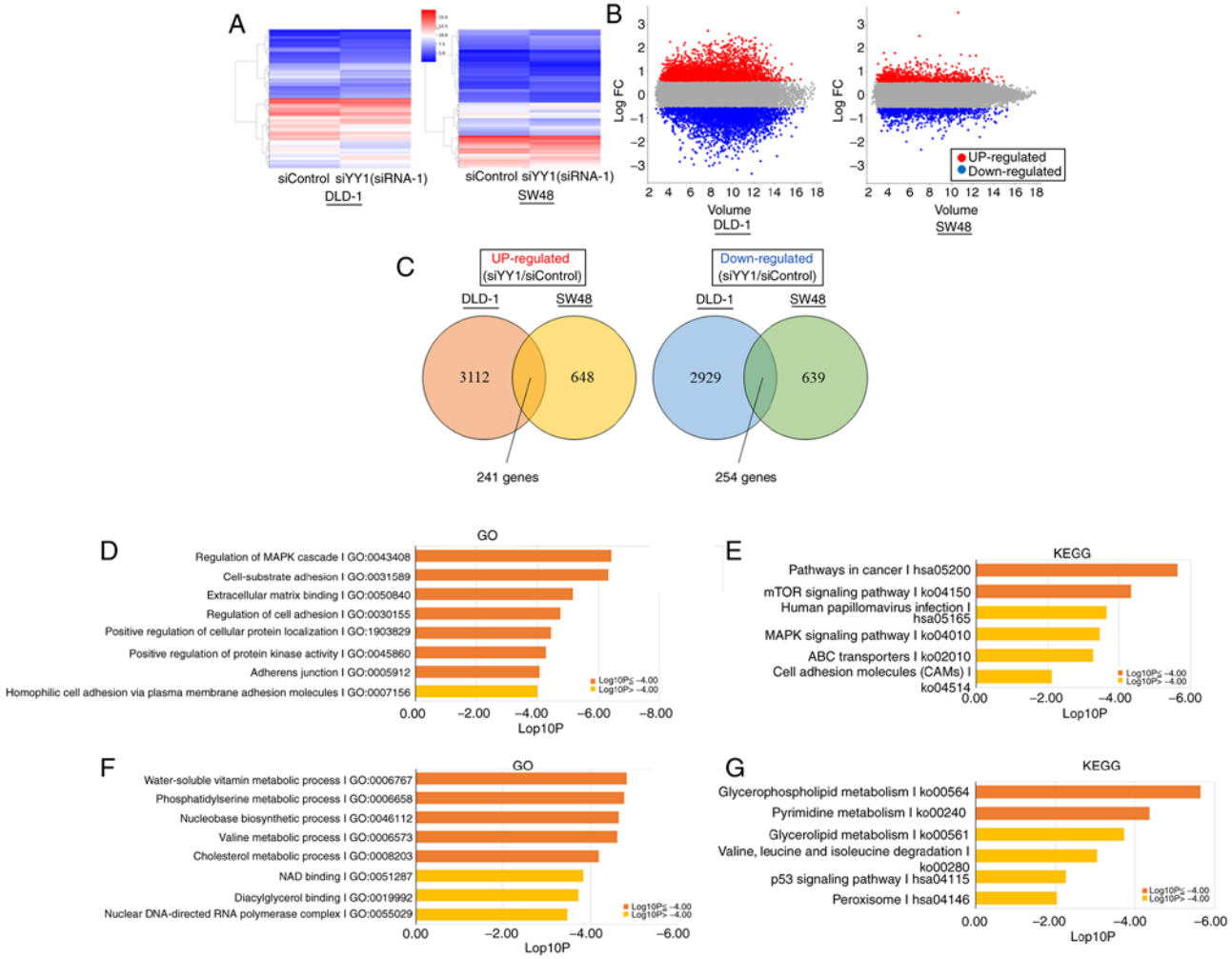

Figure 5. Extraction of DEGs and gene enrichment analysis. (A) Heat maps of DEGs between the YY1-knockdown and control cells. Red indicates upregulation and blue indicates downregulation with |fold change|≥1.5. (B) MA plot of the DEGs. Horizontal axis, the difference value of log2 converted signal; and vertical axis, the average value of log2-converted signal. Red dots are upregulated genes and blue dots are downregulated genes with |fold change| ≥1.5. (C) Venn diagram of the upregulated or downregulated DEGs in the siYY1 cells compared with those in siControl cells. (D and E) Gene enrichment analysis of the upregulated genes, based on the (D) GO terms and the (E) KEGG pathways. (F and G) Gene enrichment analysis of the downregulated genes, based on (F) GO terms and the (G) KEGG pathways. DEGs, differentially expressed genes; YY1, Yin Yang 1; si, small interfering; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

