High BAP1 expression is associated with poor prognosis in patients with colorectal liver metastases

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Abstract

BRCA-1-associated protein 1 (BAP1) is a deubiquitinating enzyme that regulates gene expression. Although previous studies have demonstrated that the role of BAP1 is either tumor promotive or suppressive, depending on the tumor and cell types, the role of BAP1 in colorectal cancer and liver metastases remains unclear. The clinical data of 81 patients who underwent hepatic resection for liver metastases after primary resection of colorectal cancer were analyzed. BAP1 expression in primary tumors and liver metastases was evaluated using immunohistochemistry, and high BAP1 expression was significantly associated with a poor prognosis. High BAP1 expression in liver metastases was an independent prognostic factor for poor overall and disease-free survival. *In vitro* studies using colon cancer cell lines have demonstrated that BAP1 overexpression increases cell proliferation, migration, invasion, and cancer stem cell-like properties. In contrast, BAP1 knockdown induced the opposite results. Western blot analyses showed that BAP1 overexpression induced the expression of vimentin, MMP2, and MMP9 but decreased E-cadherin expression. Collectively, high BAP1 expression was associated with a significantly poor prognosis in patients with colorectal cancer and liver metastasis by increasing cell proliferation, invasion, migration, and cancer stem cell-like properties.

Introduction

Colorectal cancer (CRC) is one of the leading causes of death worldwide[1]. Distant metastasis is a major prognostic factor in patients with CRC, and the liver is the most common site of CRC metastasis. Previous studies have demonstrated that approximately 30–50% of patients with CRC develop liver metastases following initial diagnosis[2–4]. Of these patients, a limited number can undergo surgical resection, and more than half of them develop recurrence even after curative resection for liver metastases[5, 6]. Therefore, appropriate treatment strategies, including surgery and systemic chemotherapy, should be considered based on accurate diagnosis to improve the clinical outcomes of patients with CRC.

BRCA-1-associated protein (BAP1) is a deubiquitinating enzyme originally characterized as a binding protein of BRCA-1[7]. BAP1 is involved in various cellular processes, including cell proliferation, apoptosis, cell cycle regulation, and chromosomal dynamics[8]. Recently, several studies have shown that BAP1 is involved in the tumor biology of solid cancers, such as uveal melanoma[9], mesothelioma[10], renal cell carcinoma[11, 12], and intrahepatic cholangiocarcinoma[13, 14]. A previous study demonstrated that BAP1 knockdown inhibits the growth and metastasis of breast cancer cells, suggesting that BAP1 is a tumor promoter[15]. Another study demonstrated that BAP1 plays a tumor-suppressive role through the ERK1/2 and JNK/c-Jun pathways[16]. Therefore, the functional role of BAP1 in tumor biology is controversial and may depend on cancer and cell types. Regarding the role of BAP1 in CRC, two studies have demonstrated conflicting evidence regarding whether BAP1 is a tumor suppressor or promoter[17, 18].

The aim of this study was to investigate whether BAP1 expression in CRC and colorectal liver metastases (CRLM) induces tumor suppression or promotion. We utilized clinical data and samples to explore the

associated underlying mechanisms.

Methods

Patients and human tissue samples

Between January 2006 and December 2017, 81 patients underwent surgical resection of primary CRC and liver metastases at Chiba University Hospital (Chiba, Japan). Primary CRC tissue samples and corresponding liver metastases were collected from these patients. Patients who underwent initial tumor excision and 2-stage hepatectomy at other institutions were excluded. Primary tumor excision was the initial treatment for patients with synchronous liver metastases. The Ethics Committee of the Department of General Surgery, Chiba University Hospital (Chiba, Japan) approved the study protocol (approval no. HK202308-07). The study protocol conformed to the provisions of the Declaration of Helsinki. Written informed consent was obtained from each patient before surgery.

Indication criteria for surgical resection of metastatic tumors

The indication criteria for resection of colorectal metastases were as follows: (i) curative resection for primary CRC has been performed or could be performed; (ii) R0 resection for CRLM could be performed; and (iii) sufficient future liver remnants (e.g., 40% or more of the total liver volume) and physiological function could be preserved.

Immunohistochemistry

Following a 24-h room temperature (15–25°C) incubation period, 10% formalin-fixed paraffin-embedded tissue samples were sliced into 4 μm-thick slices, deparaffinized using xylene, and rehydrated using a decreasing ethanol series to allow for optimal antigen-antibody binding. The slides were microwaved (500 W) in citric acid buffer (0.01 M, pH, 9.0) for 25 min at 100°C to retrieve antigens. After antigen retrieval, endogenous peroxidase activity was inhibited for 15 min at room temperature using hydrogen peroxide (3% hydrogen peroxide in methanol). Then, 5% bovine serum albumin (BSA; cat. no. 01860-36, Nacalai Tesque, Inc.) was used to block nonspecific proteins for 10 min at room temperature. The anti-BAP1 polyclonal primary antibody (1:200; cat. no. 10398-1-AP, Proteintech) was applied to the slides and incubated overnight at 4°C. After applying secondary antibodies for 60 min at room temperature (undiluted; cat. nos. K4001 and K4003, Dako EnVision™ kits, Agilent Technologies, Inc.), the slides were stained using a peroxidase DAB kit (cat. no. 25985-50, Nacalai Tesque, Inc.). Hematoxylin counterstaining was performed prior to penetration, mounting, and 1-min room temperature dehydration.

Two investigators blinded to the clinical information independently assessed the expression levels of BAP1 using an inverted light microscope (BX40, Olympus Corporation). As a negative control, a 1% BSA/phosphate-buffered saline (PBS) solution without primary antibody was used. BAP1 expression was assessed based on the intensity of stained cytoplasm in tumor cells and scored as: “0” (negative

staining), “1” (weak staining), “2” (moderate staining), and “3” (strong staining). Low and high expression levels were defined as < 2 and ≥ 2, respectively. Of the 81 primary CRC samples, 11 did not include normal tissues. Thus, 70 samples were used to assess BAP1 expression in normal tissues.

