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 (ITGA V) 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 ITGA V 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 ITGA V and ITGB1, and this transcriptional regulation may lead to the suppression of CRC cell migration and invasion.

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% H2O2 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 (ITGA5; 1:500; product code ab794975) and integrin beta 1 (ITGB1; 1:100; product code ab52971; all from Abcam). The slides were washed three times with phosphate-buffered saline (pH 7.4) and treated with biotinylated secondary antibody (EnVision™; Dako) at 37°C for 60 min and incubated at 4°C overnight with the following primary antibodies: YY1 (1:10,000), ITGA5 (1:5,000), ITGB1 (1:1,000) and beta-actin (1:5,000; 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; 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 beta-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 1.5x10^4 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.org) 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
ITGA V 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 time to surgical failure (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 and that of liver metastatic tissues was the lowest (P<0.05 and **P<0.01).

YY1, Yin Yang 1.
Table I. Associations between YY1 expression and clinicopathological features of patients with colorectal cancer.

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

*P<0.05 and *P<0.01. *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.

| 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                     | 0.102   |               |         |
| **Sex**                          |     |                          |         |               |         |
| Male                            | 93  | 84.1                     | 0.106   |               |         |
| Female                          | 51  | 100                      |         |               |         |
| **CEA, ng/ml**                  |     |                          |         |               |         |
| ≧ 5                             | 58  | 83.9                     | 0.094   |               |         |
| <5                              | 86  | 93.3                     |         |               |         |
| **CA19-9, U/ml**                |     |                          |         |               |         |
| ≧ 37                            | 26  | 74.5                     | 0.846   |               |         |
| <37                             | 118 | 92.6                     |         |               |         |
| **Site of primary tumor**       |     |                          |         |               |         |
| Left                            | 132 | 89.7                     | 0.140   |               |         |
| Right                           | 12  | 88.9                     |         |               |         |
| **Neoadjuvant chemotherapy before primary surgery** |     |                          |         |               |         |
| +                               | 16  | 73.3                     |         |               |         |
| -                               | 128 | 91.4                     |         |               |         |
| **T stage**                     |     |                          | 0.354   |               |         |
| 4                               | 35  | 87.5                     |         |               |         |
| 1-3                             | 109 | 90.2                     |         |               |         |
| **Degree of differentiation**   |     |                          | 0.049   | 3.36 (0.64-17.57) | 0.151 |
| por, muc                        | 4   | 50                       |         |               |         |
| tub, pap                        | 137 | 90.8                     |         |               |         |
| **Ly**                          |     |                          | <0.001  | 5.1 (1.31-15.53) | 0.017 |
| 2-3                             | 19  | 58.4                     |         |               |         |
| 0-1                             | 124 | 94.6                     |         |               |         |
| **V**                           |     |                          | 0.044   | 1.05 (0.36-3.09) | 0.932 |
| 2-3                             | 44  | 81.1                     |         |               |         |
| 0-1                             | 99  | 93.5                     |         |               |         |
| **Lymph node metastasis**       |     |                          | <0.001  | 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   | 1.7 (0.48-5.96) | 0.409 |
| +                               | 48  | 79.6                     |         |               |         |
| -                               | 96  | 95.6                     |         |               |         |
| **Expression of YY-1 in primary tumors** |     |                          | <0.001  | 4.54 (1.22-16.88) | 0.024 |
| Low                             | 51  | 79.7                     |         |               |         |
| High                            | 91  | 95.6                     |         |               |         |

 ULONG for International Cancer Control 8th edition, P<0.05, P<0.001 and 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.

| Clinicopathological feature, unit | Univariate | Multivariate |
|----------------------------------|------------|-------------|
|                                 | 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                       | <0.001a    | 5.29 (1.92-14.56) | 0.001b   |         |
| ≥5                               | 58         |             |                    |         |
| <5                               | 86         |             |                    |         |
| CA19-9, U/ml                     | 0.026b     | 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<sup>c</sup>              | 0.008<sup>c</sup> | 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<sup>a</sup> | 3.97 (1.1-14.35) | 0.036<sup>b</sup> |         |
| 2-3                              | 19         |             |                    |         |
| 0-1                              | 124        |             |                    |         |
| V                                | 0.024<sup>b</sup> | 1.55 (0.56-4.25) | 0.398 |         |
| 2-3                              | 44         |             |                    |         |
| 0-1                              | 99         |             |                    |         |
| Lymph node metastasis            | <0.001<sup>a</sup> | 3.09 (1.15-8.33) | 0.027<sup>b</sup> |         |
| +                                | 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<sup>a</sup> | 3.09 (1.2-7.95) | 0.020<sup>b</sup> |         |
| Low                              | 51         |             |                    |         |
| High                             | 91         |             |                    |         |