showed that YY1 forms a positive feedback loop with LINC 01578 and NF-κB, which promotes the proliferation, migration, and invasion of CRC cells (34). This discrepancy between our data and these previous studies may be due to the diversity of YY1 functions and also due to different experimental conditions and cell lines. To clarify the

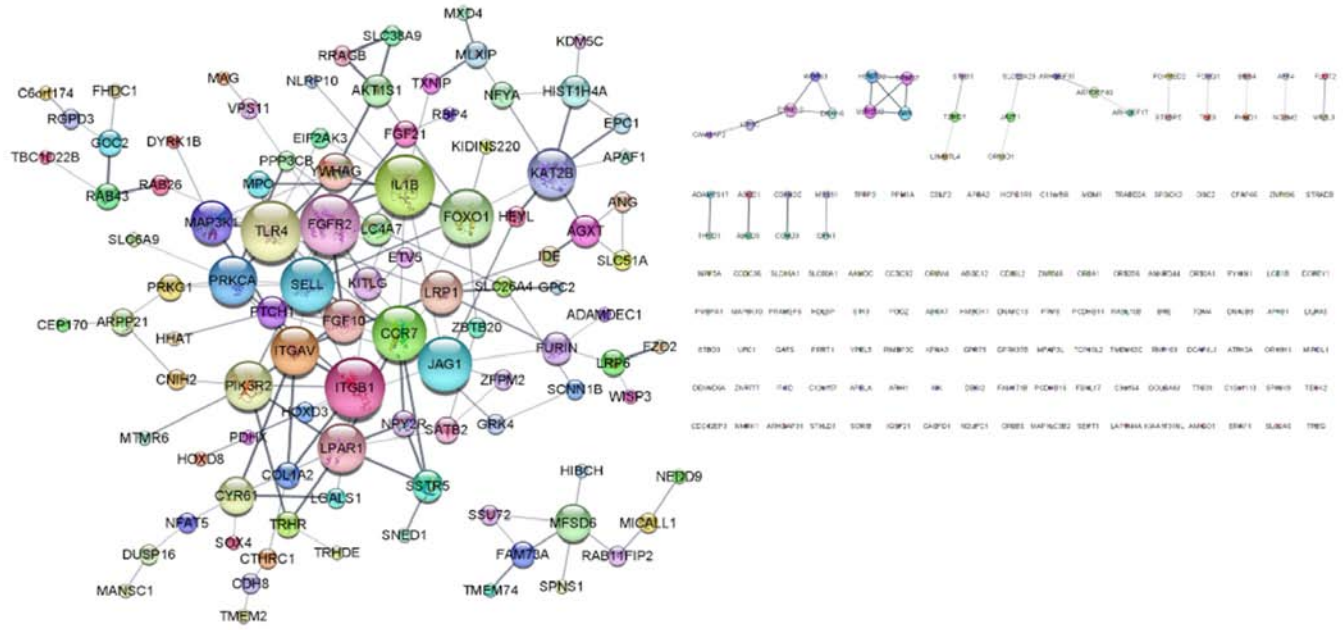

Figure 6. Protein-protein interaction network is constructed using Cytoscape. The size of the nodes represents the degree of centrality.

molecular mechanism underlying our results and identify the key genes that work downstream of YY1, a microarray analysis was conducted.