Human colon cancer cell lines and culture conditions

DLD-1 and SW620 cells were purchased from the American Type Culture Collection. We used 10% fetal bovine serum (FBS; Thermo Fisher Scientific) and RPMI-1640 medium (Gibco) to culture the DLD-1 cells, which were then incubated at 37°C with 5% CO_2. The SW620 cell line was cultured in 10% FBS (Thermo Fisher Scientific) and Leibovitz’s L-15 medium (Gibco) and incubated at 37°C without CO_2.

Construction of BAP1 overexpression or knockdown stable cell lines

Lentiviral vector transfection was used for the overexpression or knockdown of BAP1. One lentiviral vector (vector ID: VB230405-1600hbf, pLV [Exp]-Puro-CMV > EGFP (ns): hBAP1 [NM_004656.4]) was used for overexpression experiments (VectorBuilder, Yokohama, Japan). Two lentiviral vectors (1: vector ID: VB230829-1007ejj, pLV [shRNA]-EGFP: T2A: Puro-U6 > {hBAP1 [shRNA#1]}, and 2: vector ID: VB230829-1009tbp, pLV [shRNA]-EGFP: T2A: Puro-U6 > {hBAP1 [shRNA#2]}) were used for knockdown experiments (VectorBuilder). DLD-1 and SW620 cells were prepared at 30–50% confluency and then transfected at a multiplicity of infection of 10 or with a control vector, according to the manufacturer’s protocol.

Cell proliferation assay

Cell proliferation was assessed using a CCK-8 kit (Dojindo Molecular Technologies) according to the manufacturer’s instructions. DLD-1 and SW620 cells were seeded in 96-well plates at a density of 3,000 cells per well. Cell viability was measured by adding CCK-8 (10 μL/well) solution at 0, 24, 48, 72, and 96 h. Following a 2-h incubation period at 37°C, the absorbance at 450 nm was measured.

Wound-healing assay

DLD-1 (2×10^5 cells/well) and SW620 (3×10^5 cells/well) cells were seeded into 6-well plates. Then, 70 μL of cell suspension was added to each well of the plate (Culture-Insert 2 Well in μ-Dish 35 mm, high; cat. no. 80206, ibidi) when the cells reached 95–100% confluence. Following incubation for at least 24 h (DLD-1) and 96 h (SW620) at 37°C and 5% CO_2, the percentage of the wound closure area was observed under a light microscope (BX43, Olympus) at 100× magnification and analyzed using ImageJ software.

Transwell migration and Matrigel invasion assays

DLD-1 (2×10^5 cells/well) and SW620 (3×10^5 cells/well) cells were seeded in the upper chamber of the culture inserts with an 8μm pore size polyester membrane (Corning, Inc.) with RPMI-1640 and L-15 medium containing 0.1% FBS, respectively. As a chemoattractant, 500 μL of RPMI-1640 or L-15 medium with 10% FBS was added to the lower chamber. Following incubation at 37°C for 48 h, the migrating cells on the bottom of the insert membrane were stained with a dye solution consisting of 0.1% crystal violet

and 20% methanol at 37°C for 10 min. The non-migrating cells on top of the membrane were carefully removed. Each membrane was photographed three times, and the number of migrating cells was recorded each time. The Cell Biolabs CytoSelect™ 24well cell invasion assay kit (cat. no. CBA-110, Cell Biolabs, Inc.) with basement membrane-coated inserts was used for the Transwell invasion assay in accordance with the manufacturer's protocol.

Colony formation assay

DLD-1 and SW620 cells were seeded in 24-well culture plates coated with a bottom layer of medium containing 1% agar at a density of 3,000 cells per well suspended in 0.3% agar. After incubation for 14 d at 37°C, the number of colonies was counted.

Western blot analysis

Proteins were extracted from the cultured cells using RIPA buffer (cat. no. 16488-34, Nacalai Tesque, Inc.). Each protein sample was lysed in Laemmli Sample Buffer (cat. no. 1610737, Bio-Rad Laboratories, Inc.) with 5% 2-mercaptoethanol, and the samples were incubated for 10 min at 97°C. Following protein concentration measurement in each sample with the BCA Protein Assay kit (cat. no. 23225, Thermo Fisher Scientific), protein (20 μg/lane) was separated by electrophoresis on 10% XV PANTERA gels (cat. no. NXV-224P, DRC) and transferred to a polyvinylidene difluoride membrane. After incubation for 60 min at room temperature, the membranes were blocked with 5% skim milk diluted in 0.1% Tris-buffered saline containing Tween-20. The membranes underwent an overnight incubation at 4°C with primary antibodies against β-actin (1:2,000; cat. no. 5125, Cell Signaling Technology, Inc.), BAP1 (1:1,000; cat. no. sc28383, Santa Cruz Biotechnology), E-cadherin (1:1,000; cat. no. sc7870, Santa Cruz Biotechnology), Vimentin (1:500; cat. no. sc6260, Santa Cruz Biotechnology), MMP2 (1:500; cat. no. sc53630, Santa Cruz Biotechnology), and MMP9 (1:500; cat. no. sc21733, Santa Cruz Biotechnology). After that, the membranes were incubated for 60 min at room temperature with anti-mouse (1:2,000; cat. no. sc516102, Santa Cruz Biotechnology) and anti-rabbit (1:2,000; cat. no. 7074, Cell Signaling Technology, Inc.) horseradish peroxidase-conjugated secondary antibodies. The membranes were incubated with an enhanced chemiluminescence detection reagent (Chemi-Lumi One Ultra; Nacalai Tesque, Inc.) and developed with an LAS-4000UV mini luminous image analyzer (FUJIFILM Wako Pure Chemical Corporation). ImageJ software (version 1.53, National Institutes of Health) was used to quantify band intensities by densitometric analysis, and to normalize the relative protein levels to β-actin.