<sup>a</sup>P<0.001, <sup>b</sup>P<0.05 and <sup>c</sup>P<0.01. <sup>d</sup>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.
SATO et al.: YIN YANG 1 CONTRIBUTES TO IMPROVED PROGNOSIS OF COLORECTAL CANCER

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<sup>a</sup> |
| 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<sup>c</sup>              |             |             | 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<sup>c</sup>            |             |             | 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          |      |
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.

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<0.001; 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 inserted into JASPAR 2020 software to identify...
Table V. Univariate and multivariate analysis for cancer-specific survival in patients with liver metastases.

| Clinicopathological feature, unit                          | n  | 5-year survival (%) | Univariate P-value | HR (95% CI) | Multivariate 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                                     |    |                     | 0.033a              | 1.54 (0.65-3.65) | 0.332 |
| Left                                                     | 47 | 53.5                | 0.033a              | 1.54 (0.65-3.65) | 0.332 |
| Right                                                    | 19 | 78.6                |                     |             |                      |
| T stagec                                                 |    |                     | 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                                    |    |                     | 0.004b              | 3.21 (1.4-7.35) | 0.006b |
| +                                                        | 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                              |    |                     | 0.225               |             |                      |
| +                                                        | 12 | 41.7                |                     |             |                      |
| -                                                        | 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                |                     |             |                      |
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, ITGA5 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

| Clinicopathological feature, unit | n  | 5-year survival (%) | Univariate | Multivariate |
|----------------------------------|----|---------------------|------------|-------------|
| Hepatectomy                      |    |                     | 0.071      |             |
| Major                            | 10 | 44.4                |            |             |
| Minor                            | 56 | 63.2                |            |             |
| Surgical margin of liver metastasis |    |                     | 0.049*    | 0.606       |
| R1-2                             | 31 | 52                  |            |             |
| R0                               | 35 | 67.9                |            |             |
| H factor                         |    |                     | 0.023*    | 0.171       |
| H2-H3                            | 23 | 38.6                |            |             |
| H1                               | 43 | 71.6                |            |             |
| Expression of YY-1 in primary tumors |  |                     | 0.009*    | 0.030*      |
|                                  | 34 | 51.6                |            |             |
|                                  | 32 | 72.3                |            |             |

*P<0.05 and †P<0.01. ‡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.

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

| Clinicopathological feature, unit | Expression level of YY-1 in liver metastasis |  |
|----------------------------------|--------------------------------------------|----------------|
|                                  | 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.
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 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.
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 ITGA5 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). ITGA5 and ITGB1 are the members of each subunit. ITGA5 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 ITGA5 and ITGB1 on CRC have not been directly verified in the present study, several studies have suggested that integrins promote CRC progression. ITGA5 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 ITGA5 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 ITGA5 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.
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.

Acknowledgements

Not applicable.

Funding

The present study was supported (grant no. JP20K17640) by the Japan Society for the Promotion of Science KAKENHI.

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, Jamal 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, Felekorou 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 J: 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, He D, Miller LD, Kate TE, Kriel SJ, Kulik G and Sui G: Yin Yang 1 promotes mTORC2-mediated AKT phosphorylation. J Mol 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, Huang YS, He WW, Shi Y and Deng CX: Yin Yang 1 positively regulates BRCAl and inhibits mammary cancer formation. Oncogene 31: 127–138, 2012.
10. Zhang J, 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 JI, Zhu Y, Xie KL, Peng YP, Tao QJ, 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 HLL1 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-Rodriguez B, Martinez-Ruiz GU, Klunder-Klunder M, Vega MI, Lopez-Martinez B, Jimenez-Hernandez 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, Chrysoy E, Zoras O, Baritaki S and Bonavida B: Inverse correlation between the metastasis suppressor p53/KLF2 and YY1 expression in pancreatic cancer: Contrasting roles in the regulation of chemo-immuno-resistance in cancer. Drug Resist Updat 30: 28–38, 2017.
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, Elkhatabi 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, Mitisos 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, Morgeotto 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.