The microarray analysis demonstrated that YY1 may play a tumor-suppressive role through the downregulation of ITGAV and ITGB1. Integrins are known to act as major cell surface adhesion receptors (29) as well as signaling molecules (41) and have been reported to affect nearly every stage of cancer progression from primary tumor development to metastasis (42,43). Integrins are heterodimer proteins composed of the alpha and beta subunits. To date, 24 integrins with a combination of 18 alpha subunits and 8 beta subunits have been identified in mammals (41). ITGAV and ITGB1 are the members of each subunit. ITGAV forms five types of dimers, αVβ1, αVβ3, αVβ5, αVβ6, and αVβ8 (41), and are known to facilitate tumor cell adhesion to the extracellular matrix (ECM) (44). ITGB1 forms 12 types of dimers with alpha subunits (41). ITGB1 interacts with the ECM structural components, such as laminin, fibronectin, vitronectin, and collagen, and is considered to be strongly involved in the attachment of cancer cells to the basement membrane (45). Although the effects of ITGAV and ITGB1 on CRC have not been directly verified in the present study, several studies have suggested that integrins promote CRC progression. ITGAV is an important adhesion molecule for the peritoneal metastasis of CRC cells (44), and cancer-associated fibroblasts promote CRC cell invasion by depositing fibronectin in an αvβ3 integrin-dependent manner (46). In addition, the inside-out activation of ITGB1 promotes CRC cell extravasation and colonization (47) and the integrin subunits αV, α6, and β1 are involved in early events in colon cancer metastasis to the liver (48). Furthermore, there is clinical evidence that ITGAV expression is significantly associated with aggressive clinicopathological features of CRC (49) and ITGB1 expression has been significantly associated with the poor prognosis in CRC patients (50,51). Given these findings and our data, it was theorized that YY1 acts as a tumor suppressor in CRC by regulating the expression of ITGAV and ITGB1, inducing CRC cell migration and invasion. To date, the control mechanisms of integrins by YY1 remain unknown and need to be elucidated in future studies.

The present study revealed that YY1 knockdown promoted migration and invasion. This means that the lower the YY1 expression, the deeper the cancer cells infiltrate and the more the tumor metastasizes. Therefore, YY1 expression in primary tumors decreased as TNM stage progressed. Furthermore, the difference in cut-off values between 143 primary lesions and 66 primary lesions with liver metastasis may be due to the fact that the 66-lesion group includes numerous stage IV cases. Whereas, YY1 expression in primary tumors was significantly associated with aggressive metastatic behavior, YY1 expression in liver metastatic tumors was not associated with prognosis. The reason for these data may be explained by the difference in the rate of YY1 expression. The mean rate of YY1 expression in liver metastases was lower than that in the primary tumors. Therefore, it may be difficult to identify significant differences in the liver metastases. In addition, YY1 may contribute to the establishment of metastasis of CRC, but once metastasis is established, YY1 may not affect the progression of the metastatic tumor.

The present study has certain limitations. First, there may have been selection bias in the background data of the patients since all the data were collected retrospectively. Second, all the *in vitro* experiments were performed in a loss-of-function manner using siRNA transfection. Ideally, gain-of-function experiments and *in vivo* experiments should be performed to verify our data and elucidate the role of YY1 in the progression of CRC.

Collectively, low YY1 expression was significantly associated with the poor prognosis in patients with primary CRC