Statistical analysis

The relationship between BAP1 expression and patient characteristics was assessed using the χ^2 test for categorical data, unpaired Student's t-test for parametric continuous variables, or the Mann–Whitney U test for nonparametric continuous variables. Kaplan–Meier analysis was used to calculate survival rates, and the log-rank test was used to evaluate statistical significance. Overall survival (OS) and disease-free survival (DFS) were calculated from the date of hepatectomy or primary tumor resection. Cox proportional hazards regression analysis was used to assess the relationship between long-term outcomes (OS and DFS) and BAP1 expression in primary tumors or liver metastases. The *in vitro*

experiments were performed independently at least three times, and one-way or multivariate analysis of variance (ANOVA) and the unpaired Student's t-test were used to analyze the data. Differences were considered statistically significant at $P < 0.05$. The STATA® BE17 program (StataCorp LCC) was used for all statistical analyses.

Results

High expression of BAP1 in liver metastases is associated with poor prognosis

Using immunohistochemistry, BAP1 protein expression was evaluated in primary tumors and their corresponding liver metastases. BAP1 was observed in the cytoplasm of cancer cells in both primary tumors and liver metastases. However, compared to CRC cells, BAP1 expression was not observed in most adjacent normal gland cells ($P = 0.004$; Supplementary Fig. 1). The BAP1 expression profiles in primary and liver metastases and the clinicopathological characteristics of the patients are shown in Supplementary Table 1 and Table 1. High BAP1 expression in CRC cells was significantly associated with the location of primary tumors (rectum, left, and right), vascular invasion, and lymph node metastasis ($P = 0.002$, 0.046, and 0.004, respectively; Supplementary Table 1). No significant difference was found in DFS and OS after primary resection between patients with high and low BAP1 expression in primary tumors ($P = 0.678$ and 0.148, respectively; Fig. 1A, B). The DFS and OS after hepatectomy in patients with high BAP1 expression in liver metastases were significantly shorter compared to those with low BAP1 expression ($P = 0.001$ and 0.005, respectively; Fig. 1C, D). The OS after primary resection in patients with high BAP1 expression in liver metastases was significantly shorter compared to those with low BAP1 expression ($P = 0.014$; Fig. 1E). To investigate the association between BAP1 expression in primary tumors and liver metastases, we compared their respective BAP1 expression levels. The BAP1-positive score increased by 2 or more in 53 patients (65.4%) and by less than 2 in 28 patients (34.6%). OS was significantly shorter in patients who showed a score increase of more than 2 compared to patients with a score increase of less than 2 ($P = 0.003$; Fig. 1F).

Table 1
Clinicopathological features of patients with liver metastases

	BAP1expression in Liver metastases		
Features	Low (n = 31)	High (n = 50)	P-value
Age, Hepatectomy (median, range)	70.0 (38 to 84)	67 (48 to 81)	0.517
Gender (male/female)	22 / 9	34 / 16	0.466
CEA, < 5/≥5 ng/ml, before hepatectomy	12 / 19	13 / 37	0.229
CA19-9, < 37/≥37 U/ml, before hepatectomy	22 / 9	24 / 26	0.043
Liver metastasis Maximum size (cm) (median, range)	2.4 (0.5 to 10)	3.25 (0.9 to 12)	0.100
Distribution, unilobar/bilobar	17 / 14	28 / 22	0.919
Number of liver metastases (median, range)	2 (1–7)	2 (1–18)	0.807
Number of liver metastases, < 4/≥4	24 / 7	35 / 15	0.466
Hepatectomy, major/minor	3 / 28	10 / 40	0.219
R0, +/-	21 / 10	25 / 25	0.117
Other organ metastasis, +/-	5 / 26	11 / 39	0.519
Recurrence after hepatectomy, +/-	24 / 7	45 / 5	0.121
Rehepatectomy, +/-	8 / 16	10 / 33	0.372
Adjuvant chemotherapy (post-primary), +/-	6 / 25	15 / 35	0.288
Neoadjuvant before hepatectomy, +/-	12 / 19	18 / 32	0.806
Adjuvant chemotherapy (post-hepatectomy), +/-	25 / 6	38 / 12	0.625
[a]Union for International Cancer Control 8th edition. BAP1, BRCA-1 associated protein 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19 – 9; R, residual tumor;			

Univariate and multivariate analyses revealed that high BAP1 expression in liver metastases was an independent prognostic factor for poor OS and DFS after hepatectomy (P = 0.023 and 0.007, respectively; Tables 2 and 3). High BAP1 expression in liver metastases was identified as a prognostic factor for poor OS after primary resection in the univariate analysis but not in the multivariate analysis (P = 0.007 and 0.062, respectively; Supplementary Table 2). In contrast, high BAP1 expression in liver metastases was not a prognostic factor for poor DFS after primary resection (P = 0.295; Supplementary Table 3).

Table 2
Univariate and multivariate analysis for overall survival after hepatectomy

	Univariate		Multivariate	
Variable	HR (95%CI)	P-value	HR (95%CI)	P-value
Gender, Male	0.959 (0.532 to 1.728)	0.890		
CEA, < 5/≥5 ng/ml, before hepatectomy	1.406 (0.745 to 2.652)	0.292		
CA19-9, < 37/≥37 U/ml, before hepatectomy	1.492 (0.851 to 2.617)	0.162		
Site of primary tumors, colon/rectum	2.545 (1.441 to 4.495)	0.001		
Lymphatic invasion, +/-	1.701 (0.967 to 2.993)	0.065		
Vascular invasion, +/-	2.070 (0.925 to 4.634)	0.077		
T[a] stage, T1-3/T4	1.068 (0.928 to 1.229)	0.358		
Lymph node metastasis, +/-	2.423 (1.235 to 4.597)	0.010	2.423 (1.223 to 4.800)	0.011
Liver metastases maximum size	1.048 (0.938 to 1.170)	0.404		
Distribution, unilobar/bilobar	1.239 (0.706 to 2.173)	0.455		
Number of liver metastases, ≥4	2.271 (1.265 to 4.075)	0.006	2.400 (1.295 to 4.448)	0.005
R0, +/-	2.274 (1.289 to 4.009)	0.005	1.683 (0.933 to 3.034)	0.083
Adjuvant chemotherapy, +/-	1.112 (0.537 to 2.302)	0.774		
BAP1 expression (primary), high/low	1.232 (0.659 to 2.304)	0.512		
BAP1 expression (liver-meta), high/low	2.117 (1.107 to 4.047)	0.010	2.117 (1.107 to 4.047)	0.023

[a]Union for International Cancer Control 8th edition. BAP1, BRCA-1 associated protein 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19 – 9; R, residual tumor; HR, hazard ratio; CI, confidence interval.