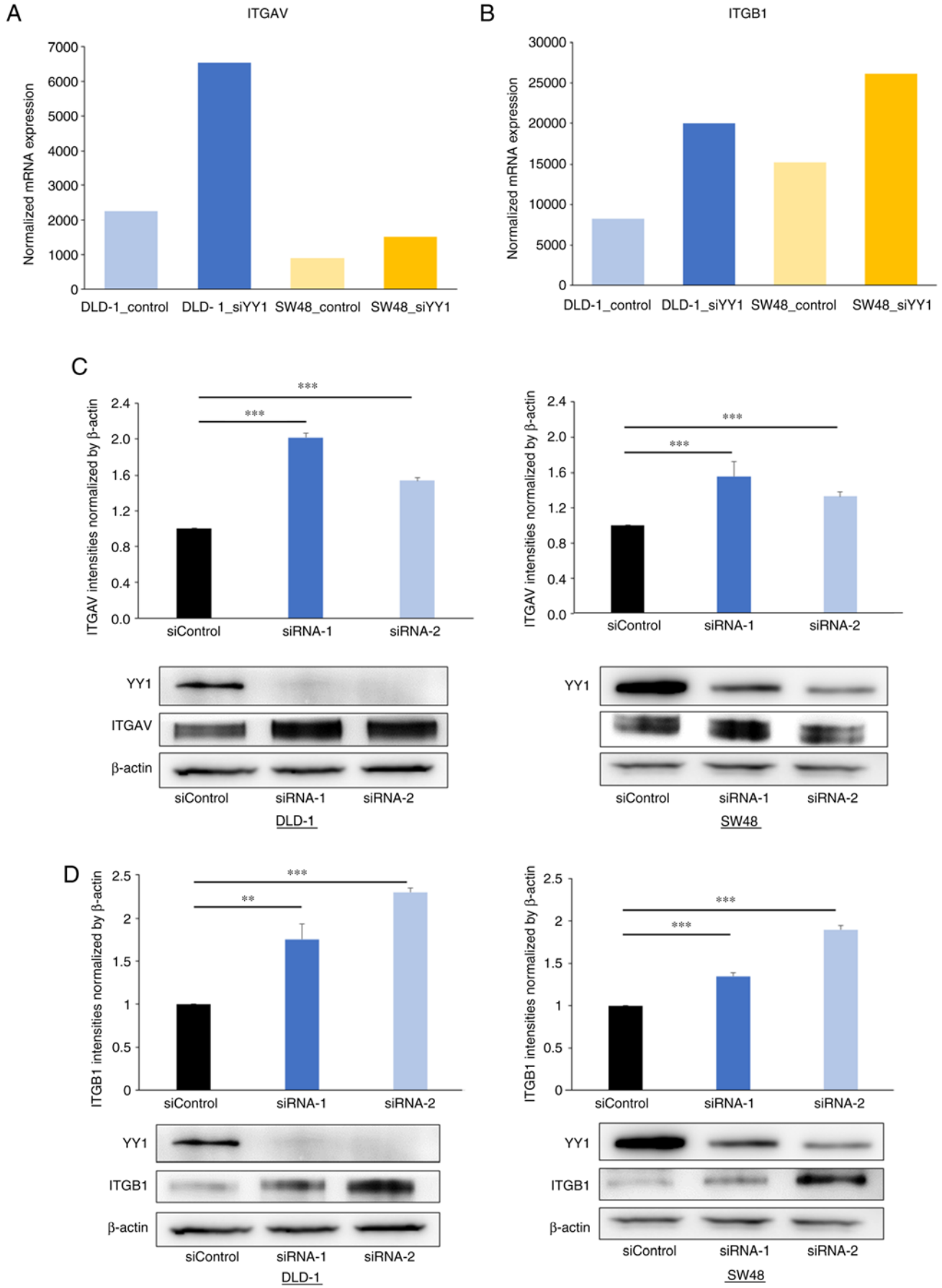

Figure 7. YY1 knockdown upregulates ITGAV and ITGB1 expression in both DLD-1 and SW48 cells. (A and B) The microarray analysis revealed that YY1 knockdown increased the mRNA expression of (A) *ITGAV* and (B) *ITGB1*. (C and D) Western blot analysis demonstrated that knockdown of YY1 significantly increased the expression of (C) ITGAV and (D) ITGB1. **P<0.01 and ***P<0.001. ITGAV, integrin alpha V; ITGB1, integrin beta 1; YY1, Yin Yang 1; si, small interfering.

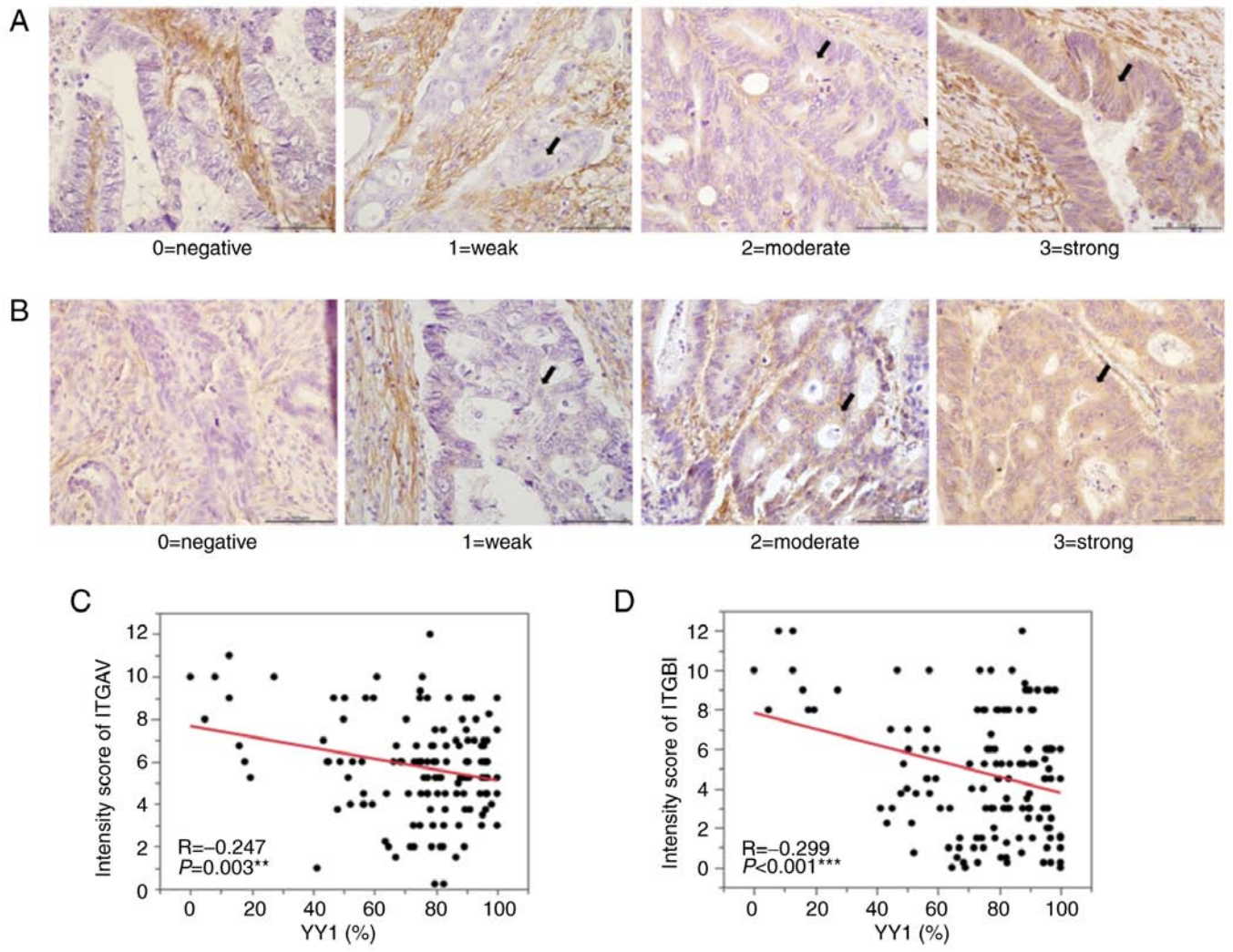

Figure 8. Expression of ITGAV and ITGB1 is negatively correlated with the expression of YY1 in the colorectal cancer tissue. (A and B) Immunohistochemistry of (A) ITGAV and (B) ITGB1. Each protein was predominantly expressed in the cytoplasm of cancer cells (arrows). The intensity score was provided as shown (magnification, x400; scale bar, 100 μm). (C and D) Regression analysis of the correlation between (C) YY1 and ITGAV and (D) ITGB1 expression. YY1 expression was negatively correlated with ITGAV (R=-0.247; P=0.003) and ITGB1 (R=-0.299; P<0.001). ITGAV, integrin alpha V; ITGB1, integrin beta 1; YY1, Yin Yang 1.

and aggressive behavior of the corresponding liver metastases. YY1 suppressed the expression of ITGAV and ITGB1, which are members of integrins playing an important role in CRC progression. This transcriptional regulation may lead to the suppression of CRC cell migration and invasion and eventually lead to the suppression of CRC cell metastasis. Overall, YY1 acted as a tumor suppressor and contributed to the survival of patients with CRC. Investigating the molecular mechanisms of YY1 in CRC metastasis may serve as a potential prognostic biomarker and therapeutic target in CRC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

NaS and NoS designed and performed the experiments. KF, TT and GO collected the data. MO and HM confirmed the authenticity of all the raw data. KF, ST and SK performed data analysis. NaS wrote and NoS revised the paper. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved (approval no. 2405) by the Ethics Committee of the Department of General Surgery of Chiba University Hospital (Chiba, Japan). Written informed consent was provided by all participants.

Patient consent for publication

Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient/parent/guardian/relative of the patient. A copy of the consent form is available for review by the Editor of this journal.

Competing interests

The authors declare that they have no competing interests.