Table 3
Univariate and multivariate analysis for disease free survival after hepatectomy

	Univariate		Multivariate	
Variable	HR (95%CI)	P-value	HR (95%CI)	P-value
Gender, Male	1.221 (0.712 to 2.094)	0.467		
CEA, < 5/≥5 ng/ml, before hepatectomy	1.085 (0.640 to 1.840)	0.760		
CA19-9, < 37/≥37 U/ml, before hepatectomy	1.165 (0.714 to 1.902)	0.539		
Site of primary tumors, colon/rectum	2.044 (1.241 to 3.369)	0.005		
T[a] stage, T1-3/T4	1.146 (1.013 to 1.296)	0.030	1.079 (0.951 to 1.225)	0.235
Lymphatic invasion, +/-	1.537 (0.942 to 2.508)	0.085		
Vascular invasion, +/-	2.168 (1.125 to 4.178)	0.021	1.650 (0.842 to 3.233)	0.144
Lymph node metastasis, +/-	1.817 (1.086 to 3.040)	0.023	1.587 (0.942 to 2.674)	0.082
Liver metastases maximum size	1.017 (0.917 to 1.128)	0.741		
Distribution, unilobar/bilobar	1.219 (0.746 to 1.993)	0.428		
Number of liver metastases, ≥4	2.308 (1.327 to 4.014)	0.003	1.880 (1.070 to 3.306)	0.028
R0, +/-	1.138 (0.690 to 1.877)	0.611		
Adjuvant chemotherapy, +/-	1.212 (0.656 to 2.237)	0.538		
BAP1 expression (primary), high/low	1.131 (0.656 to 1.951)	0.656		
BAP1 expression (liver-meta), high/low	2.171 (1.300 to 3.625)	0.003	2.056 (1.221 to 3.460)	0.007

[a]Union for International Cancer Control 8th edition. BAP1, BRCA-1 associated protein 1; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19 – 9; R, residual tumor; HR, hazard ratio; CI, confidence interval.

BAP1 promotes tumor cell proliferation

The clinical data suggested that BAP1 may have tumor-promoting roles; thus, we conducted *in vitro* experiments to clarify the underlying mechanisms of BAP1 in this context. The human colon cancer lymph node metastasis cell line SW620 and the human colon cancer cell line DLD-1 were used in the experiments. To evaluate the effect of BAP1 on tumor cell proliferation *in vitro*, we performed CCK-8 assays after BAP1 overexpression and knockdown. BAP1 overexpression significantly increased the proliferation of DLD-1 and SW620 cells (Fig. 2A, B). In contrast, BAP1 knockdown significantly decreased cell proliferation of both DLD-1 and SW620 cells (Fig. 2C, D).

BAP1 promotes tumor cell migration and invasion

Next, we assessed the effect of BAP1 on cell migration and invasion. Because BAP1 overexpressed cells were found to increase cell proliferation, mitomycin C (10 μg/mL; cat. no. 20898-21; Nacalai Tesque) was added to suppress cell proliferation. The wound-healing assays demonstrated a significant reduction in the cell-free gaps in BAP1-overexpressed DLD1 and SW620 cells compared to the control (vector) cells (Fig. 3A, B). Conversely, BAP1 knockdown suppressed this decrease in cell-free gaps (Fig. 3C, D). Transwell migration assays demonstrated that BAP1 overexpression significantly increased the number of migratory cells in both cell lines (Fig. 4A, B). In contrast, BAP1 knockdown reduced cell migration compared to the control cells (Fig. 4C, D). Matrigel invasion assays demonstrated that BAP1 overexpression significantly increased the number of invasive cells in both cell lines (Fig. 4E, F), while BAP1 knockdown reduced cell invasion compared to the control cells (Fig. 4G, H).

BAP1 promotes cancer stem cell (CSC)-like properties

A colony formation assay was used to examine the self-renewal potential of colon cancer cells *in vitro*. BAP1 overexpression significantly increased colony formation in DLD1 and SW620 cells (Fig. 5A, B). In contrast, BAP1 knockdown significantly suppressed colony formation in both cell lines (Fig. 5C, D).

BAP1 is associated with epithelial–mesenchymal transition (EMT)-related protein expression in CRC cells

Western blotting was performed to assess the expression of EMT-related proteins. BAP1 overexpression in DLD-1 and SW620 cells reduced E-cadherin expression and increased vimentin and matrix metalloproteinase (MMP2 and MMP9) expression (Fig. 5E).

Discussion

The present study demonstrated that BAP1 plays a tumor-promoting role in CRC with liver metastases. To the best of our knowledge, this is the first study to demonstrate the prognostic effect of BAP1 expression in CRC and its liver metastases and to explore the underlying mechanisms revealed in the clinical data. Our data shows that high BAP1 expression in liver metastases is significantly associated with shorter OS and DFS. Furthermore, high BAP1 expression in liver metastases was an independent prognostic factor for poor OS and DFS. Our *in vitro* experiments revealed that BAP1 promotes cell proliferation, migration,

invasion, and CSC-like properties, which could lead to shorter survival times in patients with high BAP1 expression.