References

1. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 71: 209-249, 2021.
2. Zacharakis M, Xynos ID, Lazaris A, Smaro T, Kosmas C, Dokou A, Felekouras E, Antoniou E, Polyzos A, Sarantonis J, *et al*: Predictors of survival in stage IV metastatic colorectal cancer. Anticancer Res 30: 653-660, 2010.
3. Field K and Lipton L: Metastatic colorectal cancer-past, progress and future. World J Gastroenterol 13: 3806-3815, 2007.
4. Shi Y, Lee JS and Galvin KM: Everything you have ever wanted to know about Yin Yang 1. Biochim Biophys Acta 1332: F49-F66, 1997.
5. Khachigian LM: The Yin and Yang of YY1 in tumor growth and suppression. Int J Cancer 143: 460-465, 2018.
6. Zhang Q, Wan M, Shi J, Horita DA, Miller LD, Kute TE, Kridel SJ, Kulik G and Sui G: Yin Yang 1 promotes mTORC2-mediated AKT phosphorylation. J Mol Cell Biol 8: 232-243, 2016.
7. Liu D, Zhang J, Wu Y, Shi G, Yuan H, Lu Z, Zhu Q, Wu P, Lu C, Guo F, *et al*: YY1 suppresses proliferation and migration of pancreatic ductal adenocarcinoma by regulating the CDKN3/MdM2/P53/P21 signaling pathway. Int J Cancer 142: 1392-1404, 2018.
8. Wu S, Wang H, Li Y, Xie Y, Huang C, Zhao H, Miyagishi M and Kasim V: Transcription factor YY1 promotes cell proliferation by directly activating the pentose phosphate pathway. Cancer Res 78: 4549-4562, 2018.
9. Lee MH, Lahusen T, Wang RH, Xiao C, Xu X, Hwang YS, He WW, Shi Y and Deng CX: Yin Yang 1 positively regulates BRCA1 and inhibits mammary cancer formation. Oncogene 31: 116-127, 2012.
10. Zhang JJ, Zhu Y, Yang C, Liu X, Peng YP, Jiang KR, Miao Y and Xu ZK: Yin Yang-1 increases apoptosis through bax activation in pancreatic cancer cells. Oncotarget 7: 28498-28509, 2016.
11. Zhang JJ, Zhu Y, Xie KL, Peng YP, Tao JQ, Tang J, Li Z, Xu ZK, Dai CC, Qian ZY, *et al*: Yin Yang-1 suppresses invasion and metastasis of pancreatic ductal adenocarcinoma by downregulating MMP10 in a MUC4/ErbB2/p38/MEF2C-dependent mechanism. Mol Cancer 13: 130, 2014.
12. Wang CC, Tsai MF, Hong TM, Chang GC, Chen CY, Yang WM, Chen JJ and Yang PC: The transcriptional factor YY1 upregulates the novel invasion suppressor HLJ1 expression and inhibits cancer cell invasion. Oncogene 24: 4081-4093, 2005.
13. Chen Q, Zhang JJ, Ge WL, Chen L, Yuan H, Meng LD, Huang XM, Shen P, Miao Y and Jiang KR: YY1 inhibits the migration and invasion of pancreatic ductal adenocarcinoma by downregulating the FER/STAT3/MMP2 signaling pathway. Cancer Lett 463: 37-49, 2019.
14. Antonio-Andrés G, Rangel-Santiago J, Tirado-Rodríguez B, Martinez-Ruiz GU, Klunder-Klunder M, Vega MI, Lopez-Martinez B, Jiménez-Hernández E, Torres Nava J, Medina-Sanson A and Huerta-Yepez S: Role of Yin Yang-1 (YY1) in the transcription regulation of the multi-drug resistance (MDR1) gene. Leuk Lymphoma 59: 2628-2638, 2018.
15. Wottrich S, Kaufhold S, Chrysos E, Zoras O, Baritaki S and Bonavida B: Inverse correlation between the metastasis suppressor RKIP and the metastasis inducer YY1: Contrasting roles in the regulation of chemo/immuno-resistance in cancer. Drug Resist Updat 30: 28-38, 2017.
16. Vega MI, Valencia-Hipolito A, Hernandez-Atenogenes M, Vega GG, Mayani H, Mendez-Tenorio A, Martinez-Maza O, Huerta-Yepez S and Bonavida B: High expression of Kruppel-Like Factor 4 (KLF4) and its regulation by Yin Yang 1 (YY1) in non-Hodgkin's B-cell lymphomas: Clinical implication. Cancer Res 73 (Suppl 8): S5450, 2013.
17. Cho AA and Bonavida B: Targeting the overexpressed YY1 in cancer inhibits EMT and metastasis. Crit Rev Oncog 22: 49-61, 2017.
18. Palmer MB, Majumder P, Cooper JC, Yoon H, Wade PA and Boss JM: Yin yang 1 regulates the expression of snail through a distal enhancer. Mol Cancer Res 7: 221-229, 2009.
19. Matsumura N, Huang Z, Baba T, Lee PS, Barnett JC, Mori S, Chang JT, Kuo WL, Gusberg AH, Whitaker RS, *et al*: Yin yang 1 modulates taxane response in epithelial ovarian cancer. Mol Cancer Res 7: 210-220, 2009.
20. Naidoo K, Clay V, Hoyland JA, Swindell R, Linton K, Illidge T, Radford JA and Byers RJ: YY1 expression predicts favourable outcome in follicular lymphoma. J Clin Pathol 64: 125-129, 2011.
21. de Nigris F, Botti C, de Chiara A, Rossiello R, Apice G, Fazioli F, Fiorito C, Sica V and Napoli C: Expression of transcription factor Yin Yang 1 in human osteosarcomas. Eur J Cancer 42: 2420-2424, 2006.
22. Wang W, Yue Z, Tian Z, Xie Y, Zhang J, She Y, Yang B, Ye Y and Yang Y: Expression of Yin Yang 1 in cervical cancer and its correlation with E-cadherin expression and HPV16 E6. PLoS One 13: e0193340, 2018.
23. Xu W, Banerji S, Davie JR, Kassie F, Yee D and Kratzke R: Yin Yang gene expression ratio signature for lung cancer prognosis. PLoS One 8: e68742, 2013.
24. Seligson D, Horvath S, Huerta-Yepez S, Hanna S, Garban H, Roberts A, Shi T, Liu X, Chia D, Goodglick L and Bonavida B: Expression of transcription factor Yin Yang 1 in prostate cancer. Int J Oncol 27: 131-141, 2005.
25. Kang W, Tong JH, Chan AW, Zhao J, Dong Y, Wang S, Yang W, Sin FM, Ng SS, Yu J, *et al*: Yin Yang 1 contributes to gastric carcinogenesis and its nuclear expression correlates with shorter survival in patients with early stage gastric adenocarcinoma. J Transl Med 12: 80, 2014.
26. Sanchez-Carbayo M, Socci ND, Lozano J, Saint F and Cordon-Cardo C: Defining molecular profiles of poor outcome in patients with invasive bladder cancer using oligonucleotide microarrays. J Clin Oncol 24: 778-789, 2006.
27. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, *et al*: Gene ontology: Tool for the unification of biology. The gene ontology consortium. Nat Genet 25: 25-29, 2000.
28. Ogata H, Goto S, Sato K, Fujibuchi W, Bono H and Kanehisa M: KEGG: Kyoto encyclopedia of genes and genomes. Nucleic Acids Res 27: 29-34, 1999.
29. Berrier AL and Yamada KM: Cell-matrix adhesion. J Cell Physiol 213: 565-573, 2007.
30. Caswell PT and Norman JC: Integrin trafficking and the control of cell migration. Traffic 7: 14-21, 2006.
31. Missan DS and DiPersio M: Integrin control of tumor invasion. Crit Rev Eukaryot Gene Expr 22: 309-324, 2012.
32. Chinnappan D, Xiao D, Ratnasari A, Andry C, King TC and Weber HC: Transcription factor YY1 expression in human gastrointestinal cancer cells. Int J Oncol 34: 1417-1423, 2009.
33. Zhang N, Li X, Wu CW, Dong Y, Cai M, Mok MT, Wang H, Chen J, Ng SS, Chen M, *et al*: microRNA-7 is a novel inhibitor of YY1 contributing to colorectal tumorigenesis. Oncogene 32: 5078-5088, 2013.
34. Liu J, Zhan Y, Wang J, Wang J, Guo J and Kong D: Long noncoding RNA LINC01578 drives colon cancer metastasis through a positive feedback loop with the NF-κB/YY1 axis. Mol Oncol 14: 3211-3233, 2020.
35. Yokoyama NN, Pate KT, Sprowl S and Waterman ML: A role for YY1 in repression of dominant negative LEF-1 expression in colon cancer. Nucleic Acids Res 38: 6375-6388, 2010.
36. Chen Z, Han S, Huang W, Wu J, Liu Y, Cai S, He Y, Wu S and Song W: MicroRNA-215 suppresses cell proliferation, migration and invasion of colon cancer by repressing Yin-Yang 1. Biochem Biophys Res Commun 479: 482-488, 2016.
37. Tauriello DV, Calon A, Lonardo E and Batlle E: Determinants of metastatic competency in colorectal cancer. Mol Oncol 11: 97-119, 2017.
38. Dmello RS, To SQ and Chand AL: Therapeutic targeting of the tumour microenvironment in metastatic colorectal cancer. Int J Mol Sci 22: 2067, 2021.