Although several studies have demonstrated that BAP1 plays an important role in cancer progression, it remains unclear whether BAP1 is a tumor promoter or suppressor[19, 20]. Previous studies have provided conflicting evidence, and the role of BAP1 seems to be context-dependent. Regarding the role of BAP1 in CRC, only two studies have explored the association between BAP1 expression and prognosis, demonstrating conflicting results[17, 18]. One study demonstrated that low BAP1 expression was an independent factor for poor prognosis in patients with CRC[18]. However, another study demonstrated that elevated BAP1 expression was observed in colon cancer and was significantly associated with poor prognosis, probably through the regulation of cell proliferation, apoptosis, and DNA replication[17]. In the present study, BAP1 expression was rarely observed in normal gland cells of the colon. However, BAP1 expression was observed at a significantly higher frequency in the cancer cells of primary and metastatic tumors. When assessing the association between BAP1 expression in primary and liver metastases and prognosis in patients with CRC, both OS and DFS were significantly shorter in patients with high BAP1 expression. Moreover, an increase in the BAP1 staining score from primary to metastatic tumors was significantly associated with a shorter OS, suggesting that high BAP1 expression is associated with malignant potential.

During progression, cancer cells acquire a phenotypic change from epithelial to mesenchymal and exhibit aggressive behavior. This phenomenon, the so-called EMT, plays a critical role in cancer progression, metastasis, and recurrence after curative treatment for primary lesions[21, 22]. Our *in vitro* data demonstrate that BAP1 promotes cell migration and invasion. Cell invasion and migration are fundamental steps in the cancer metastatic cascade induced by EMT[23]. Upon overexpression of BAP1, the expression of E-cadherin, an epithelial marker, decreased, whereas that of vimentin, a mesenchymal marker, increased. In addition, the expression of MMP2 and MMP9, which are essential proteases for cell migration, increased.

Our data also demonstrate that BAP1 expression is associated with colony formation in CRC cells, suggesting that BAP1 is involved in CSC-like properties. Cancer stemness is an important mechanism underlying the malignant behavior of cancers[24]. We previously demonstrated that CSC-like properties are significantly associated with long-term outcomes in patients with CRC and CRLM[25]. In this study, our data indicated that patients with high CSC-like properties developed unresectable recurrences earlier than those with low CSC-like properties. With regard to the relationship between EMT and CSC-like properties, previous studies indicate that cancer cells acquire CSC-like properties during EMT in several cancers, including CRC[26–29]. Collectively, our data indicated that BAP1 may promote cell migration and invasion through EMT-related genes, at least in part, and increase CSC-like properties. These processes may lead to early recurrence and poor OS in patients with high BAP1 expression.

Another hypothesis may be proposed regarding the molecular mechanisms underlying the oncogenic roles of BAP1: Qin et al. found that BAP1 is a KLF5 deubiquitinase. Stabilization of KLF5 by BAP1 induces tumorigenicity and distant metastasis, suggesting an oncogenic role for BAP1 in breast cancer[15]. Our previous study demonstrated that KLF5 promotes cell proliferation and CSC-like properties, resulting in shorter survival of patients with CRLM[30]. Therefore, the interactions between BAP1 and KLF5 should be assessed in future studies.

The present study has some limitations. First, all clinical data were retrospectively collected from the database of a single institution. Therefore, selection bias may have affected the clinical data analysis. Moreover, all patients included in the survival analyses in the present study had liver metastases. Therefore, the role of BAP1 in CRC patients without liver metastases has not yet been determined. Second, although our *in vitro* experiments partially demonstrated the mechanisms behind the clinical data, the molecular pathways of our results remain unclear and require further analysis, including *in vivo* experiments.

In conclusion, high BAP1 expression in liver metastases is an efficient predictor of a poor prognosis. The malignant potential of cells with high BAP1 expression may be due to their high proliferative, migratory, invasive, and CSC-like properties. Further studies are necessary to explore the precise mechanisms through which BAP1 accelerates CRC cell malignancy.

Declarations

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Author contributions

Y.O. and N.S. designed the study and acquired data. Y. O., N. S., T. T., S. T., and D. S. analyzed and interpreted the data. Y.O. drafted the manuscript. G. O., H. M., and M. O. critically revised the manuscript. All authors have reviewed and approved the manuscript for publication.

Additional information

Competing interests: The authors declare no competing interests.