39. Chen Q, Yang C, Chen L, Zhang JJ, Ge WL, Yuan H, Meng LD, Huang XM, Shen P, Miao Y and Jiang KR: YY1 targets tubulin polymerisation-promoting protein to inhibit migration, invasion and angiogenesis in pancreatic cancer via p38/MAPK and PI3K/AKT pathways. Br J Cancer 121: 912-921, 2019.
40. Zheng L, Chen Y, Ye L, Jiao W, Song H, Mei H, Li D, Yang F, Li H, Huang K and Tong Q: miRNA-584-3p inhibits gastric cancer progression by repressing Yin Yang 1-facilitated MMP-14 expression. Sci Rep 7: 8967, 2017.
41. Hynes RO: Integrins: Bidirectional, allosteric signaling machines. Cell 110: 673-687, 2002.
42. Hamidi H and Ivaska J: Every step of the way: Integrins in cancer progression and metastasis. Nat Rev Cancer 18: 533-548, 2018.
43. Seguin L, Desgrosellier JS, Weis SM and Cheresh DA: Integrins and cancer: Regulators of cancer stemness, metastasis, and drug resistance. Trends Cell Biol 25: 234-240, 2015.
44. Lepsenyi M, Algethami N, Al-Haidari AA, Algaber A, Syk I, Rahman M and Thorlacius H: CXCL2-CXCR2 axis mediates αV integrin-dependent peritoneal metastasis of colon cancer cells. Clin Exp Metastasis 38: 401-410, 2021.
45. Brakebusch C and Fässler R: Beta 1 integrin function in vivo: Adhesion, migration and more. Cancer Metastasis Rev 24: 403-411, 2005.
46. Attieh Y, Clark AG, Grass C, Richon S, Pocard M, Mariani P, Elkhatib N, Betz T, Gurchenkov B and Vignjevic DM: Cancer-associated fibroblasts lead tumor invasion through integrin-β3-dependent fibronectin assembly. J Cell Biol 216: 3509-3520, 2017.
47. Kato H, Liao Z, Mitsios JV, Wang H-Y, Deryugina EI, Varner JA, Quigley JP and Shattil SJ: The primacy of β1 integrin activation in the metastatic cascade. PLoS One 7: e46576, 2012.
48. Mook OR, van Marle J, Jonges R, Vreeling-Sindelárová H, Frederiks WM and Van Noorden CJ: Interactions between colon cancer cells and hepatocytes in rats in relation to metastasis. J Cell Mol Med 12: 2052-2061, 2008.
49. Waisberg J, De Souza Viana L, Affonso Junior RJ, Silva SR, Denadai MV, Margeotto FB, De Souza CS and Matos D: Overexpression of the ITGAV gene is associated with progression and spread of colorectal cancer. Anticancer Res 34: 5599-5607, 2014.
50. Liu QZ, Gao XH, Chang WJ, Gong HF, Fu CG, Zhang W and Cao GW: Expression of ITGB1 predicts prognosis in colorectal cancer: A large prospective study based on tissue microarray. Int J Clin Exp Pathol 8: 12802-12810, 2015.
51. Zhang J, Liu K, Peng P, Li S, Ye Z, Su Y, Liu S, Qin M and Huang J: Upregulation of nectin-4 is associated with ITGB1 and vasculogenic mimicry and may serve as a predictor of poor prognosis in colorectal cancer. Oncol Lett 18: 1163-1170, 2019.