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Figures

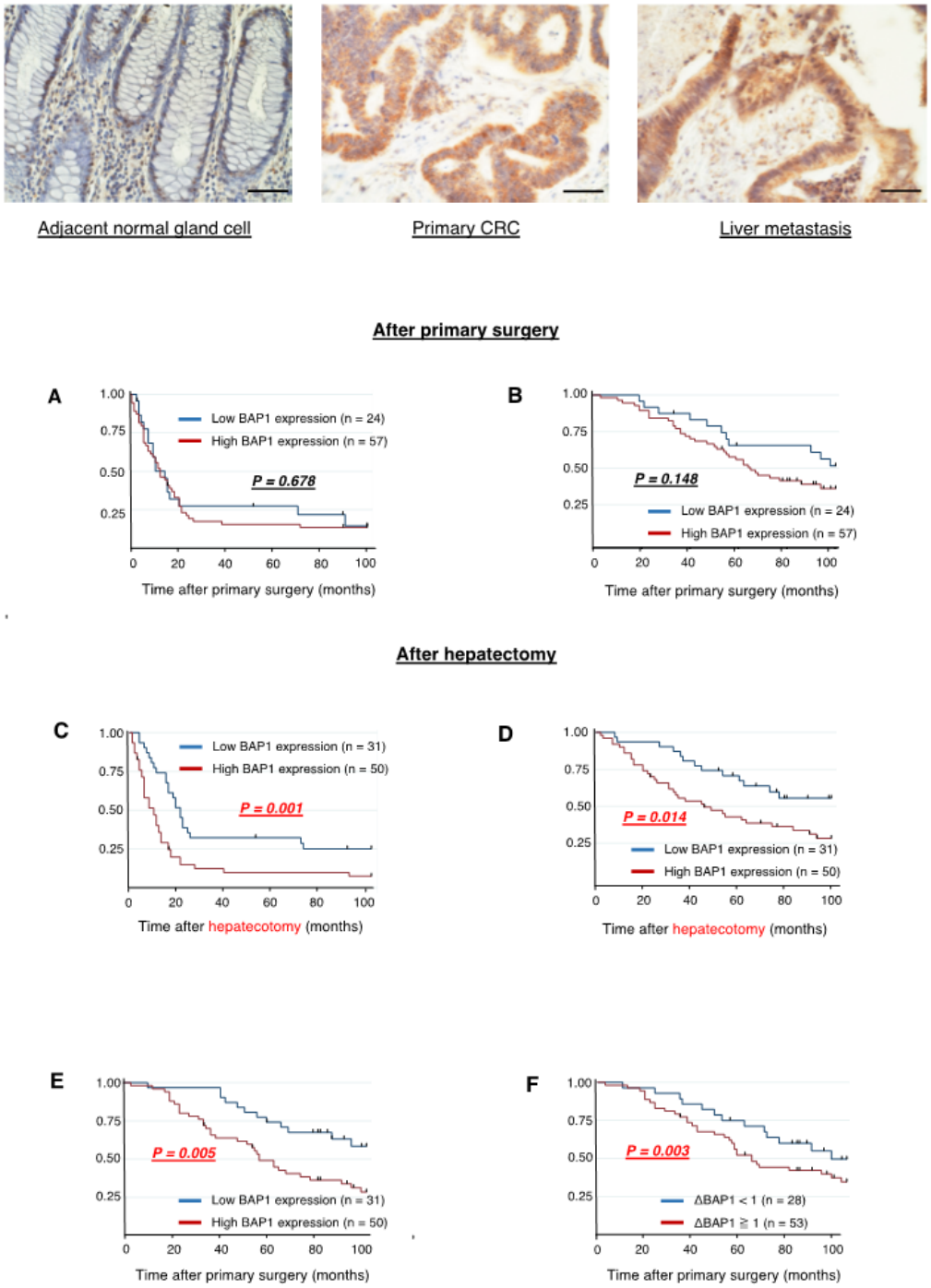

Fig.1

Figure 1

Immunohistochemistry analysis for BRCA-1 associated protein 1 (BAP1) expression in adjacent normal gland, colorectal cancer (CRC), and colorectal liver metastases cells. Scale bar = 20 μm. Kaplan–Meier analysis for disease-free survival (A, C) and overall survival (B, D, E, F) based on BAP1 expression in primary tumors (A, B) and liver metastases (C, D, E).

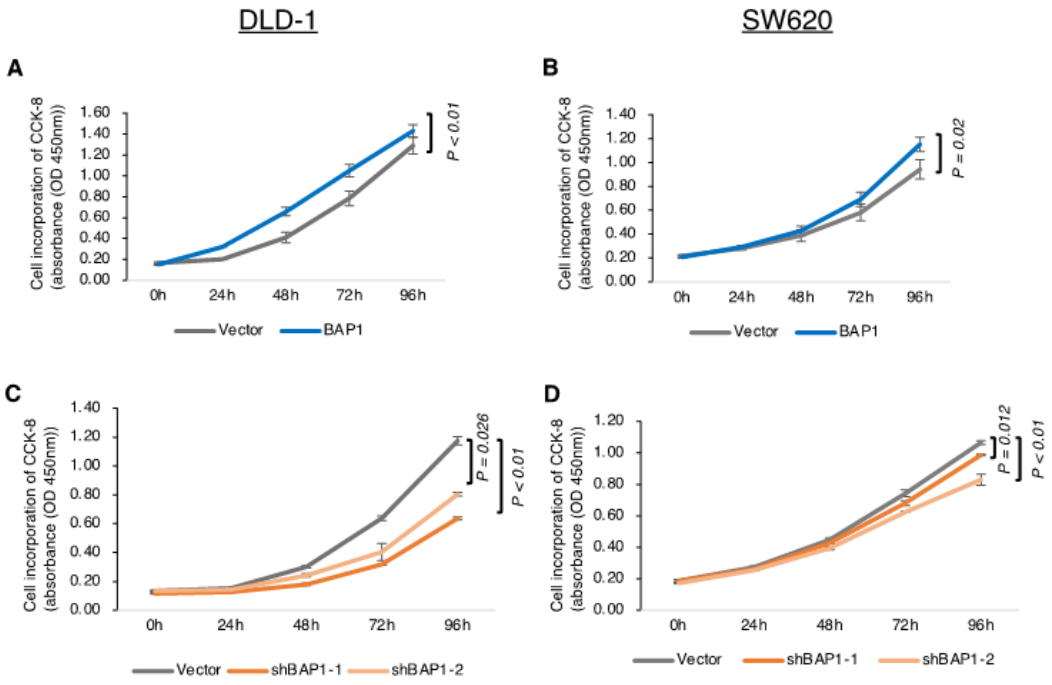

Fig.2

Figure 2

Cell proliferation assay using the CCK-8 assay in DLD-1 and SW620 cells with BAP1 overexpression (A, B) or knockdown (C, D). Values are expressed as the mean ± SD of three independent experiments.

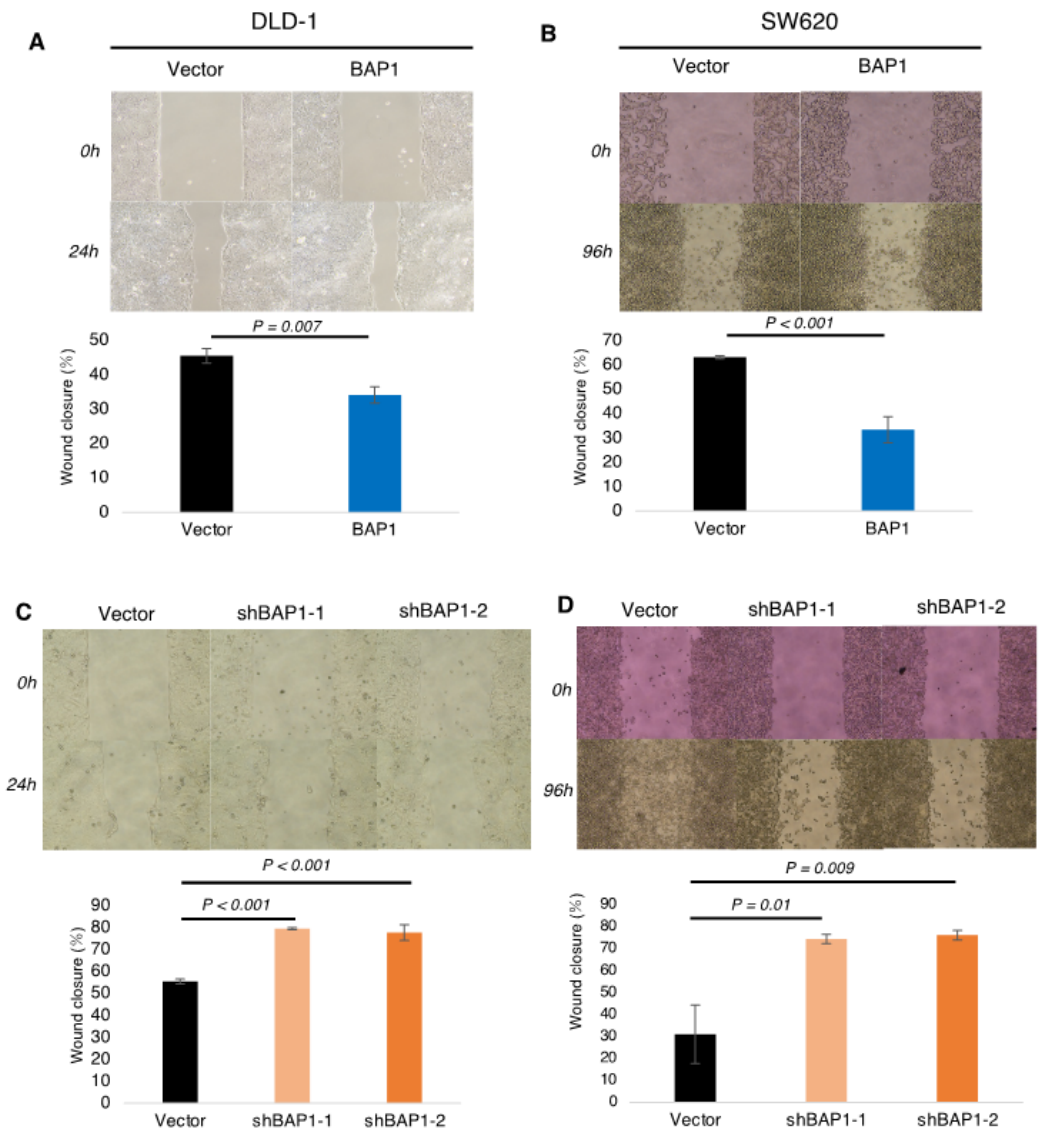

Fig.3

Figure 3

Wound-healing, Transwell migration, and Matrigel invasion assays in DLD-1 and SW620 cells with BAP1 overexpression (A, B) or knockdown (C, D). Values are expressed as the mean ± SD of three independent experiments.

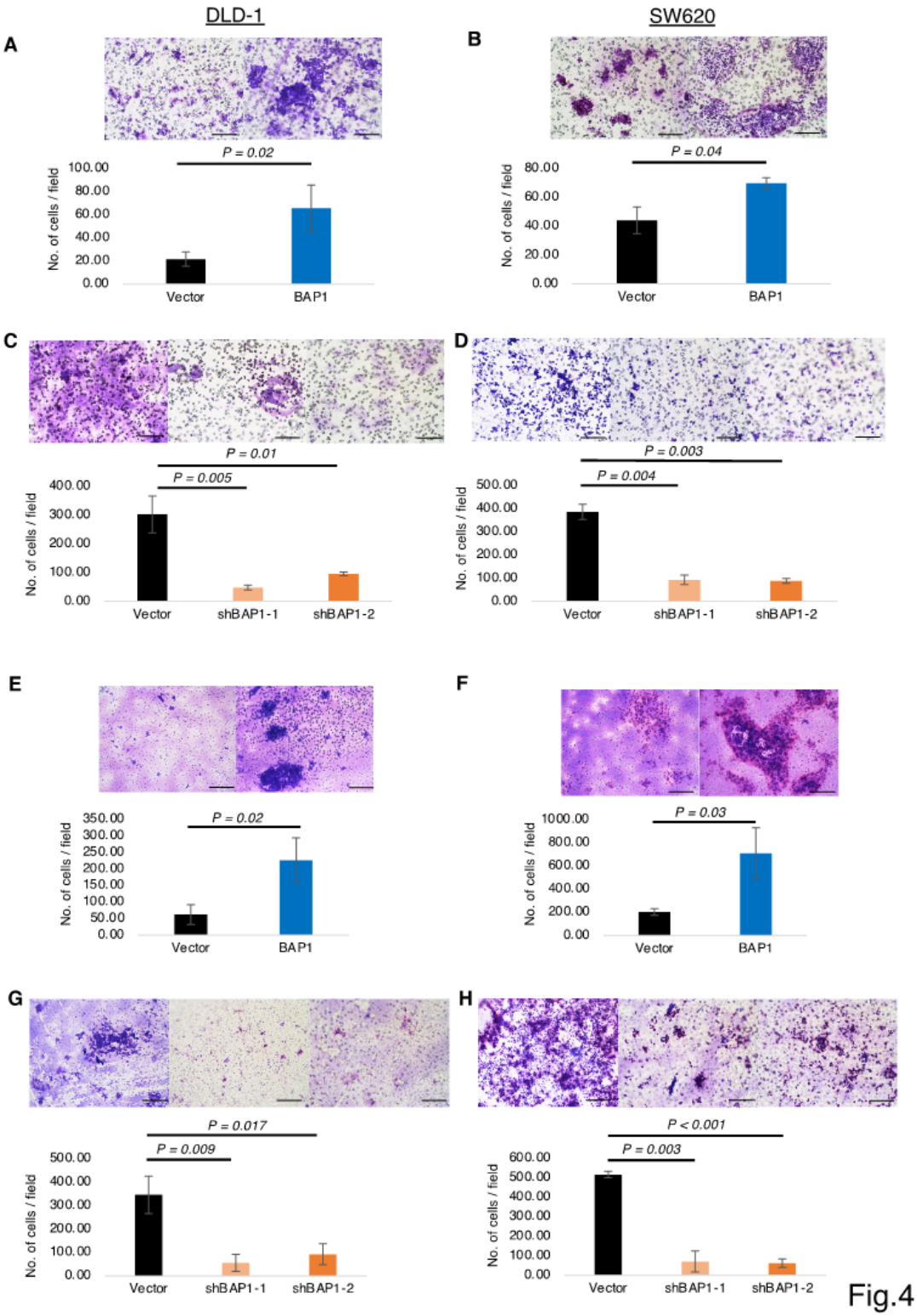

Figure 4

Transwell migration and Matrigel invasion assays in DLD-1 and SW620 cells with BAP1 overexpression (A, B), (E, F) or knockdown (C, D), (G, H). Values are expressed as the mean ± SD of three independent experiments. Scale bar = 40 μm.

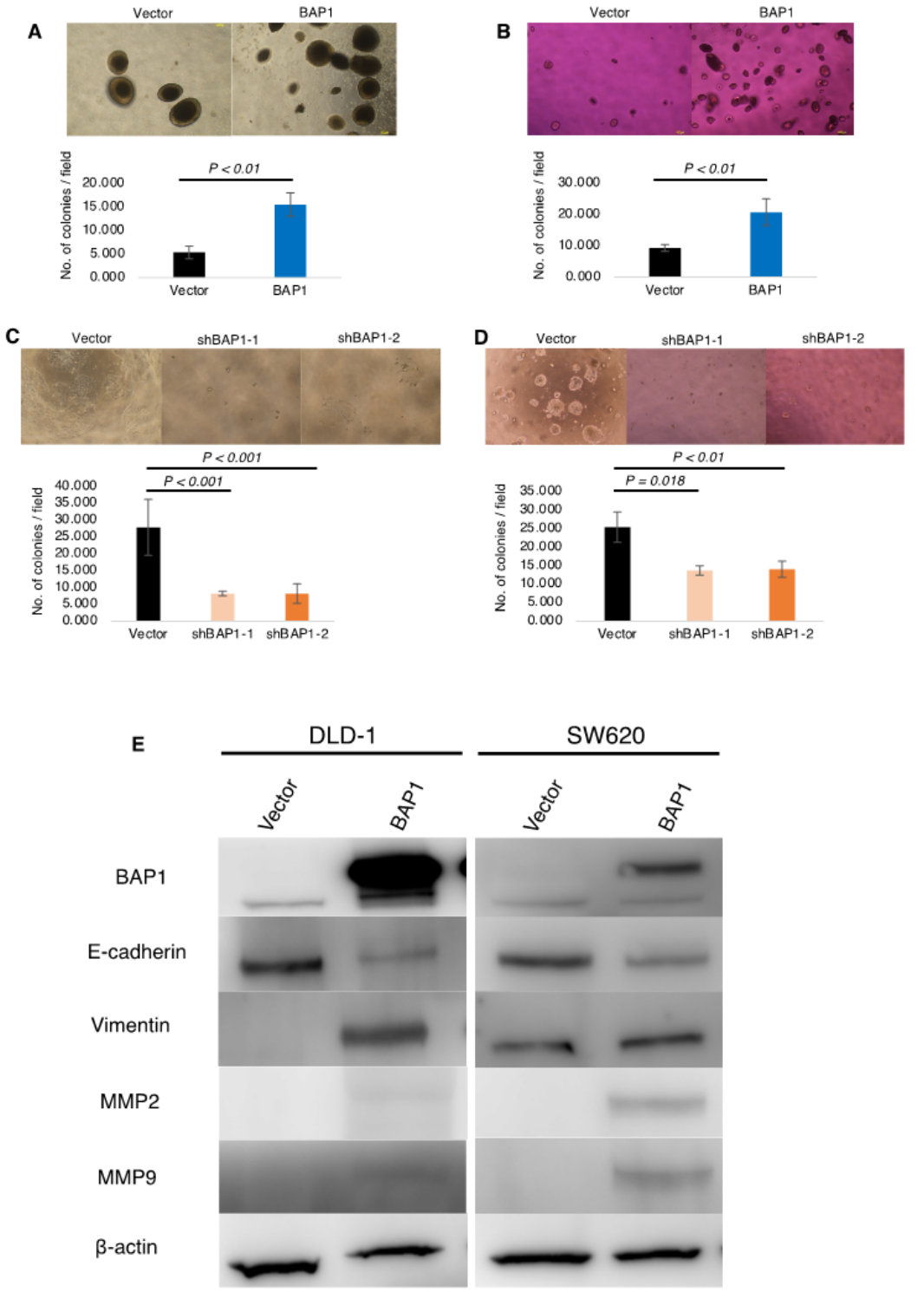

Fig.5

Figure 5

Colony formation assays on DLD-1 and SW620 cells with BAP1 overexpression (A, B) or knockdown (C, D). Values are expressed as the mean ± SD of three independent experiments. The effects of BAP1 overexpression were evaluated using western blot analysis in DLD-1 and SW620 cells (E). The band intensities were normalized to that of β-actin.

Supplementary Files

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- TableFigureSupplementary.pdf